Increase in Serum Amylase and Resistive Index After Kidney Transplant Are Biomarkers of Delayed Graft Function

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Abstract. Background: Both amylase and resistive index (RI) are routinely measured after kidney transplant and proposed as markers of delayed graft function (DGF). Material and Methods: This retrospective cross-sectional study analyzed amylase and RI in 269 renal transplant recipients before and after transplantation, and at discharge. An increase above 20% of total amylase with/without RI>0.7 were evaluated as prognostic markers of DGF, hospitalization length and risk of rejection. Results: Serum amylase increase >20% was found in 103/269 (38.3%) patients who showed DGF (45.6% vs. 25.3%, p=0.001) and had lower estimated glomerular filtration rate compared to those with an amylase increase <20% (42.0±21.7 vs. 49.8±23.2 ml/min, p=0.007). The double condition consisting of concomitant amylase increase >20% and RI>0.7 was associated with higher DGF occurrence (65% vs. 24%, p<0.001), longer hospital stay, lower eGFR at discharge, and higher risk of rejection. Conclusion: Patients with concomitant amylase increase >20% and RI>0.7 might require closer monitoring to diagnose DGF early and modify the therapeutic approach accordingly.

Kidney transplant is the treatment of choice for patients with end-stage renal disease (1). It guarantees higher survival rates than hemodialysis or peritoneal dialysis for all patients, including the elderly (2, 3) and reduces the death risk compared to patients in waiting list for renal transplant by up to 41% (4). Surgical techniques and postoperative procedures have been significantly improved, but long-term graft survival is still a clinical challenge. In particular, biological markers that specifically reflect the presence of early lesions are lacking. Creatinine and cystatin C are commonly used to follow renal function after transplant. However, creatinine levels increase only when at least 50% of graft function is lost, without providing any information about the site or type of lesions (5), and cystatin C levels may be affected by high doses of steroids used during perioperative procedures (6). The early identification of cellular and tissue damage is pivotal in order to determine the rejection risk and promptly modify immunosuppressive therapy (7). Therefore, many efforts have been made to identify novel biomarkers that could accurately and sensitively reveal early renal damage after transplantation. Recently, additional molecules, including gelatinase-associated lipocalin (8, 9), kidney injury molecule 1 (10), and interleukin 18 have been investigated to discriminate renal lesions after transplant and to evaluate the prognosis and efficacy of the therapeutic regimen (11).

Serum amylase has been proposed as a novel marker to ascertain early renal function immediately after transplant (12). During chronic renal failure with substitutive treatment and in the absence of acute pancreatitis, amylase levels increase by up to 3-fold over the normal threshold and tend to decrease after transplantation (13). Consistently, it has been reported that in 67% of patients with graft failure and in 60% with acute cellular rejection amylase remains high and is inversely correlated with creatinine clearance (12).

The resistive index (RI) is a hemodynamic parameter obtained by Doppler sonography commonly used to predict kidney transplant outcome (14). Postoperative RI evaluation confirmed good allograft status after 24 hours from transplantation (15) and a lower RI after 1 week was associated with satisfying graft function at 6 months (16). Conversely, RI>0.8 after 3, 12 and 24 months was associated

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transplantation and combined liver and kidney transplantation, but not those who underwent combined heart and kidney transplantation.

Both amylase and RI are routinely measured after kidney transplantation. The changes in these parameters may provide further clinical information about the occurrence of delayed graft function (DGF), defined as the need for dialysis during the first post-transplant week. Therefore, in this study we investigated whether the combined increase of serum amylase and RI may be indicative of renal function recover in the early post-transplant period.

Patients and Methods

Study design. This retrospective cross-sectional study analyzed clinical data of 269 patients who received renal transplant at the Nephrology Unit of S. Orsola Hospital, Bologna, Italy, from January 2005 to December 2008. The analysis included patients who had single kidney transplantation, from living or deceased donor, double kidney transplantation and combined liver and kidney transplantation, but not those who underwent combined heart and kidney transplantation.

Measurements. Clinical and demographic data were collected during the waiting period and hospital stay for kidney transplantation. Total amylase levels were assessed before transplantation, immediately after transplantation, after 5-7 days and at discharge. Before transplantation, at day 1 and at discharge, creatinine levels were also recorded. These values were used to calculate the estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (18). Peak systolic velocity (Vmax) and minimal diastolic velocity (Vmin) were determined at day 1, 2, 3, 4, 7 and 14 and at discharge. RI was calculated as (Vmax−Vmin)/Vmax. RI ≤0.7 was considered as normal (19).

Other clinical information, including anthropometric parameters, previous kidney diseases, previous dialysis treatment, and immunosuppressive therapy were collected from electronic health records of the waiting list procedure and the hospital stay.

Variables. An increase above 20% of total amylase after transplantation with/without RI>0.7 were evaluated as prognostic markers of DGF, length of hospital stay and risk of rejection.

Statistical analysis. Continuous variables are expressed as the mean±standard deviation and compared with Student t-test. Categorical variables are presented as absolute numbers and percentages, and compared by chi-square test or Fisher test (if

### Table I. Baseline characteristics of the total population of patients (n=302). Continuous variables are presented as mean±standard deviation, categorical variables as absolute numbers and percentages.

| Characteristic                                  | Value       |
|-------------------------------------------------|-------------|
| Age, years                                      | 51.1±12.5   |
| Female                                          | 99 (32.8%)  |
| Nephropathy, N (%)                              | 89 (29.4%)  |
| Glomerulonephritis                              | 61 (20.2%)  |
| Cystic nephropathy                              | 30 (9.9%)   |
| Chronic tubular-interstitial nephropathy        | 28 (9.3%)   |
| Chronic vascular nephropathy                    | 7 (2.3%)    |
| SLE-associated nephropathy                      | 6 (2.0%)    |
| Vascularitis                                    | 6 (2.0%)    |
| Alport’s syndrome                               | 3 (1.0%)    |
| Diabetic nephropathy                            | 25 (8.3%)   |
| Other causes                                    | 42 (13.9%)  |
| Pre-transplantation dialysis, N (%)             | 242 (80.1%) |
| Hemodialysis                                    | 51 (16.9%)  |
| Peritoneal dialysis                             | 9 (3.0%)    |
| Transplantation, N (%)                          | 252 (83.4%) |
| Single renal transplant from deceased donor     | 9 (3.0%)    |
| Single renal transplant from living donor       | 22 (7.3%)   |
| Double renal transplant                         | 20 (6.6%)   |
| Combined liver-kidney                           |             |
| After transplantation, N (%)                    |             |
| Patients who received at least one substitutive |             |
| treatment during the hospital stay              |             |
| Delayed graft function                          | 99 (32.8%)  |
| Primary non function                            | 9 (3.0%)    |

SLE: Systemic lupus erythematosus.

### Table II. Baseline characteristics of patients with total amylase increase above 20% (Group 1) and below 20% (Group 2). Continuous variables are presented as mean±standard deviation, categorical variables as absolute numbers and percentages.

| Characteristic                | Group 1 (N=103) | Group 2 (N=166) | p-Value |
|------------------------------|-----------------|-----------------|---------|
| Female                       | 35 (33.9%)      | 53 (31.9%)      | n.s.    |
| Age, years                   | 53.2±11.0       | 50.2±12.4       | 0.045   |
| Dialysis history, months     | 52.7±2.2        | 56.1±44.7       | n.s.    |
| Pre-transplantation dialysis |                 |                 |         |
| Hemodialysis                 | 70 (68.0%)      | 143 (86.1%)     | <0.001  |
| Peritoneal dialysis          | 27 (26.2%)      | 20 (12.0%)      |         |
| Type of donor                |                 |                 |         |
| Deceased                     | 101 (98.1%)     | 159 (95.8%)     | n.s.    |
| Living                       | 2 (1.9%)        | 7 (4.2%)        |         |
| Number of patients with DGF  | 47 (45.6%)      | 42 (25.3%)      | 0.001   |
| Diuresis, ml/day             | 1640±636.1      | 1878±453.8      | 0.001   |
| Creatinine, mg/dl            | 2.2±1.7         | 1.9±1.6         |         |
| eGFR at discharge, ml/min    | 42.0±21.7       | 49.8±23.2       | 0.007   |

DGF, Delayed graft function; eGFR, estimated glomerular filtration rate; RI, resistive index. A p-value of less than 0.05 was considered statistically significant (n.s.: not significant).
sample size was less than 5). Ordinal variables were compared with the non-parametric Mann-Whitney test. A p-value lower than 0.05 was considered as significant. All statistical analyses were performed with SPSS software (IBM SPSS Statistics, version 20.0; IBM Corp., Armonk, NY, USA).

Results

Patient characteristics. From January 2005 to December 2008, 302 patients underwent kidney transplant. Baseline characteristics, transplantation procedure and parameters related to the post-transplant clinical course are described in Table I. Median age was 51.1 years and 32.8% of patients were females; overall 97.0% of patients were treated with either hemodialysis or peritoneal dialysis before transplant. Two hundred and seventy-four patients received an organ from a deceased donor (252 of them single, and 22 double renal transplant) and nine from living donor; 20 patients had a combined transplant of liver and kidney.

Amylase determination. Data on total amylase levels before and immediately after transplantation were available for 269 out of 302 patients. Among them, 106/269 patients (39.4%) had amylase <110 U/l, and 163/269 (60.6%) were hyperamylemic with amylase ≥110 U/l. After transplantation a rise in amylase of more than 20% was observed in 51/106 (48.1%) patients with normal baseline level and in 52/163 (31.9%) hyperamylemic patients (p=0.006).

Therefore, two groups were identified in the study population: Group 1: patients who showed an amylase rise above 20% (n=103); and group 2: patients without this increase (n=166).

In group 1, six patients were affected by acute pancreatitis and eight had biliary lithiasis; in group 2, eight cases of biliary lithiasis and none of acute pancreatitis were reported.

As shown in Table II, gender distribution, the type of donor and duration of dialysis prior to transplantation were similar for the two groups; however, patients without 20% rise of serum amylase level were more frequently treated with hemodialysis (p<0.001). After transplantation, the percentage of patients who had DGF was significantly higher in group 1 than in group 2 (p=0.001) and eGFR at discharge was lower in group 1 than in group 2 (p=0.007). The percentage of patients with RI>0.7 was significantly higher in group 1 at days 1, 7 and 14 (p=0.002, p=0.004, and p=0.011, respectively).

Resistive index. Considering the overall population of 269 renal transplant recipients, we found that the patients with DGF were 89 (33.1%) and showed the highest RI. In particular, among patients with DGF, RI>0.7 was reported in 59/89 (66.3%) of them at day 1 (p=0.011), 62/89 (69.7%) at day 4 (p=0.014), 57/89 (64.0%) at day 7 (p=0.001), 58/89 (65.2%) at day 14 (p<0.001) and 53/89 (59.6%) at discharge (p=0.0039).

A value of p<0.05 was considered statistically significant.

Table III. Length of hospital stay and serum creatinine at discharge according to resistive index (RI) >0.7. Continuous variables are presented as mean±standard deviation, categorical variables as absolute number.

|                  | Patients, N | Length of hospital stay, days | p-Value | Serum creatinine, mg/dl | p-Value |
|------------------|-------------|-------------------------------|---------|-------------------------|---------|
| Day 1            | RI≤0.7      | 178                           | 18.7±6.6| 1.6±1.16                | 0.01    |
|                  | RI>0.7      | 91                            | 23.9±8.8| 2.5±2.18                |         |
| Day 4            | RI≤0.7      | 170                           | 20.3±7.8| <0.001                  | 1.5±0.58| <0.001 |
|                  | RI>0.7      | 99                            | 25.8±11.3| <0.001                  | 2.7±2.20|         |
| Day 7            | RI≤0.7      | 159                           | 18.5±6.0| <0.001                  | 1.5±0.81| 0.001  |
|                  | RI>0.7      | 40                            | 25.0±10.1| <0.001                  | 2.1±1.53|         |
| Day 14           | RI≤0.7      | 159                           | 19.2±6.3| <0.001                  | 1.5±0.61| <0.001 |
|                  | RI>0.7      | 40                            | 25.8±9.6| <0.001                  | 2.2±1.58|         |
| At discharge     | RI≤0.7      | 204                           | 20.3±8.5| 0.0410                  | 1.6±0.76| 0.0416 |
|                  | RI>0.7      | 65                            | 22.8±8.7| <0.001                  | 1.9±1.11|         |

A p-value less than 0.05 was considered statistically significant (n.s.: not significant).

Table IV. Patients with total amylase increase of >20% and resistive index (RI) >0.7 after transplantation (double condition). Categorical variables are presented as absolute numbers and percentages (calculated on the overall population of 269 renal transplant recipients).

|                  | Patients with total amylase increase >20% and RI>0.7 | Patients with total amylase increase <20% and RI≤0.7 | p-Value |
|------------------|----------------------------------------------------|----------------------------------------------------|---------|
| Day 1            | 26/269 (9.7%)                                      | 24/269 (8.9%)                                      | n.s.    |
| Day 4            | 22/269 (8.2%)                                      | 20/269 (7.4%)                                      | n.s.    |
| Day 7            | 41/269 (15.2%)                                     | 43/269 (16.0%)                                     | n.s.    |
| Day 14           | 37/269 (13.8%)                                     | 40/269 (14.9%)                                     | n.s.    |
| At discharge     | 22/269 (8.2%)                                      | 35/269 (13.0%)                                     | n.s.    |

A p-value less than 0.05 was considered statistically significant (n.s.: not significant).
The relationship of RI>0.7 with the length of hospital stay, serum creatinine levels at discharge and the need for hemodialysis were also analyzed. Patients with RI>0.7 at postoperative day 1, 4, 7 and 14 and at discharge had significantly longer hospital stay and serum creatinine level after transplant (Table III). The need for hemodialysis was also significantly more prevalent in these patients (data not shown).

### Double condition

The number and percentages (calculated on the whole population of 269 renal transplant recipients) for those patients who concomitantly experienced an increase above 20% in amylase level and a RI>0.7, defined as ‘double condition’, at each experimental time are detailed in Table IV.

DGF occurrence was evaluated in patients with the double condition at day 1, when the need for additional treatment is more likely: among these patients, 65% had DGF compared to 24% of patients without the double condition \((p<0.001)\).

Table V compares eGFR, length of hospital stay, rate of transplant rejection at days 1, 4, 7, 14 post-transplant and at discharge in patients with the double condition \(\textit{versus}\) those without the double condition. The patients with the double condition had significantly poorer graft function, longer hospital stays, and higher rejection rates at all the experimental time points compared to those without the double condition.

### Discussion

In this cross-sectional study, we investigated whether the increase of amylase and RI are useful parameters for predicting early graft function. A rise above 20% of serum amylase after transplantation was observed in patients with DGF and lower eGFR at discharge. The majority of these patients also had an elevated RI. In particular, we found a RI>0.7 in patients who needed hemodialysis after transplant, had increased serum creatinine at discharge, and who experienced longer hospital stays. The concomitant presence of an increase in amylase level above 20% and high RI, the double condition, was described in patients with DGF and lower eGFR at discharge. These patients had longer hospital stays compared with patients without the double condition and had a greater graft rejection rate. Therefore, serum amylase increase and high RI, both singularly and in combination, were significantly related to postoperative DGF, length of hospitalization and poorer graft function at discharge.

In our population, the prevalence of hyperamylasemia before transplant was 60.6% and serum amylase levels were increased 1.5 times compared to the normal value. Poor and discordant data are reported in literature about hyperamylasemia prevalence in patients with end-stage renal disease. In a study by Borazan \textit{et al.}, hyperamylasemia was noted in 21% of patients on hemodialysis and 33% of those on peritoneal dialysis \((20)\). Yilmaz \textit{et al.} described a prevalence of 67% in 21 hemodialyzed patients \((12)\); Dardamanis \textit{et al.} reported hyperamylasemia before transplant in 100% of 74 patients \((21)\). The discrepancy may be ascribable to the limited sample size of the currently available studies. After transplantation, amylase levels tend to normalize and the prevalence of hyperamylasemia decreases \((12, 22)\). However, we observed a rise above 20% in 51 out of 106 (48.1%) patients with normal levels before transplantation. This increase may be considered as being independent from the pre-transplant amylase level and specific for each patient. In six patients, hyperamylasemia was caused by acute pancreatitis, while in other patients, it may have been related to lack of renal catabolism.

RI, the second parameter considered in our analysis, has been proposed as non-invasive parameter for evaluating

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Table V. Estimated glomerular filtration rate (eGFR), length of hospital stay and rejection rate in patients with and without the double condition (concomitant amylase increase of >20% and resistive index >0.7). Continuous variables are presented as mean±standard deviation, categorical variables as percentages. A value of \(p<0.05\) was considered statistically significant.

|                     | Double condition | Patients, N | eGFR, ml/min | p-Value | Hospital stay, days | p-Value | Rejection rate, % | p-Value |
|---------------------|------------------|-------------|--------------|---------|--------------------|---------|------------------|---------|
| Day 1               | No               | 243         | 49.3±22.1    | 0.002   | 19.3±6.9           | <0.001  | 6.3              | 0.011   |
|                     | Yes              | 26          | 34.6±21.8    | 0.007   | 20.1±7.7           | <0.001  | 7.4              | <0.001  |
| Day 4               | No               | 247         | 48.8±21.8    | 0.006   | 19.5±7.1           | <0.001  | 6.5              | 0.004   |
|                     | Yes              | 22          | 34.4±27.4    | 0.001   | 27.5±11.7          | 21.9    |                  |         |
| Day 7               | No               | 228         | 48.4±22.3    | 0.006   | 19.2±8.3           | <0.001  | 8.2              | 0.420   |
|                     | Yes              | 41          | 38.2±19.5    | <0.001  | 29.2±10.4          | 18.9    |                  |         |
| Day 14              | No               | 232         | 48.1±22.6    | <0.001  | 20.2±8.3           | <0.001  | 27.1±10.2        | 13.6    |
|                     | Yes              | 37          | 33.6±19.3    | 0.001   | 29.2±10.4          | 18.9    |                  |         |
| At discharge        | No               | 247         | 48.1±22.6    | <0.001  | 20.2±8.3           | <0.001  | 8.2              | 0.420   |
|                     | Yes              | 22          | 34.9±13.7    | <0.001  | 27.1±10.2          | 13.6    |                  |         |
allograft dysfunction but there is controversial evidence on its clinical utility. In the first 24 hours after transplant, renal Doppler ultrasonography was useful in the diagnosis of primary graft dysfunction (23); indeed, RI and power Doppler correlated well with DGF, time of renal function recovery and number of dialysis sessions (24). Recipient age, previous diabetes mellitus and DGF were the main determinants of high RI immediately after kidney transplant (25). Our results indicated that the prevalence of RI>0.7 even at day 1 after transplant was significantly higher in patients with DGF and compromised renal function at discharge. The length of hospital stay was higher in patients with high RI and was further extended in those with the double condition at each experimental time point compared to patients with only high RI. The rejection rate was also significantly increased in patients with the double condition on postoperative days 1, 4, 7 and 14. Therefore, monitoring changes in both serum amylase level and RI immediately after transplantation may be useful to identify the potential risk of graft dysfunction early and modify the therapeutic approach. In the presence of normal amylase and RI, it may be possible to reduce the need to recourse to biopsy in order to examine the graft status (26).

It should be noted that the number of patients who had the double condition was too low to be exhaustive and a larger multicentric study should be conducted to confirm the predictive value of combined amylase and RI examination. Furthermore, the time points of serum amylase determination before and after transplant should be exactly defined in order to standardize the protocol.

In conclusion, the detection of concomitant increased serum amylase and RI>0.7 was predictive of DGF early after kidney transplant. A low GFR at discharge and longer hospital stay were noted when both serum amylase and RI were altered. Therefore, patients with the double condition might be specifically monitored in order to diagnose DGF early and modify the therapeutic approach accordingly.

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