Regular Article

Evaluation of Proteinuria Using Urine Protein : Creatine Ratio in Treatment with Molecular Targeted Agents for Advanced Renal Cell Carcinoma

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The usefulness of the urine protein : creatine ratio (UPCR) in management of molecular targeted therapy and immunotherapy has not been studied, although urine protein dipstick testing (uPr) is widely used in the clinical setting. The aim of this study was to investigate the usefulness of UPCR as compared to uPr in patients undergoing molecular targeted therapy for advanced renal cell carcinoma (RCC). A total of 25 patients (median age 68 years) with advanced RCC were included. Sunitinib, pazopanib, axitinib, sorafenib, everolimus, and nivolumab were administered to 15, 9, 16, 3, 7, and 13 patients, respectively, with duplication. Proteinuria was managed according to the grade determined by UPCR. Data at each treatment visit were retrospectively collected and uPr and UPCR were compared. The overall incidences of any grade of proteinuria associated with sunitinib, pazopanib, axitinib, sorafenib and everolimus were 86.7, 88.9, 93.8, 100, and 85.7%, respectively. There were discordances between the uPr-based grade and UPCR-based grade. UPCR did not meet the criteria of Grade 3 in 70.6, 100, 83.3, and 83.3% at visits in cases with uPr 3+ for sunitinib, pazopanib, sorafenib, and everolimus, respectively. In axitinib treatment, UPCR did not meet the criteria for withholding in 46.2% of the cases of uPr 2+ and more. Our study suggests that UPCR may be useful tool in management of adverse events associated with tyrosine kinase inhibitors, everolimus and can provide patients with optimal opportunities for receiving treatment.

Key words  molecular targeted drug therapy; renal cell carcinoma; proteinuria; urine protein : creatine ratio

INTRODUCTION

In daily clinical practice for treatment of advanced renal cell carcinoma (RCC), tyrosine kinase inhibitors (TKIs), mammalian target of rapamycin (mTOR) inhibitors and immune checkpoint inhibitors are used sequentially. Proteinuria has been reported as one of the most frequent adverse events (AEs) associated with TKIs and mTOR inhibitors, which have antiangiogenic activity. The proteinuria occurs as a result of increased permeability of vascular endothelial cells caused by inhibition of the binding of vascular endothelial growth factor (VEGF) secreted from podocytes around the renal tubular tubules to VEGF receptors on vascular endothelial cells, and focal glomerular sclerosis originating from cell damage. Twenty-four-hours urine collection is the gold standard method for quantitative evaluation of proteinuria. However, this procedure requires time and effort and is troublesome in the daily clinical setting. Instead, urine protein dipstick testing (uPr) is widely used in management of molecular targeted therapy because of its convenience, although its potential inaccuracy is not negligible. On the other hand, the single urine protein : creatinine ratio (UPCR) is widely used as an alternative quantitative examination for proteinuria in patients with chronic kidney disease, including diabetic nephropathy. However, the usefulness of UPCR in management of molecular targeted therapy has not been studied. Therefore we compared uPr and UPCR to investigate the usefulness of UPCR measurement in patients undergoing molecular targeted therapy for RCC.

MATERIALS AND METHODS

Patients who were treated with sunitinib, pazopanib, axitinib, sorafenib, everolimus and nivolumab for advanced RCC and interviewed at a pharmacist outpatient service during the period from April 1, 2015 through March 31, 2019 at the Sapporo Medical University Hospital were enrolled in this study. Data were collected by retrospective review of medical records. uPr and UPCR testing were routinely conducted at every treatment visit. In the Common Terminology Criteria for Adverse Events (CTCAE) v4.0, proteinuria Grades 1–3 were determined as uPr 1+ or urinary protein <1.0 g/24 h, uPr 2+ or urinary protein 1.0 to <3.5 g/24 h and uPr 3+ or urinary protein ≥3.5 g/24 h, respectively. In our series, UPCR was used instead of 24-urine protein, although proteinuria was evaluated based on CTCAE v4.0. With regard to proteinuria, Grade 2 needed dose reduction and Grade 3 needed withholding of the drug until recovery to Grade 1 in management of patients receiving molecular targeted agents other than axitinib. In axitinib therapy, criteria for withholding were determined to be uPr 2+ and urinary protein ≥2.0 g/24 h.
### Table 1. Patient Characteristics

| No. | Gender | Age  | BMI  | Histology | IMDC risk group | Comorbidity | eGFR before treatment (mL/min/1.73 m²) | Agent (the highest grade of proteinuria) | Treatment duration (months)/total dosage or cycles of the drug |
|-----|--------|------|------|-----------|-----------------|-------------|----------------------------------------|---------------------------------------------|---------------------------------------------------------------|
| 1   | M      | 71   | 20.1 | Clear cell| Intermediate    | n           | 53.6                                   | Sunitinib (G1) Axitinib (G2) Nivolumab (ND) | 4.4/2437.5 mg/130900 mg 19.6/1206mg 6.8/1050mg |
| 2   | M      | 71   | 21.4 | Clear cell| Favorable       | y           | 47.4                                   | Sunitinib (G3) Axitinib (G0) Nivolumab (ND) | 18.9/626.5 mg/130900 mg 33.8/162.5 mg 6.9/122.5 mg |
| 3   | M      | 79   | 18.1 | Clear cell| Intermediate    | n           | 46.2                                   | Sunitinib (G0) Axitinib (G0) Nivolumab (ND) | 3.2/122.5 mg/130900 mg 21.3/125 mg 6.9/122.5 mg |
| 4   | F      | 67   | 21.1 | Clear cell| Intermediate    | y           | 50.4                                   | Sunitinib (G0) Axitinib (G3) Nivolumab (ND) | 6.7/1427.5 mg/130900 mg 6.4/3950 mg 12.2/3540 mg |
| 5   | M      | 65   | 24.4 | Clear cell| Intermediate    | y           | 77.9                                   | Sunitinib (G2) Axitinib (G3) Nivolumab (ND) | 14.7/10800 mg/130900 mg 12.2/3540 mg 9.4/ND |
| 6   | M      | 68   | 24.7 | Clear cell| Favorable       | n           | 46.3                                   | Sunitinib (G1) Axitinib (G2) Nivolumab (ND) | 28.3/3475 mg/130900 mg 28.3/3475 mg 26.1/ND |
| 7   | M      | 76   | 27.1 | Clear cell| Intermediate    | y           | 25.9                                   | Sunitinib (G0) Axitinib (G1) Nivolumab (ND) | 6.4/525 mg/130900 mg 6.4/3950 mg 12.2/3540 mg |
| 8   | M      | 59   | 21.1 | Clear cell| Intermediate    | n           | 50.7                                   | Sunitinib (G1) Axitinib (G1) Nivolumab (ND) | 6.7/1427.5 mg/130900 mg 6.4/3950 mg 12.2/3540 mg |
| 9   | M      | 63   | 23.7 | Clear cell| Intermediate    | n           | 51.4                                   | Sunitinib (G2) Axitinib (G3) Nivolumab (ND) | 29.9/13125 mg/130900 mg 18.2/2448 mg 1.6/520 mg |
| 10  | M      | 77   | 18.6 | Clear cell| Intermediate    | n           | 66.6                                   | Sunitinib (G0) Axitinib (G1) Nivolumab (ND) | 2.8/21000 mg/130900 mg 2.8/21000 mg 9.4/ND |
| 11  | M      | 66   | 32.6 | Clear cell| Intermediate    | y           | 43.4                                   | Sunitinib (G0) Axitinib (G1) Nivolumab (ND) | 20.9/14350 mg/130900 mg 20.9/14350 mg 9.4/ND |
| 12  | F      | 66   | 21.6 | Clear cell| Favorable       | n           | 88.9                                   | Sunitinib (G0) Axitinib (G1) Nivolumab (ND) | 28.3/3475 mg/130900 mg 28.3/3475 mg 26.1/ND |
| 13  | F      | 62   | 22.2 | Clear cell| Poor            | n           | 37.3                                   | Sunitinib (G0) Axitinib (G1) Nivolumab (ND) | 13.6/9312.5 mg/130900 mg 3.9/600 mg 2.1/140 mg |
| 14  | M      | 55   | 21.3 | Clear cell| Intermediate    | y           | 64.6                                   | Sunitinib (G0) Axitinib (G2) Nivolumab (ND) | 13.6/9312.5 mg/130900 mg 3.9/600 mg 2.1/140 mg |
| 15  | M      | 70   | 19.8 | Clear cell| Intermediate    | y           | 69.4                                   | Sunitinib (G0) Axitinib (G0) Nivolumab (ND) | 19.6/1206mg/130900 mg 19.6/1206mg 6.4/1110 mg |
| 16  | F      | 76   | 21.1 | Clear cell| Favorable       | n           | 40.9                                   | Sunitinib (G0) Axitinib (G3) Nivolumab (ND) | 2.8/67200mg/130900 mg 2.8/67200mg 7.4/821 mg |
| 17  | M      | 80   | 26.5 | Clear cell| Intermediate    | y           | 74.7                                   | Sunitinib (G0) Axitinib (G0) Nivolumab (ND) | 12.4/5175 mg/130900 mg 12.4/5175 mg 6.4/1110 mg |
| 18  | M      | 68   | 22.1 | Clear cell| Poor            | n           | 42.5                                   | Sunitinib (G0) Axitinib (G0) Nivolumab (ND) | 2.8/67200mg/130900 mg 2.8/67200mg 7.4/821 mg |
| 19  | F      | 59   | 19.9 | Clear cell| Intermediate    | y           | 54.1                                   | Sunitinib (G0) Axitinib (G3) Nivolumab (ND) | 8.3/9500 mg/130900 mg 8.3/9500 mg 4.6/140 mg |
| 20  | M      | 64   | 18.8 | Clear cell| Intermediate    | n           | 46.5                                   | Sunitinib (G0) Axitinib (G0) Nivolumab (ND) | 6.9/13040mg/130900 mg 6.9/13040mg 4.6/140 mg |
| 21  | M      | 70   | 27.8 | Clear cell| Intermediate    | y           | 43.6                                   | Sunitinib (G0) Axitinib (G3) Nivolumab (ND) | 24.1/1432mg/130900 mg 24.1/1432mg 15.4/430mg |
| 22  | M      | 77   | 21.5 | Clear cell| Intermediate    | y           | 48.3                                   | Sunitinib (G0) Axitinib (G3) Nivolumab (ND) | 4.4/ND/130900 mg 4.4/ND/130900 mg 15.4/430mg |
| 23  | M      | 49   | 18.4 | Clear cell| Intermediate    | y           | 38.8                                   | Sunitinib (G0) Axitinib (G3) Nivolumab (ND) | 4.4/ND/130900 mg 4.4/ND/130900 mg 15.4/430mg |
| 24  | M      | 69   | 26.3 | Clear cell| Favorable       | y           | 44.7                                   | Sunitinib (G0) Axitinib (G3) Nivolumab (ND) | 1.8/1040mg/130900 mg 1.8/1040mg 7.1/4025 mg |
| 25  | M      | 49   | 20.5 | Clear cell| Poor            | n           | 99.0                                   | Sunitinib (G0) Axitinib (G3) Nivolumab (ND) | 1.8/1040mg/130900 mg 1.8/1040mg 7.1/4025 mg |

The shaded part of the table was not included in this analysis because no pharmacist outpatient intervention was performed.
Statistical examination was performed using EZR with the Spearman's rank correlation coefficient.\textsuperscript{12} \( p \)-Values of \(< 0.05\) were considered statistically significant. This study was approved by the Institutional Review Board of Sapporo Medical University Hospital (25-188).

**RESULTS**

**Patient Characteristics**  Patient characteristics are shown in Table 1. Twenty-five patients (20 males, 5 females) were evaluated. Their median age was 68 years (IQR: 49–80 years). The numbers of patients treated with sunitinib, pazopanib, axitinib, sorafenib, everolimus, and nivolumab were 15, 9, 16, 3, 7, and 13, respectively, with duplication. Sunitinib was used as the first, third and fourth treatment lines in 13, 1, and 1 patients, respectively. Pazopanib was used as the first, second, third, and fourth lines or later in 4, 1, 3, and 2, respectively. Axitinib was used as the second and fourth lines or later in 14 and 2, respectively. Everolimus was used as the second, third and fourth lines or later in 1, 3, and 3, respectively. Sorafenib was used as the fourth line or later in 3. Nivolumab was used as the second, third and fourth lines or later in 2, 9 and 2 patients, respectively. Four patients (16.0\%) received a single regimen, whereas 24 (84.0\%) were treated with multiple regimens.

**Prevalence and Grade of Proteinuria**  Figure 1 shows the prevalence of proteinuria in patients receiving molecular targeted therapy according to the agent used. The overall incidences of any grade and grade 3 or higher of proteinuria associated with sunitinib, pazopanib, axitinib, sorafenib and everolimus were 86.7, 88.9, 93.8, 100, and 85.7\%, and 26.7, 22.2, 25.0, 33.3, and 14.3\%, respectively. All instances of Grade 3 proteinuria were reversible after drug withholding, and there was no case with progression to nephrotic syndrome or deterioration in renal function.

**Association between uPr and UPCR**  There was a significant correlation between the values of uPr and UPCR for all drugs, whereas there were discordances between the uPr-based grade and UPCR-based grade (Fig. 2). UPCR did not meet the criteria of Grade 3 in 70.6, 100, 83.3, and 83.3\% for those with uPr 3+ for sunitinib, pazopanib, sorafenib, and everolimus, respectively. In axitinib treatment, UPCR did not meet the criteria for withholding in 46.2\% of cases of uPr 2+ and more.

**DISCUSSION**

The introduction of TKIs has drastically improved the prognoses of patients with advanced and metastatic RCC.\textsuperscript{13–15} mTOR inhibitor (mTORi) also plays a critical role in treatment for metastatic RCC.\textsuperscript{16} Furthermore, the emergence of immune checkpoint inhibitors has revolutionized treatment of malignant diseases, including RCC.\textsuperscript{17} Specific AEs associated with each drug are known and their management is critical for adequate treatment. Proteinuria is known as a representative AE associated with TKIs targeting VEGF and mTORi. According to a meta-analysis, the incidences of grade 3 or higher proteinuria were 2.2, 4.6 and 0.9\% with pazopanib, axitinib, and sorafenib, respectively.\textsuperscript{18} In clinical trials, significant proteinuria was rarely associated with sunitinib\textsuperscript{19} or everolimus.\textsuperscript{20,21} In our series, the incidences of grade 3 or higher proteinuria associated with each drug were relatively high. The high incidence may be explained by the small number of samples. The influence of the previous treatment cannot be excluded. Another possible explanation is potential overestimation of urine protein by UPCR because its accuracy is limited, although it may be more accurate than uPr.

A significant number of cases of uPr-based proteinuria needing drug withholding did not meet the criteria determined by UPCR in all drug treatments. In our series, the grade of proteinuria was determined by using UPCR and there was
no irreversible event with regard to renal function, although the sample size was small. These results suggest that uPr overestimates urine protein, which can lead to unnecessary drug withholding. UPCR is strongly correlated to 24-h urine protein in healthy individuals and patients with chronic kidney disease. However, UPCR is not always correlated to 24-h urine protein in patients with glomerulonephritis or nephrotic syndrome. There are only a few reports about the correlation between UPCR and 24-h urine protein in patients receiving molecular targeted therapy. Although the validity of UPUR for evaluation of TKI-associated proteinuria has not been established, Evans et al. reported promising data. They evaluated the correlation in patients with hepatic cell carcinoma who were randomized to receive sorafenib or lenvatinib.
a multikinase inhibitor of VEGF receptors 1–3, fibroblast growth factor receptors 1–4, platelet-derived growth factor-alpha, KIT, and RET. In their study, the two parameters had a strong significant correlation.

This study has some limitations. As described above, the number of samples was small. Its retrospective nature cannot exclude biases in management of treatment for RCC. In addition, UPCR usage in this setting should be validated by comparing it with 24-h urine collection. However, 24-h urine collection requires time and effort and is not practical in outpatient management of patients with malignant disease receiving medical therapy. UPCR can be used simply in the daily clinical setting and provides useful information for adequate management of molecular targeted therapy.

CONCLUSIONS

UPCR may be appropriate for evaluating critical proteinuria in patients with advanced RCC receiving molecular targeted therapy as compared to uPr. UPCR may be a useful tool for management of adverse events associated with TKIs, mTORi that can provide patients with optimal opportunities for receiving treatment.

Conflict of Interest The authors declare no conflict of interest.

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