Impact of dialysis practice patterns on outcomes in acute kidney injury in Intensive Care Unit

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Abstract

Aim: Recent advances in dialysis therapy have made an impact on the clinical practice of renal replacement therapy (RRT) in acute kidney injury (AKI) in Intensive Care Unit (ICU). We studied the impact of RRT practice changes on outcomes in AKI in ICU over a period of 8 years. Subjects and Methods: AKI patients requiring RRT in ICU referred to a nephrologist during two different periods (period-1: Between May 2004 and May 2007, n = 69; period-2: Between August 2008 and May 2011, n = 93) were studied. The major changes in the dialysis practice during the period-2, compared to period-1 were introduction of prolonged intermittent RRT (PIRRT), early dialysis for metabolic acidosis, early initiation of RRT for anuria and positive fluid balance and use of bicarbonate-based fluids for continuous RRT (CRRT) instead of lactate buffer. The primary study outcome was 28-day hospital mortality. Results: The mean age was 53.8 ± 16.1 years and 72.6% were male. Introduction of PIRRT resulted in 37% reduction in utilization of CRRT during period-2 (from 85.5% to 53.7%). The overall mortality was high (68%) but was significantly reduced during period-2 compared to period-1 (59% vs. 79.7%, P = 0.006). Metabolic acidosis but not the mode of RRT, was the significant factor which influenced mortality. Conclusions: Adaption of PIRRT resulted in 37% reduction of utilization of CRRT. The mortality rate was significantly reduced during the period of adaption of PIRRT, possibly due to early initiation of RRT in the latter period for indications such as anuria and metabolic acidosis.

Keywords: Acute kidney injury, continuous renal replacement therapy hemodialysis, prolonged intermittent renal replacement therapy

Introduction

Acute kidney injury (AKI) is common in critically ill patients and occurs in 18–65% of adult patients admitted to Intensive Care Unit (ICU).[1-3] Up to 20% of patients who develop AKI require renal replacement therapy (RRT) in ICU[3,4] and carry high mortality, in excess of 50%. While the incidence of AKI requiring dialysis is increasing,[6] the mortality in them is decreasing over the last several years in the developed world.[9]

The practice of dialysis in critically ill patients with AKI varies widely across the world. There has been a rapid and striking increase in our knowledge of application of RRT in AKI in the last decade, culminating in publication of guidelines for RRT in AKI.[10] However, there is still a lack of consensus regarding timing, general indications to initiate RRT and the choice of...
modality of RRT, which partly account for the wide variability in practice of RRT in AKI across the world. In contrast to the developed world, the practice patterns of RRT in ICU for AKI in developing countries are likely to be different, but studied inadequately. Several factors such as different epidemiology, time to seeking of medical help, resource and economic constraints and lack of technical expertise may contribute to this wide difference.

The RRT in ICU is provided either as a continuous therapy in the form of continuous RRT (CRRT) or intermittently in the form of intermittent hemodialysis (IHD). In recent years, hybrid therapy, namely, prolonged intermittent RRT (PIRRT) or slow low-efficiency dialysis has evolved as an alternate to CRRT to provide intermittent RRT in ICU. PIRRT incorporates the advantages of CRRT such as increased duration of dialysis, reduced blood and dialysate flow rates, which improves the hemodynamic tolerance. PIRRT is practiced in several ICUs across the world and their experience has been reported in the last decade. The major factor determining the choice of RRT modality in ICU is hemodynamic tolerance and, hence, CRRT by virtue of its better hemodynamic tolerance is the preferred modality in most ICU’s in the developed world, despite a lack of survival advantage in clinical studies. In contrast to the developed world, CRRT is not widely used to dialyze patients of AKI in the developing world due to lack of finances, resource, and trained personnel.

We studied patients of AKI requiring RRT over one decade and explored the changes in the dialysis practice patterns during this period and its impact on outcomes in AKI in ICU.

Subjects and Methods

The setting of the study was an ICU of a tertiary referral hospital in South India. The ICU admitted heterogeneous group of critically adult patients and the bed strength was 30 in 2004, which increased to 74 in 2011. The Hospital Ethics Committee approval was obtained to conduct the study.

Study population and data collection

All patients of AKI in ICU requiring dialysis referred to a single nephrologist (first author) during two different periods (Period-1: Between May 2004 and May 2007, Period-2: Between August 2008 and May 2011) were included in the study. The patients who had a baseline serum creatinine (Scr) of >1.3 mg/dL or thought to have underlying preexisting renal dysfunction based on the clinical investigations and those who were discharged against medical advice were excluded from the study. The data was collected prospectively. The baseline demographic data was collected at the time of initiation of RRT and included age, gender, urine output in the preceding 24 h, type of initial RRT, presence of sepsis, setting of AKI (medical, surgical, or obstetrical), requirement of ventilator and inotrope support and blood pressure. The severity of illness was assessed by Acute Physiology and Chronic Health Evaluation (APACHE) II score at the time of initiation of RRT. The laboratory variables done within 8 h before initiation on RRT were blood urea nitrogen (BUN), Scr, hemoglobin, serum albumin, arterial blood gas analysis, and blood glucose.

Definitions

AKI was defined as per risk, injury, failure, loss, and end-stage kidney criteria. Sepsis was defined according to criteria proposed by the American College of Chest Physicians/Society of Critical Care Consensus Conference. Anuria was defined as urine output of less than 100 mL/day. When RRT was initiated within 24 h of admission to ICU, the urine output preceding the commencement of RRT was extrapolated to 24-h to define anuria.

RRT was administered at the discretion of a single nephrologist, who also decided the timing, initial modality, dose and anticoagulation for RRT.

Indications for initiation of renal replacement therapy

Broadly, the indications to apply RRT during period-1 were (1) severe azotemia with BUN >100 mg/dL, (2) metabolic acidosis, when arterial pH was <7.2 despite administration of intravenous bicarbonate, (3) dyselectrolytemia commonly serum potassium of more than 6 mEq/L despite antikalemic therapy, and (4) fluid overload causing pulmonary edema not responding to large doses of intravenous furosemide (60–120 mg). During period-2, some modifications were done for initiation of RRT based on the emergence of new knowledge that severe organ edema caused increased mortality and acidosis was an independent risk factor for death in our population during the study period-1 (unpublished results). During study period-2, RRT was initiated when pH was <7.25 and when severe fluid retention was present despite diuretic trial. Prolonged anuria was less frequently endured during period-2.

Mode of dialysis

The choice of RRT was based on the clinical judgment and hemodynamic stability was the prime determinant of choice of RRT modality. In addition, other factors such as

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degree of fluid overload, degree of acidosis and financial constraint of patient family also influenced the choice of RRT modality, but to a much lesser extent. In general, during period-1, all hemodynamically unstable patients were initiated on CRRT, whereas during period-2 only the patients with more severe hemodynamic instability such as patients with MAP <70 mmHg or requirement of one or more inotropes, were initiated on CRRT. In general, IHD was used for hemodynamically stable patients (MAP >80 mmHg and no inotropes) during both periods. PIRRT was used only during period-2 for patients with mild hemodynamic instability (MAP >70 mmHg or need for one inotrope). When patients initiated on CRRT became hemodynamically stable after more than 48 h, they were transferred to either PIRRT or IHD. Similarly if patients on PIRRT or IHD became hemodynamically unstable while on therapy were switched to CRRT. CRRT was performed as continuous venovenous hemodiafiltration and a four-pump CRRT machine (Infomed, Germany, between 2004 and 2008, Prisma, Minneapolis, MN, USA, between 2006 and 2010) was used to deliver therapy.

**Dialysis settings**

The standard settings of CRRT were blood flow rate ($Q_b$) of 150 mL/min, dialyzate flow rate ($Q_d$) of 1000 mL/h and ultrafiltration rate (replacement fluid [RF]) of 500–1000 mL/h. Net fluid removal rate was based on the need and hemodynamic status of the individual patient. During period-1, the dialysis fluid was lactate-based solution used for peritoneal dialysis (Dianeal, Baxter) with 1.5% glucose concentration, where as during period-2 the dialysis fluid used was bicarbonate-based commercial fluid (Hemosol, Gambro). During period-1, the RF used was custom-made and was an isonatrenc fluid with or without bicarbonate, depending on the arterial pH. The RF during period-2 was bicarbonate-based fluid (Hemosol, Gambro). RF was administered by predilution method during both time periods. IHD was given for 4 h each time and was given 3–6 times a week and PIRRT was given for 6–8 h 3–6 times a week. The $Q_b$ was 200 mL/min and $Q_d$ was 500 mL/min in IHD whereas $Q_b$ was 150 mL/min and $Q_d$ was 200–300 mL/min in PIRRT. High-efficiency dialysis filter (F5 or F6, Fresenius) was used to deliver IHD and PIRRT. IHD and PIRRT were performed with no anticoagulant. Similarly continuous venovenous hemodialysis was done without anticoagulation and only in an exceptional case where the dialysis filter clotted prematurely (defined as within 24 h), heparin was used for anticoagulation. The aim of RRT was to keep BUN below 100 mg/dL and preferably below 60 mg/dL at all times after 24 h of initiation of dialysis, and the dose and frequency of dialysis was targeted to achieve these levels and was tailored to the individual needs.

**Outcomes**

The primary outcome was 28-day hospital mortality. The secondary outcomes were duration of ICU stay and dialysis dependency at discharge.

**Statistical analysis**

Student’s $t$-test for quantitative variables and Chi-square ($\chi^2$) test for qualitative variables were used for comparison. Survival analysis was performed using Kaplan–Meier curves and log-rank test was used for comparison. Univariate and multivariate logistic regression was performed to determine the factors associated with 28-day hospital mortality. A $P < 0.05$ was considered statistically significant. Statistical analysis was done using the Statistical Package for Social Science version 17.0 (SPSS Inc., Chicago, IL, USA).

**Results**

The mean age was 53.8 ± 16.1 years and 72.6% were male. Table 1 shows baseline demographic, clinical and laboratory parameters of study population. The tropical

| Table 1: Baseline demographic, clinical and laboratory parameters of the study population |
| --- |
| **Age (years)** | All patients (n=162) | Period-1 (n=69) | Period-2 (n=93) | $P$ |
| Gender (male, %) | 53.8±16.1 | 52.7±15.8 | 54.7±16.5 | 0.43 |
| Setting of AKI (%) | 122 (72.6) | 55 (79.7) | 66 (71) | 0.36 |
| Medical | 142 (87.7) | 58 (84.1) | 84 (90.3) | 0.315 |
| Surgical | 19 (11.7) | 10 (14.5) | 9 (9.7) | 0.61 |
| Obstetric | 1 (0.6) | 1 (1.4) | 0 | 0.19 |
| Pathology of AKI (%) | 7 (0.8) | 8 (1.2) | 0 | 0.98 |
| Septic ATN | 57 (33.9) | 30 (43.5) | 27 (29) | 0.069 |
| Ischemic/toxic ATN | 72 (44.4) | 43 (61.9) | 28 (30.3) | 0.085 |
| Glomerulonephritis | 2 (1.2) | 1 (1.4) | 1 (0.9) | 1 |
| Urine output (mL/24 h) | 430±578 | 306±381 | 523±676 | 0.18 |
| Anuria at initiation of RRT (%) | 57 (35.2) | 20 (28.8) | 37 (40.1) | 0.98 |
| APACHE II score | 23.9±6.7 | 24.1±6.8 | 23.9±6.9 | 0.85 |
| Mean arterial pressure (mmHg) | 78.2±13.6 | 75.8±15.3 | 79.5±11.8 | 0.085 |
| Ventilator (%) | 134 (82.7) | 75 (82.6) | 59 (62.8) | 0.97 |
| Mode of initial RRT (%) | 109 (67.2) | 59 (85.5) | 50 (53.7) | <0.001 |
| CRRT | 16 (10) | 10 (14.5) | 6 (6.5) | 0.15 |
| IHD | 37 (22.8) | 0 | 37 (39.8) | <0.001 |
| PIRRT | 57.0±29.9 | 54.82±27.9 | 58.65±31.5 | 0.42 |
| Serum creatinine (mg/dL) | 3.35±1.95 | 3.35±1.7 | 3.36±2.2 | 0.98 |
| Serum albumin (g/dL) | 2.75±0.75 | 2.67±0.71 | 2.82±0.79 | 0.22 |
| Arterial pH | 7.23±0.75 | 7.215±0.14 | 7.271±0.05 | 0.021 |
| Serum bicarbonate (mEq/L) | 16.7±5.1 | 14.4±4.7 | 18.4±4.7 | <0.001 |
| Hemoglobin (g/dL) | 10.03±2.9 | 10.5±3.2 | 9.7±2.7 | 0.07 |

AKI: Acute kidney injury; ATN: Acute tubular necrosis; RRT: Renal replacement therapy; APACHE: Acute Physiology and Chronic Health Evaluation; CRRT: Continuous renal replacement therapy; PIRRT: Prolonged intermittent renal replacement therapy; IHD: Intermittent hemodialysis; BUN: Blood urea nitrogen
diseases such as malaria, leptospirosis and dengue infection were seen in 4 (7.6%) patients during study period-1 and in 5 (5.2%) patients during study period-2. The details of the indications for dialysis during two periods are shown in Figure 1. Anuria was more common at the time of initiation of RRT during period-1 compared to period-2, but was not statistically significant (43.5% vs. 29%, \( P = 0.069 \)). Arterial pH of <7.25 was significantly more during period-1 compared to period-2 (53.6% vs. 31%, \( P = 0.007 \)). The results of the study outcomes are shown in Table 2. The mortality was significantly reduced during period-2 compared to period-1 (79.7% vs. 59%, \( P = 0.006 \)). Table 3 shows details of hemodynamic parameters between different modalities of RRT at the time of initiation of dialysis. The MAP (mmHg) at initiation of dialysis in CRRT was significantly lower than in PIRRT (74.9 ± 11.9 vs. 80.1 ± 12.1 mmHg, \( P = 0.024 \)). The number of patients who required inotropes was significantly higher in CRRT compared to that of PIRRT (90% vs. 62%, \( P < 0.001 \)). Figure 2 shows the mortality in different modalities of RRT during two periods. During period-2, there was no significant difference in the mortality between CRRT and PIRRT (72% vs. 55%, \( P = 0.32 \)). The Kaplan–Meir survival curves for the two periods are shown in Figure 3. The log-rank test was significant for \( P = 0.007 \). The results of the study outcomes are summarized below.

**Patient population**

The mean age of patients in our study population was 53.8 ± 16.1 years, which is higher than reported

| Table 2: Results of primary and secondary outcomes |
|-----------------------------------------------|
| \( 28\)-day hospital mortality (%) | All patients | Period-1 | Period-2 | \( P \) |
|---------------------------------|-------------|----------|----------|---|
| 110 (67.9) | 55 (79.7) | 55 (59.1) | 0.004 |
| Duration of ICU stay (days) | 12.4 ± 13.9 | 11.8 ± 13.8 | 13 ± 13.9 | 0.62 |
| Dialysis dependent at discharge (%) | 4 (2.5) | 2 (2.8) | 2 (2.2) | 0.76 |

ICU: Intensive Care Unit

| Table 3: Details of hemodynamic parameters at renal replacement therapy initiation in different modalities of renal replacement therapy |
|-----------------------------------------------|
| \( \text{OR} \) | CRRT | PIRRT | \( P \) value between CRRT and PIRRT | IHD |
|---------------------------------|-------------|----------|---------------------------------|-----|
| Systolic blood pressure (mmHg) | 106.8 ± 18.6 | 114.1 ± 22.5 | 0.053 | 135.5 ± 27.3 |
| Diastolic blood pressure (mmHg) | 58.8 ± 12.7 | 63.2 ± 11.8 | 0.066 | 72.5 ± 13.4 |
| MAP (mmHg) | 74.9 ± 11.9 | 80.1 ± 12.1 | 0.024 | 93.5 ± 15.6 |
| Inotropes (%) | 11 (10%) | 14 (37.8%) | <0.001 | 9 (56.2%) |
| None | 39 (35.8%) | 20 (54%) | 0.055 | 3 (18.8%) |
| 1 | 34 (31.2%) | 2 (5.4%) | 0.002 | 4 (25%) |
| >2 | 25 (23%) | 1 (2.7%) | 0.005 | 0 |

CRRT: Continuous renal replacement therapy; PIRRT: Prolonged intermittent renal replacement therapy; IHD: Intermittent hemodialysis; MAP: Mean arterial pressure

| Table 4: Results of univariate and multivariate logistic regression analysis showing variables affecting hospital mortality |
|-----------------------------------------------|
| \( \text{OR} \) | 95% CI | \( P \) | \( \text{OR} \) | 95% CI | \( P \) |
| Age | 1.0 | 0.98–1.02 | 0.97 | 0.96–1.02 | 0.51 |
| Gender (male) | 2.13 | 1.01–4.49 | 0.046 | 2.27 | 0.85–6.1 | 0.10 |
| CRRT versus others | 2.87 | 1.41–5.8 | 0.004 | 0.95 | 0.34–2.68 | 0.92 |
| Anuria | 2.38 | 1.07–5.23 | 0.032 | 1.97 | 0.69–5.6 | 0.2 |
| Serum creatinine | 0.69 | 0.56–0.85 | <0.001 | 0.85 | 0.65–1.1 | 0.22 |
| BUN | 0.98 | 0.97–0.99 | 0.02 | 0.99 | 0.98–1.01 | 0.55 |
| pH | 0.001 | 0.0001–0.84 | <0.001 | 0.001 | 0.000–1.04 | 0.005 |
| MAP | 0.96 | 0.94–0.99 | 0.009 | 0.99 | 0.96–1.04 | 0.95 |
| Inotropes | 2.12 | 1.43–3.22 | <0.001 | 1.39 | 0.82–2.4 | 0.24 |
| Ventilator | 4.62 | 1.97–10.8 | <0.001 | 2.15 | 0.73–6.3 | 0.16 |

CRRT: Continuous renal replacement therapy; BUN: Blood urea nitrogen; MAP: Mean arterial pressure; OR: Odds ratio; CI: Confidence interval
The etiology of AKI was similar during two study periods and sepsis was the major cause of AKI, observed in 54.3%. The severity of illness of patients as assessed by APACHE II score at the time of initiation of RRT was similar during the two periods. The setting of AKI was also similar and vast majority of AKI was in the medical setting (87.7%). Tropical infections such as malaria, leptospirosis, and dengue hemorrhagic fever contributing to AKI in our population were seen in only 5.4%. However, the epidemiology of AKI requiring RRT may not necessarily reflect the epidemiology of AKI in general. Very few studies from India explore the epidemiology of dialysis-dependent AKI in ICU and they have reported variable epidemiology.[22‑25]

The pattern of dialysis-dependent AKI in our study resembled that in the developed world and not the previous reports from India,[22‑24] with the exception of study by Sankarasubbiayan et al.,[25] who reported AKI pattern similar to ours, indicating the changing pattern of AKI requiring dialysis in urban population in India.

Mortality and other outcomes

The overall mortality in dialysis-dependent AKI in ICU was high (68%) in our study and similar high mortality rates of dialysis-dependent AKI have been previously reported from India.[2,23,25,26] In our study, mortality was significantly reduced during period-2 compared to period-1 (79% vs. 59%, \(P = 0.006\)). This improvement was not accounted by epidemiology of AKI, severity of illness or dose of dialysis, since these factors were similar during the two periods. The dose of dialysis was not quantified but we used the level of azotemia control on RRT as the surrogate of dose, which was similar at 48 h in surviving patients during two periods. We analyzed the impact of changes in dialysis practice during the period-2 for the improved mortality. The major changes in the dialysis practice during the later period (period-2) were: (1) Introduction of PIRRT, (2) early dialysis for metabolic acidosis, (3) early initiation of RRT for anuria and large amount of fluid accumulation, and (4) use of bicarbonate-based fluids for CRRT. The mode of dialysis did not influence hospital mortality in our study populations. This is in agreement with all the studies, which showed similar mortality rates with CRRT and intermittent RRT.[16,17] The bicarbonate-based fluids are preferred over lactate-based fluids for CRRT since they result in better correction of acidosis, lower lactate levels, and improved hemodynamic tolerance.[27] However, their patient survival benefit in CRRT remains uncertain. We doubt that the utilization of bicarbonate-based fluids for CRRT contributed significantly to the improved mortality during period-2. The multivariate regression analysis showed that degree of metabolic acidosis, but not modality of RRT influenced mortality. Based on these observations, we speculate that the change in our policy to dialyze patients with anuria and metabolic acidosis early during period-2 may have influenced the better outcome during this period. Significantly higher proportion of patients had anuria prior to initiation of dialysis in period-1 compared to period-2. This may just imply more severe AKI or delayed RRT. Anuric patients are more likely to have fluid overload which has been shown to have adverse impact on survival in critically ill patients[21,28] and in patients with AKI.[29,30] The impact of metabolic acidosis and its correction on outcomes in critically ill patients has not been studied adequately. Correction of acidosis in critically ill patients is a controversial topic and there are no clear guidelines to intervene in terms of RRT for metabolic acidosis are possible due to paucity of data on this important topic.[10] The general guideline to initiate RRT to supplement bicarbonate, when pH is below 7.2 is arbitrary and not based on interventional studies in AKI in ICU.[33]
Our results suggested that severe metabolic acidosis is an independent risk factor of hospital mortality in dialysis-dependent AKI in ICU and early intervention to corrected acidosis during period-2 may have contributed to improved survival noted during this period. However, it was not possible to single out one factor for the improved outcome during period-2. It is likely that better overall care and integrated approach taking in to account several aspects of uremic complications simultaneously may have been the major contributing factor in better outcome during the later period.

**The choice of modality of renal replacement therapy in acute kidney injury**

In the absence of evidence for survival advantage of CRRT over PIRRT or IHD, the choice of RRT in AKI is influenced by several factors such as hemodynamic instability, need for large amount of fluid removal, cost of therapy and ease of operation. Our approach has been to choose CRRT over PIRRT in (1) moderate to severe hemodynamic instability and (2) severe metabolic acidosis. The correction of acidosis is rapid with intermittent RRT such as IHD and PIRRT. However, such rapid correction is undesirable especially when acidosis is severe, since it may aggravate intracellular acidosis, lactic acidosis and reduces ionized calcium all of which may worsen cardiovascular instability. The correction of metabolic acidosis is slower and more sustained in CRRT and hence may be preferred over intermittent RRT in severe metabolic acidosis.[31]

With the introduction of PIRRT during period-2, the utilization of CRRT was reduced by 37% (from 85.5% to 54%), despite no change in the demography and severity of illness during two periods. PIRRT was effective, safe and was tolerated in most patients (95%). Only 2 (5%) patients did not tolerate PIRRT at the initiation and hence were transferred to CRRT within 24 h, due to worsening hemodynamic instability. Our experience with PIRRT is in agreement with recent observational studies, which incorporated PIRRT successfully to dialyze patients with AKI in ICU.[33,32] Recently, Marshall et al. reported experience from 3 centers from Australia, New Zealand, and Italy, which changed dialysis practice adapting PIRRT in place of CRRT without any adverse impact on mortality.[33]

**Strengths and limitations**

There are several strengths to our study. First, ours is the largest study from developing countries comparing change in the pattern of RRT over the period of 8 years reflecting the modification of dialysis practice based on the emergence of new knowledge. Second, our study is the first to report a large experience of CRRT and PIRRT from India. Third, our study has evaluated objective outcomes and has incorporated all the likely factors that could affect mortality in our setting. Our study convincingly demonstrates the safety, efficacy, and tolerance of PIRRT in the Indian setting. PIRRT use in our study decreased CRRT utilization by 37%. In a setting where a major barrier for dialysis in AKI is the cost incurred, our study suggests PIRRT as an equally efficacious cheaper alternative. Our study has several limitations. First, our study is from a single center and a single nephrologist managed the dialysis. A decision of dialysis practice by a single nephrologist may restrict the generalizability of our results, but also could be an advantage in that different dialysis practice patterns as seen across several nephrologists could introduce a confounder making it difficult to identify the factors influencing outcomes. Second, the mode of dialysis was decided based on clinical judgment rather than objective hemodynamic data. However, no clear guidelines exist for choice of RRT based on objective hemodynamic parameters. The choice of RRT in our study was consistent and was based on broad hemodynamic parameters such as blood pressure and need for inotropes. Third, we did not quantify fluid overload based on objective parameters such as cumulative fluid balance or weight gain and fluid overload assessment was subjective in our study and was based on degree of peripheral edema. Fourth, our study was a retrospective analysis of the data, which has inherent limitations. Finally, differences in outcomes between the 2 time periods may be a function of differences in care and support provided to other organs.

**Conclusions**

Adaptation of PIRRT resulted in 37% reduction of utilization of CRRT in our patients. The mortality was better during the period of adaptation of PIRRT, but this could not be attributed to PIRRT use. Early initiation of RRT for indications of anuria and metabolic acidosis may have resulted in better survival during later period.

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**Conflicts of interest**

There are no conflicts of interest.

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