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Biomarkers, Clinical Features, and Rechallenge for Immune Checkpoint Inhibitor Renal Immune-Related Adverse Events

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Introduction: Immune checkpoint inhibitors (ICIs) are effective in treating several cancers; however, acute kidney injury (AKI) can occur as part as an immune-related adverse event (iRAE). Biomarkers at the time of AKI diagnosis may help determine whether they are ICI- related and guide therapeutic strategies.

Methods: In this retrospective study, we reviewed patients with cancer treated with ICI therapy between 2014 and 2020 who developed AKI (defined as a ≥1.5-fold increase in serum creatinine [SCr]) that was attributed to ICI (ICI-AKI) and compared them with an adjudicated non–ICI-AKI group. Clinical and laboratory features, including SCr, serum C-reactive protein (CRP), and urine retinol binding protein/urine creatinine (uRBP/Cr) levels at AKI event were evaluated.

Results: There were 37 patients with ICI-AKI and 13 non–ICI-AKI referents in the cohort for analysis. At time of AKI, SCr, CRP, and uRBP/Cr were significantly higher in the ICI-AKI compared with the non–ICI-AKI patients (median [interquartile range (IQR)] SCr 2.0 [1.7, 2.9] vs. 1.5 [1.3, 1.6] mg/dl, serum CRP 54.0 [33.7, 90.0] vs. 3.5 [3.0, 7.9] mg/l, and uRBP/Cr 1927 [1174, 46,522] vs. 233 [127, 989] μg/g Cr, respectively, $P < 0.05$ for all). Compared with the referent group, time from ICI initiation to AKI was shorter in the ICI-AKI patients. Among the ICI-AKI group, complete renal recovery occurred in 39% of patients by 3 months; rechallenge occurred in 16 (43%) of patients, of whom 3 (19%) had recurrence of AKI.

Conclusion: Our findings suggest that serum CRP and uRBP/Cr may help to differentiate AKI due to ICI from other causes.

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ICIs are monoclonal antibodies that can unleash the immune system by blocking surface molecules that serve as important breaks (or checkpoints) that mediate negative regulation of T cells.[1] ICIs have substantially improved the prognosis for patients with a wide range of malignancies.[2–4] Approved agents include anti–cytotoxic T lymphocyte antigen 4 (anti–CTLA-4) antibody (ipilimumab) and anti–programmed death 1 (anti–PD-1) antibodies (nivolumab, pembrolizumab, cemiplimab), and anti–programmed death 1 ligand (anti–PD-L1) antibodies (atezolizumab, avelumab, durvalumab).

They may induce a variety of iRAEs, including nephrotoxicity.[5,6] The incidence of iRAE in patients receiving ICI can be as high as 85% depending on the target and the use of mono- or combination therapy.[7–9] The most commonly affected organs are skin, endocrine glands, gastrointestinal tract, lungs, and liver.[5] Kidney toxicity while on ICI therapy occurs in up to 17% in reported series, but only a fraction is related to ICI, varying from 1% to 5% (depending on the type of ICI or use of combined ICI agents).[10–15] Acute interstitial nephritis (AIN) is the most common histopathological lesion, and depending on the severity of the renal or

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Table 1. Demographic patient characteristics at time of ICI initiation

Patient characteristic	Non–ICI-AKI (n = 13)	ICI-AKI (n = 37)	Total (n = 50)	P value
Age at time of AKI (y), mean (SD)	67.5 (4.4)	66.8 (11.4)	67.0 (10.0)	0.83
Gender, n (%)	—	—	—	0.33
Male	5 (38.5)	20 (54.1)	25 (50.0)	—
Female	8 (61.5)	17 (45.9)	25 (50.0)	—
White race, %y	13 (100.0)	37 (100.0)	50 (100.0)	—
eGFR, median [IQR] ml/min per 1.73 m^2	76.8 [68.1, 80.7]	77.9 [59.9, 89.5]	77.9 [62.6, 85.6]	0.61
HTN, %y	9 (69.2)	23 (62.2)	32 (64.0)	0.75
DM, %y	1 (7.7)	5 (13.5)	6 (12.0)	>0.99
CKD, %y	2 (15.4)	7 (18.9)	9 (18.0)	>0.99
COPD, %y	0 (0.0)	7 (18.9)	7 (14.0)	0.17
ICI type[a, b, c, d, e], n (%)				0.41
CTLA-4	0 (0.0)	1 (2.7)	1 (2.0)	
PD-1	10 (76.9)	23 (62.2)	33 (66.0)	
PD-L1	3 (23.1)	7 (18.9)	10 (20.0)	
Combo	0 (0.0)	6 (16.2)	6 (12.0)	
History of autoimmune disease, %y	1 (7.7)	2 (5.4)	3 (6.0)	0.77
Asthma, %y	1 (7.7)	1 (2.7)	2 (4.0)	0.46
Psoriasis, %y	0 (0.0)	1 (2.7)	1 (2.0)	>0.99
Malignancy treated with ICPi, n (%)				0.24
Melanoma	1 (7.7)	13 (35.1)	14 (28.0)	
Lung adenocarcinoma	5 (38.5)	10 (27.0)	15 (30.0)	
Lung small cell	2 (15.4)	4 (10.8)	6 (12.0)	
Head and neck cancer	1 (7.7)	1 (2.7)	2 (4.0)	
Renal cell	3 (23.1)	3 (8.1)	6 (12.0)	
Bladder/Urothelial	1 (7.7)	1 (2.7)	2 (4.0)	
Other	0 (0.0)	5 (13.5)	5 (10.0)	
PD-L1 tumor marker, n (%)	—	—	—	0.44
Not done	8 (61.5)	27 (73)	35 (70)	—
Done	5 (38.5)	10 (27)	15 (30)	—
Percent PD-L1 among tests done, median [IQR]	70.0 [0.0, 90.0]	37.5 [5.0, 80.0]	55.0 [5.0, 80.0]	0.85

AKI, acute kidney injury; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; CTLA-4, cytotoxic T lymphocyte–associated antigen 4; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HTN, hypertension; ICI, immune checkpoint inhibitors; PD-1, programmed cell death 1; PD-L1, programmed death-ligand 1; Combo, combination.
No patients had chronic heart failure or chronic liver disease.
Unless otherwise indicated, timing is at initiation of ICI therapy.
[a]Denotes all immune checkpoint inhibitors ever received.
[b]Ipilimumab was the ICI in 100% of those who received an anti–CTLA-4 antibody.
[c]Nivolumab or pembrolizumab or cemiplimab were the anti–PD-1 antibodies.
[d]Atezolizumab, avelumab, durvalumab were the anti–PD-L1 antibodies.
[e]Ipilimumab/nivolumab was the combination therapy regimen.

extra-renal iRAE, further treatment with immunotherapy may be limited.[16] In general, the use of systemic cancer agents is associated with AKI of diverse etiologies, and it is important to understand the likely cause so as to not unnecessarily limit future chemotherapeutic treatment options.[17] Therefore, prompt diagnosis of AKI as well as identification of its cause is paramount in the ever-evolving arena of chemotherapeutics and target immunotherapies.

The goal of the current study was to define the association between ICI-related AKI status and serum and urine biomarkers, as well as other clinical and laboratory characteristics. In addition, we investigated the timing and incidence of renal recovery, rechallenge, and death in ICI-AKI patients during follow-up.

METHODS

Study Population

This is a single-center, retrospective observational study. We performed a search of our electronic medical records for all patients who had received an ICI and suffered an AKI event between January 2014 and June 2020 at Mayo Clinic, Rochester. ICIs were defined as the following: cytotoxic T lymphocyte antigen 4 inhibitors (ipilimumab), PD-1 inhibitors (pembrolizumab, nivolumab and cemiplimab), and PD-L1 antibodies (atezolizumab, avelumab, durvalumab). AKI events and their likely causes, including iRAE, were identified either by clinical documentation by the consulting nephrologist at the time of the clinical event, or when this was not clearly stated after retrospective chart review and mutual consensus by the authors (SM, SH, and NL) performing the retrospective chart review. Biomarkers were not part of the adjudication process to distinguish ICI-AKI from non–ICI-AKI individuals. Patients who did not provide research authorization were excluded. This study was approved by Mayo Clinic Institutional Review Board.

Data Collection

Clinical characteristics, cancer subtype, comorbidities, and concurrent medications use before ICI drug use were collected manually. Both prior and concurrent non-kidney iRAE as documented by care providers was also collected. Baseline creatinine was defined as the last stable serum creatinine value before initiating ICI. AKI events were defined as the patient experiencing a ≥1.5-fold increase in serum creatinine from baseline (Grade 1 kidney toxicity).[18] AKI cases directly attributable to other recognizable reasons (e.g., obstruction, sepsis, systemic hemodynamic changes) were excluded from analysis. Patients were considered to have ICI-related AKI (ICI-AKI) if they were either biopsy-confirmed AIN, kidney function was responsive to steroids, or progressed without steroids. Patients considered to have AKI not related to ICI (non–ICI-AKI) were either biopsy-confirmed alternative causes or did not receive steroids and did not progress on resuming with ICI. Measures of kidney function (serum creatinine and estimated glomerular filtration rate, estimated using the Chronic Kidney Disease Epidemiology Collaboration equation), as well as the biomarkers CRP and uRBP/Cr were also collected at ICI initiation, at the AKI event, and up to 1-year follow-up.

Data were also obtained on the management of AKI, as well as kidney function and adverse events throughout follow-up. AKI severity was staged

Table 2. ICI and AKI characteristics over time

	Non–ICI-AKI (n = 13)	ICI-AKI (n = 37)	Total (n = 50)	P value
At time of ICI initiation				
TNI drug, %y	3 (23.1%)	21 (56.8%)	24 (48.0%)	**0.037**
Type of medication, %y	—	—	—	—
Proton pump inhibitors	3 (23.1)	20 (54.1)	23 (46.0)	0.10
Antibiotics	0 (0.0)	0 (0.0)	0 (0.0)	—
NSAIDs	0 (0.0)	2 (5.4)	2 (4.0)	>0.99
Other	0 (0.0)	1 (2.7)	1 (2.0)	>0.99
Within 1 mo before AKI				
Cisplatin, %y	0 (0.0)	3 (8.1)	3 (6.0)	0.56
TKI/VEGF, %y	3 (23.1)	2 (5.4)	5 (10.0)	0.10
>14 days before AKI[a]				
Any iRAE (1+)	3 (23.1)	16 (43.2)	19 (38.0)	0.20
Subtype, %y	—	—	—	—
Rash	1 (7.7)	5 (13.5)	6 (12.0)	>0.99
Colitis	0 (0.0)	3 (8.1)	3 (6.0)	0.56
Hepatitis	1 (7.7)	3 (8.1)	4 (8.0)	>0.99
Thyroid disease	1 (7.7)	7 (18.9)	8 (16.0)	0.66
Hypophysitis	0 (0.0)	1 (2.7)	1 (2.0)	>0.99
Type 1 DM	0 (0.0)	1 (2.7)	1 (2.0)	>0.99
Other	0 (0.0)	7 (18.9)	7 (14.0)	0.17
Within 14 days before AKI[b]				
TNI drug, %y	6 (46.2)	28 (75.7)	34 (68.0)	**0.0497**
Type of medication, %y	—	—	—	—
Proton pump inhibitors	3 (23.1)	25 (67.6)	28 (56.0)	**0.009**
Antibiotics	4 (30.8)	2 (5.4)	6 (12.0)	**0.033**
NSAIDs	2 (15.4)	8 (21.6)	10 (20.0)	>0.99
Any iRAE (1+)	0 (0.0)	13 (35.1)	13 (26.0)	**0.013**
Subtype, %y	—	—	—	—
Rash	0 (0.0)	2 (5.4)	2 (4.0)	>0.99
Colitis	0 (0.0)	3 (8.1)	3 (6.0)	0.56
Hepatitis	0 (0.0)	2 (5.4)	2 (4.0)	>0.99
Pneumonitis	0 (0.0)	5 (13.5)	5 (10.0)	0.31
Myocarditis	0 (0.0)	1 (2.7)	1 (2.0)	>0.99
Other	0 (0.0)	4 (10.8)	4 (8.0)	0.56
Time of AKI				
AKI stage[c]**, %n**	—	—	—	**<0.001**
Stage 1	11 (84.6)	9 (24.3)	20 (40.0)	—
Stage 2	1 (7.7)	16 (43.2)	17 (34.0)	—
Stage 3	1 (7.7)	12 (32.4)	13 (26.0)	—
After AKI				
Complete renal recovery within 3 mo, %y	6 (46.2%)	17 (45.9%)	23 (46.0%)	0.99

AKI, acute kidney injury; DM, diabetes mellitus; ICI, immune checkpoint inhibitors; iRAE, immune-related adverse event; NSAID, nonsteroidal anti-inflammatory drug; VEGF, vascular endothelial growth factor.
[a]There were no cases of pneumonitis, primary adrenal insufficiency, or myocarditis >14 days before AKI.
[b]There were no cases of thyroid disease, hypophysitis, primary adrenal insufficiency, or Type 1 DM within 14 days before AKI.
[c]AKI severity according to the Kidney Disease Improving Global Outcomes Work Group (KDIGO) criteria.

according to the Kidney Disease Improving Global Outcomes Work Group criteria.[19] Complete response was defined as return of kidney function back to baseline or <25% from baseline at 3 months.

Statistical Methods

Summary statistics were presented as mean (SD) for continuous normally distributed variables, median [IQR] for continuous variables with skewed distributions, and as n (%) for categorical variables. Comparisons between the ICI-AKI and non–ICI-AKI groups were evaluated using the equal variance t-test for continuous normally distributed variables, the nonparametric Wilcoxon rank sum test for non-normally distributed continuous variables, the χ^2 test for categorical variables where the expected cell counts were greater than 5, and the Fisher exact test for categorical variables where the expected cell counts were less than 5. Additional analysis of covariance analysis was also used to compare kidney function and biomarker levels between ICI-AKI and non–ICI-AKI groups after separately adjusting for medication use at ICI initiation and within 14 days before AKI, using the natural log transformation of kidney function and biomarker measures due to the skewedness of their distributions (Supplementary Table S1). Comparisons between clinical and laboratory characteristics at rechallenge and recurrent AKI status were evaluated using the Wilcoxon rank sum test and the Fisher exact test. Time to event analyses were conducted to assess the endpoint of survival, using Kaplan-Meier methods and the log-rank test to evaluate equality over strata, as well as time to renal recovery at 3 months, using cumulative incidence curves to take into account the competing risks of rechallenge and death. Because levels of missing data were variable across different outcome measures, we performed available case analysis for each outcome to best retain power. Because most patients did not have a CRP and/or uRBP/Cr laboratory measure at their AKI event, we introduced a variable indicating missing CRP and/or uRBP/Cr labs and compared this variable with patient characteristics from Tables 1 and 2 using the same statistical methods to highlight any distributional differences between patients with versus without these biomarkers (Supplementary Table S2).

RESULTS

Patient Population and Characteristics

A total of 2143 unique patients received ICI therapy between January 2014 and June 2020. Among these patients, 365 (17%) developed AKI; of these patients, 313 (85.8%) patients had AKI clearly attributable to non-ICI causes (e.g., obstruction, infection), and were excluded from further analysis. A total of 52 patients remained that had possible ICI-related AKI. Of these, 37 (71%) patients had clinically suspected or biopsy proven ICI-AKI (exposed); 2 (4%) patients did not resume ICI and so were excluded; and the remaining 13 (25%) non–ICI-AKI patients served as referents (Figure 1). Baseline characteristics are detailed in

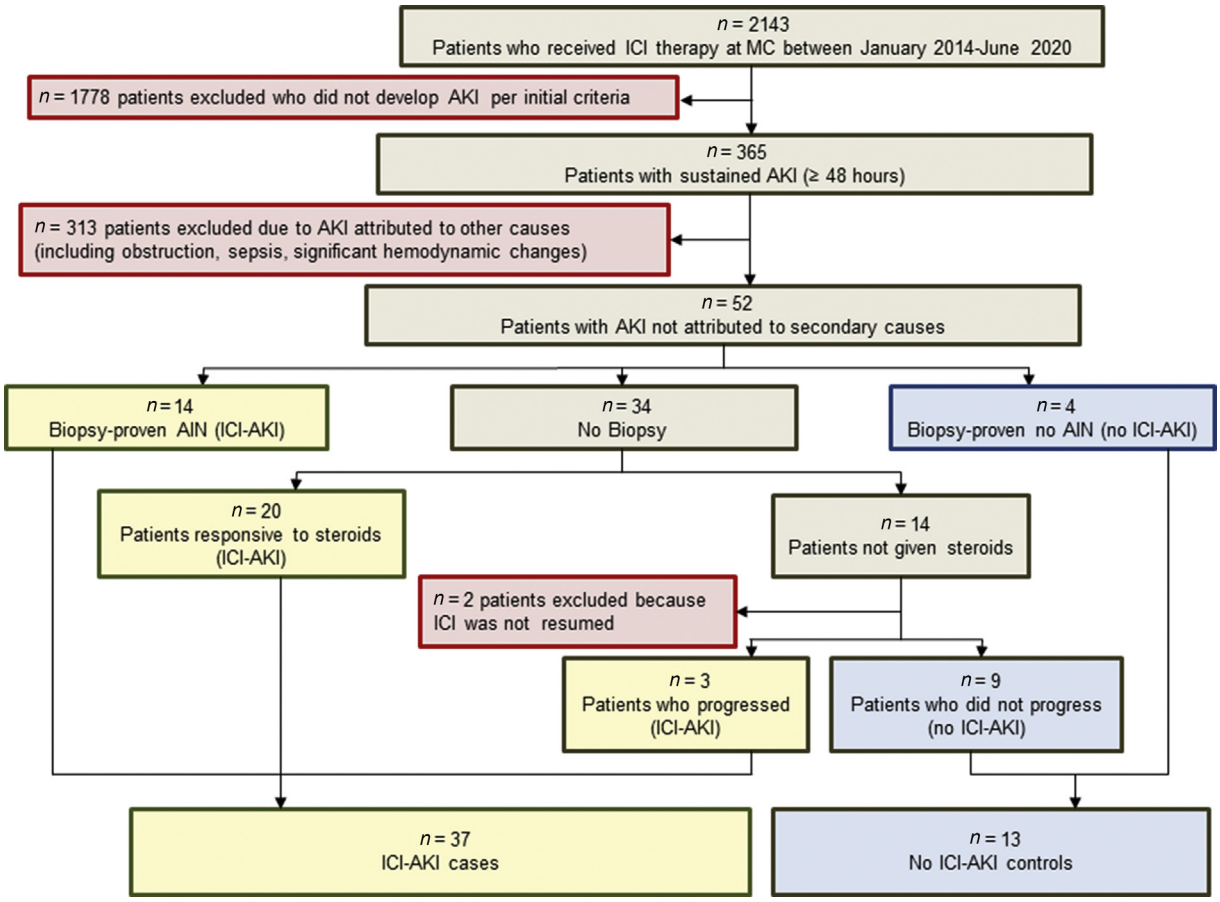

Figure 1. Study flow chart of inclusion criteria. AKI, acute kidney injury; ICI, immune checkpoint inhibitors; MC, Mayo Clinic.

Table 1. Age at AKI, sex, and kidney function at time of ICI initiation was similar in ICI-AKI and non–ICI-AKI patients. The most common malignancies in both groups were lung cancer, melanoma, and renal cell carcinoma. Medical comorbidities, class of ICI treatment, PD-L1 tumor marker, and history of autoimmune diseases were also similar between groups at baseline.

Clinical Characteristics of Patients With ICI-AKI Versus Non–ICI-AKI

AKI and ICI characteristics over time are reported in Table 2. Patients with ICI-AKI experienced a more severe stage of AKI compared with non–ICI-AKI (43.2% vs. 7.7% for stage 2 and 32.4% vs. 7.7 % for stage 3, respectively, $P < 0.001$). The most common iRAEs before AKI were related to skin and the endocrine system, with rash and thyroid disease mostly reported in this cohort, whereas pneumonitis and colitis were the most common iRAEs that occurred concomitantly with the AKI. There was no significant difference observed in overall iRAE rates between the groups (43.2% vs. 23.1% for ICI-AKI vs. non–ICI-AKI) more than 14 days before AKI diagnosis. However, within 14 days of AKI, iRAE rates were higher in the ICI-AKI group compared with the referents (35.1% vs. 0%, respectively, $P = 0.013$) (Figure 2a and b). Patients with ICI-AKI also had higher rates of proton pump inhibitors (PPIs) administered within 14 days before the AKI compared with those with non–ICI-AKI (67.6% vs. 23.1%, $P = 0.009$). There were no significant differences observed in administration of other cancer therapeutic agents (e.g., platinum and vascular endothelial growth factor signaling pathways inhibitor agents) within 1 month before the AKI diagnosis.

AKI was found to develop earlier in the ICI-AKI patients compared with the non–ICI-AKI patients (median [IQR] 4 [1.2, 11.4] vs. 8.5 [5.3, 10.4] months, respectively, $P = 0.026$). Within each group, we performed further analyses assessing both overall AIN medication uses and each subtype on time to AKI, but no significant differences were observed (data not shown).

Assessment of urinary laboratory measures at the time of AKI revealed a higher protein-to-creatinine ratio in the ICI-AKI compared with the non–ICI-AKI patients (median [IQR] 0.8 [0.4, 1.8] vs. 0.3 [0.2, 0.6], respectively, $P = 0.020$), although the proteinuria was subnephrotic in both groups (Table 3). Blood white blood cell count and urine red blood cell count were also elevated in the ICI-AKI group compared with the

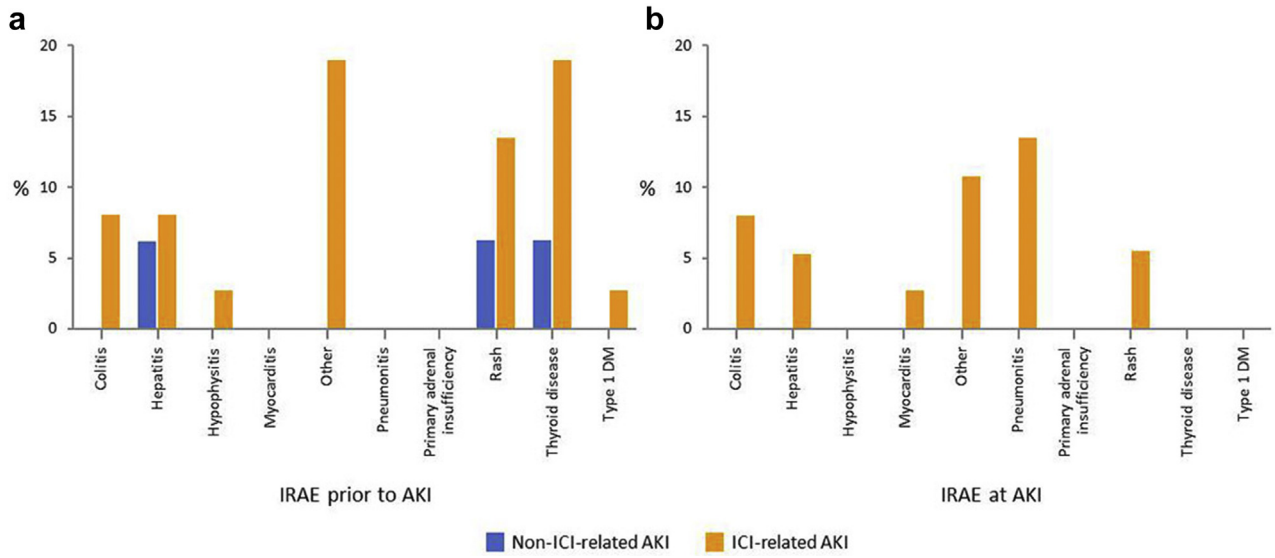

Figure 2. Bar charts of iRAE events (a) before and (b) at time of AKI, by cause of AKI. AKI, acute kidney injury; DM, diabetes mellitus; ICI, immune checkpoint inhibitors; iRAE, immune-related adverse event.

non–ICI-AKI patients (median [IQR] white blood cell of 8.3 [6.7, 9.5] vs. 6.0 [5.2, 7.5], $P = 0.028$ and red blood cell 0 in 53.1% vs. 91.7%, 1–3 in 37.5% vs. 0% and 4–10 in 9.4% vs. 8.3%, $P = 0.036$ in ICI-AKI vs. non–ICI-AKI, respectively). Blood eosinophil numbers were similar between groups.

Kidney biopsy was performed in 14 (38%) ICI-AKI patients. Among these, AIN was the predominant acute lesion found in 14 (100%), whereas in the non–ICI-AKI group kidney biopsy was performed in 4 (27%) and the predominant acute lesion was ATI (acute tubular injury) in 2 (50%) with mild to moderate interstitial fibrosis/tubular atrophy in 4 (100%). In the ICI-AKI patient group, 12 (86%) also had ATI and 6 (43%) manifest tissue eosinophilia. One of the cases had evidence of endotheliitis and none had tubular basement membrane deposits by immunofluorescence microscopy or electron microscopy. Histologic features of these patients are presented in Supplementary Table S1.

Kidney Function and Biomarkers

At time of AKI, SCr, serum CRP, and uRBP/Cr measures were increased in the ICI-AKI patients compared with non–ICI-AKI patients (median [IQR] serum creatinine 2.0 [1.7, 2.9] vs. 1.5 [1.3, 1.6] mg/dl, CRP 54.0 [33.7, 90.0] vs. 3.5 [3.0, 7.9] mg/l, and uRBP/Cr 1927 [1174, 46,522] vs. 233 [127, 989] μg/g Cr, respectively, $P < 0.05$ for all) (Figure 3a–d). Among the 16 patients with both CRP and uRBP/Cr biomarkers available at time of AKI ($n = 6$ ICI-AKI and $n = 10$ non–ICI-AKI), we also evaluated the association between the product of these 2 biomarkers (CRP*uRBP/Cr) and ICI-AKI status, and found this measure also to be elevated in the ICI-AKI group compared with the non–ICI-AKI patients (median [IQR]: 212,955 [7,922, 359,862] vs. 1088 [624, 2967], respectively, $P = 0.008$) (Supplementary Figure S1). As the patients were managed for ICI-AKI, SCr, CRP, and uRBP/Cr values progressively decreased over time, with all values attenuated at 1 year

Table 3. Urine and serum laboratory measures at time of AKI

	Non-ICI-AKI ($n = 13$)	ICI-AKI ($n = 37$)	Total ($n = 50$)	*P* value
Urine				
Spot U protein:Osmo[a]	—	—	—	**0.020**
n	12	31	43	
Median [IQR]	0.3 [0.2, 0.6]	0.8 [0.4, 1.8]	0.6 [0.3, 1.5]	
WBC/hpf, *n* (%)	—	—	—	0.26
Missing	1	5	6	
0	4 (33.3)	9 (28.1)	13 (29.5)	
1–3	6 (50.0)	10 (31.3)	16 (36.4)	
4–10	1 (8.3)	4 (12.5)	5 (11.4)	
11–20	0 (0.0)	2 (6.3)	2 (4.5)	
21–30	1 (8.3)	4 (12.5)	5 (11.4)	
31–40	0 (0.0)	1 (3.1)	1 (2.3)	
41–50	0 (0.0)	1 (3.1)	1 (2.3)	
>100	0 (0.0)	1 (3.1)	1 (2.3)	
RBC/hpf, *n* (%)				**0.036**
Missing	1	5	6	
0	11 (91.7)	17 (53.1)	28 (63.6)	
1–3	0 (0.0)	12 (37.5)	12 (27.3)	
4–10	1 (8.3)	3 (9.4)	4 (9.1)	
Serum				
Eosinophil count × 10^9/l	—	—	—	0.77
n	8	33	41	
Median [IQR]	0.1 [0.0, 0.3]	0.2 [0.0, 0.3]	0.1 [0.0, 0.3]	
WBC count × 10^9/l	—	—	—	**0.028**
n	9	34	43	
Median	6.0 [5.2, 7.5]	8.3 [6.7, 9.5]	7.5 [5.9, 9.0]	

Bold values indicate statistical significant.
hpf, high power field; RBC, red blood cell; WBC, white blood cell count.
[a]If prot:Osmo was missing, prot:creat ratio was used instead.

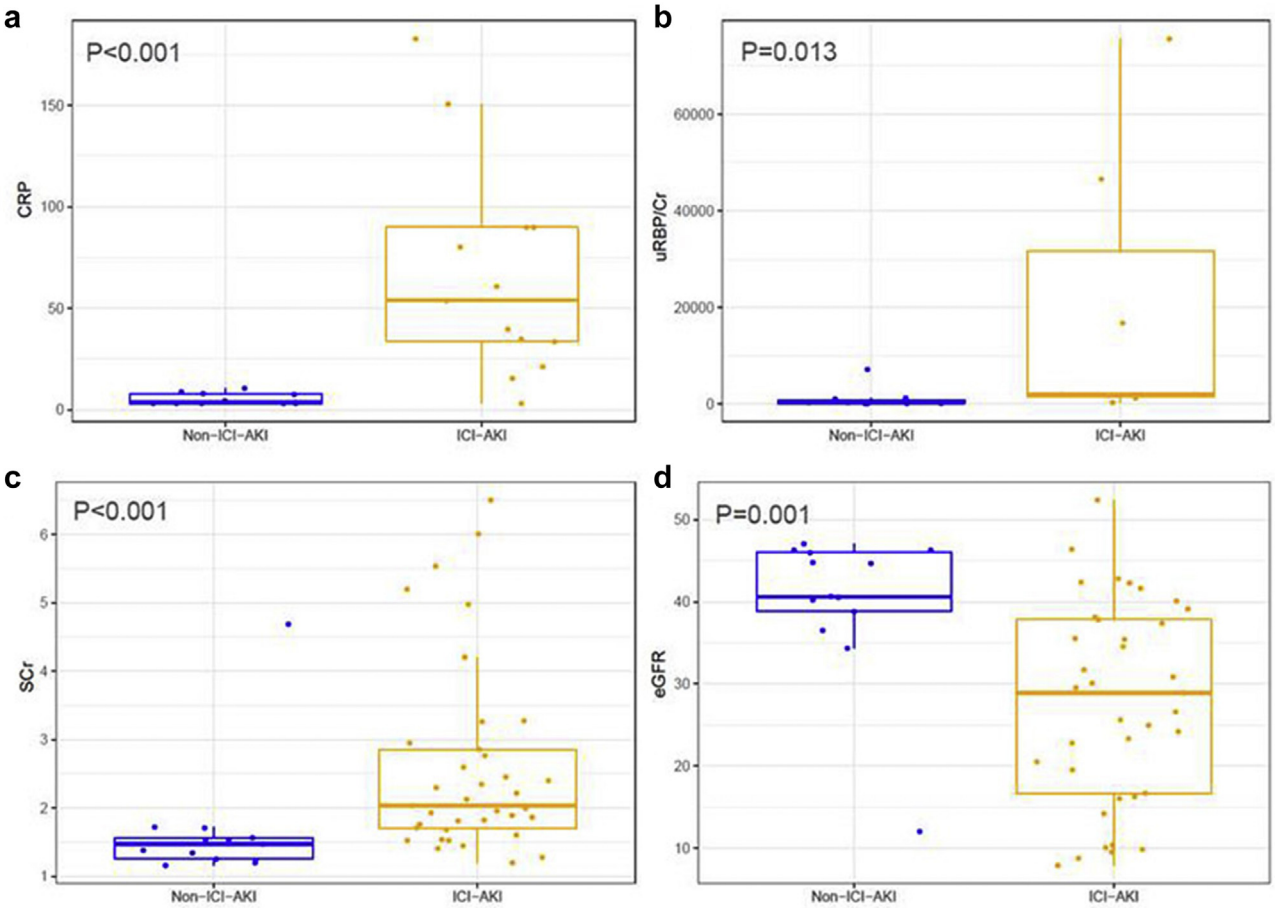

Figure 3. Boxplots overlaid with jitterplots of (a) CRP, (b) urine RBP/Cr, (c) SCr, and (d) eGFR measures at time of AKI, by cause of AKI. The boxes extend from the 25th to the 75th percentile and are bisected by the median; the whiskers extend to the most extreme value within 1.5 of the interquartile range. *P* values are derived from between-group comparisons using the nonparametric Wilcoxon rank sum test. AKI, acute kidney injury; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; ICI, immune checkpoint inhibitors; uRBP/Cr, urine retinol binding protein/urine creatinine; SCr, serum creatinine.

(Supplementary Figure S2 and Supplementary Table S4); however, due to the large number of missing kidney function and biomarker values over follow-up, we did not attempt any statistical testing. A similar pattern was observed with estimated glomerular filtration rate, which decreased in the ICI-AKI group as compared with the non–ICI-AKI group at the time of AKI (median 28.9 [16.7, 37.8] vs. 40.6 [38.9, 46] ml/min per 1.73 m^2, respectively, $P = 0.001$). Results remained significant after adjusting for medication use both at ICI initiation and within 14 days before AKI (Supplementary Table 1).Patient characteristics were shown to be similar in patients with missing versus nonmissing CRP and/or uRBP/Cr labs, with the exception of patients with missing labs having later stages of AKI and fewer antibiotic drugs prescribed than patients with present labs (Supplementary Table S1).

Treatment of ICI-AKI

ICI therapy was held or completed in 36 (97%) versus 2 (15%) of the patients with ICI-AKI and non–ICI-AKI, respectively. A total of 34 (92%) patients received corticosteroids in the ICI-AKI group. Among the ICI-AKI patients with available data ($n = 33$), median [IQR] initial prednisone dose was 60 [40, 60] mg/d approximately (1 mg/kg), and median time from initiation of glucocorticoid therapy to prednisone tapered to ≤10 mg/d was 1.55 [1.12, 2.30] months. There was no significant association observed between initial prednisone dose and time to tapering (correlation coefficient = 0.10, *P* value = 0.57). Median intravenous pulse steroids was 2 [0.75, 4] g/d among the 11 (30%) patients with ICI-AKI who were treated with this method. At initial AKI episode, none of the patients received additional immunosuppression beyond steroids. Only 3 patients required renal replacement therapy in the ICI-AKI group at initiation of corticosteroids.

Cumulative Incidence of Renal Recovery After ICI-AKI

Among the ICI-AKI patients, the cumulative incidence of renal recovery by 3 months (SCr <25% from baseline) was calculated after accounting for the competing

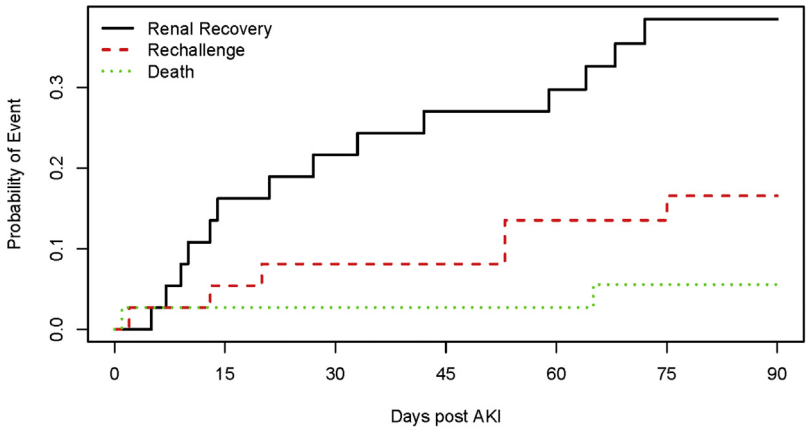

Figure 4. Cumulative incidence of renal recovery, with the competing risks of rechallenge and death. AKI, acute kidney injury.

risks of rechallenge or death. Patients who were lost to follow-up before 3 months were censored at their contact date. Over the course of 3 months of follow-up, 14 (39%) patients first experienced renal recovery, 6 (17%) patients first experienced rechallenge, and 2 (6%) patients died without experiencing renal recovery or undergoing rechallenge (Figure 4). At 1 month, the probabilities of renal recovery, rechallenge, and death were 22%, 8%, and 3%, respectively; at 2 and 3 months, they were 30%, 14%, and 3% and 39%, 17%, and 6%, respectively.

Rechallenge of Patients With ICI-AKI and Recurrent AKI

Rechallenge with an ICI was attempted in 16 (43%) of the ICI-AKI patients at a median [IQR] of 2.1 [0.87, 8.2] months after their AKI event. Most patients (15 [97%]) were rechallenged with the same ICI agent implicated in the initial AKI episode; among these, 3 (20%) had the ICI reduced to monotherapy (nivolumab) from previous combined therapy with (ipilimumab). There was only 1 (6%) patient who switched drugs (pembrolizumab to atezolizumab). A total of 13 (81%) patients were on corticosteroids at rechallenge. Survival in the rechallenged group was compared with the $n = 15$ patients in the ICI-AKI group who were not rechallenged due to the following reasons: remission ($n = 2$), because of ICI-AKI and fear of recurrence ($n = 11$), or another more severe immune-related adverse event ($n = 2$). Patients who were not rechallenged due to death or transition to hospice ($n = 5$) or progression of disease on ICI ($n = 1$) were excluded from the analysis, as they were not eligible for rechallenge. A total of 9 patients died during follow-up after the decision of whether or not to rechallenge was made. Survival tended to be higher in the group not rechallenged compared with rechallenged; however, results were not statistically significant (log-rank test P value = 0.06) (Supplementary Figure S3). Results were similar when restricted to the melanoma malignancy subtype (data not shown). Other subtypes had too few patients and events to obtain reliable estimates.

Recurrent ICI-AKI occurred in 3 (19%) of rechallenged patients (Supplementary Figure S4). With the exception of 1, all these patients had been rechallenged with the same ICI (pembrolizumab). The latency period between the initial AKI episode and rechallenge was similar between those with and without recurrent AKI (median [IQR] of 6.2 [0.69, 15] months vs. 3.8 [1.3, 4.3] months, respectively; $P = 0.59$). AIN drug, subtype, and prednisone dose at rechallenge were also found to not significantly differ between patients with recurrent AKI and those without a recurrent AKI (Supplementary Table S4).

DISCUSSION

In this retrospective study, we investigated the levels of serum and urine biomarkers over time, as well as clinical and laboratory features in patients who developed ICI-AKI and patients who received immunotherapy but developed AKI due to reasons unrelated to the immunotherapy. First, we discovered that patients with ICI-AKI developed more severe kidney injury at an earlier time than the non–ICI-AKI patients, and they were also more likely to be on a PPI as compared with the referent group at inception and throughout the course of therapy. As previously reported by our group and others, AIN is the most common histopathological dominant lesion seen in patients with ICI-AKI, found in 100% of biopsied patients in our institution at the time of AKI.[10,12–14]

As ICIs have been shown to improve survival of patients with several types of advanced cancers, overall survival may be affected by development of AKI.[16] There is a critical need to identify biomarkers that can predict risk of nephrotoxicity in patients receiving ICIs, especially in those for whom kidney biopsy is

contraindicated. Our study is the first to identify 2 protein biomarkers, serum CRP and uRBP/Cr, as identifiers of ICI-AKI, with AIN as the dominant lesion in the kidney biopsy. Currently, major efforts in biomarker studies are ongoing in patients using ICIs.[20] Several types have been proposed to identify different organs affected by iRAE[21] while many of these biomarkers are still in early phases of development, CRP and uRBP/Cr are already routinely available for clinical use. Previous studies have shown that increased CRP levels after baseline may indicate increased iRAE risk,[22] but it can be nonspecific for individual iRAEs. However, CRP used in conjunction with other biomarkers and in the right clinical context, could be a highly sensitive diagnostic of presence of iRAE when other infectious and inflammatory causes are ruled out. We found that elevated serum CRP concentrations, particularly in conjunction with an elevated uRBP/Cr ratio, may indicate the presence of renal iRAE. RBP is a low-molecular-weight protein that is reabsorbed by renal proximal tubule cells where it is catabolized and under normal conditions very little of the filtered RBP is excreted in the urine.[23] Therefore, RBP is a biomarker of proximal tubular dysfunction and is being used as a diagnostic tool in some proximal tubulopathies and interstitial kidney diseases like AIN.[24,25] Presumably, the urinary RBP excretion is increased due to nonspecific effects of the interstitial inflammation. In our study, cases of ICI-AKI were associated with elevated levels of CRP and uRBP/Cr, specifically with occurrence of other concomitant iRAE and/or presence of associated AIN medication. Conversely, when both biomarkers are within normal limits, the presence of renal iRAE is unlikely. In our study, only 1 patient in the ICI-AKI group had CRP levels within normal limits. This patient had been on corticosteroid therapy for 4 weeks to treat other iRAEs, but creatinine had not returned to baseline, and therefore kidney biopsy was performed to confirm this patient's diagnosis of resolving AIN and ATI.

These biomarker findings may be of particular value when ICIs are used in association with other potential nephrotoxic cancer agents, as it can be helpful to distinguish ICI-AKI from other forms of AKI related to platinum therapy or other targeted therapies because usually these other cancer agents are not associated with the plethora of unique iRAEs. Particularly, if CRP is normal, it would make an ICI-AKI diagnosis less likely compared with AKI from other conventional cytotoxic chemotherapy. This also applies when differentiating AKI in the setting of hemodynamic changes or iodine contrast exposure. Moreover, the use of these biomarkers can be useful to guide therapy when kidney biopsy cannot be obtained. However, if at any point results of kidney biopsy implicate change in management, then it is paramount to have kidney biopsy performed.

As previously reported, subnephrotic proteinuria and pyuria were present in patients with ICI-AKI[26,27] and they had greater number of urinary white blood cells, red blood cells, and blood leucocyte count as compared with non–ICI-AKI. Hansel stain was not performed or was negative in most of the patients, as it is well known that its sensitivity/specificity is quite low for AIN diagnosis.[28]

Similar with other reports, we also confirmed that PPI use is associated with ICI-AKI.[13] We specifically reviewed patients who were on PPI or any other drug associated with AIN at inception of ICI therapy, and found that the time to development of AKI occurred earlier in the ICI-AKI group as compared with the non–ICI-AKI group. These results suggest that when PPIs are prescribed at the initiation of immunotherapy, these patients should be followed particularly closely for AKI events; however, larger confirmatory prospective studies are needed. Other risk factors associated with ICI-AKI that have been previously described include presence of chronic kidney disease and combination ICI therapy; however, we were not able to verify these findings, possibly due to limited statistical power.[16]

Among patients who developed ICI-AKI, approximately 40% presented with complete renal recovery by 3 months of follow-up. Once rechallenged, approximately 80% of the patients were free of AKI recurrence at last follow-up. However, for those who had recurrence of AKI, none of them achieved complete renal recovery from the second AKI, with 1 requiring renal replacement therapy (Supplementary S3). Survival was not different between patients who were rechallenged or not.

Our study is the first to identify potential biomarkers and specific clinical and laboratory characteristics to distinguish development of ICI-AKI compared with idiopathic AKI. However, we do acknowledge several limitations. First, because this is a retrospective study, laboratory measures and follow-up were limited by availability, resulting in a relatively small sample size and therefore reduced power to detect statistically significant differences. Moreover, it is possible that some nonbiopsied patients either in the ICI-AKI or non–ICI-AKI groups may have been misadjudicated by the treating nephrologists as having one or the other diagnosis; however, kidney function did not deteriorate in the referent group. AKI was also milder in this group, which could potentially be explained by the fact that AKI stage 1 is a common scenario for nephrology referral. Therefore, for early stages of AKI, CRP and uRBP/Cr can serve as useful adjuvant

screening tools for the diagnosis of ICI-AKI, because all exposed and referent patients were selected from the same at-risk population. Although some studies have described ATI as one of the tissue findings for the diagnosis of ICI-AKI,[29] we considered biopsies with predominant ATI findings on the kidney biopsy not to be related to ICI-AKI, and this was confirmed by improvement of kidney function with conservative management (i.e., without glucocorticoids) in practically all the referents with reinstitution of ICI without further detriment of kidney function.

In conclusion, we provide novel, important data for clinicians regarding the use of biomarkers in the routine evaluation of cause of AKI in patients on ICI therapy. These biomarkers could assist with discriminating ICI-AKI from other causes and may also help aid clinical decision-making related to both management and recurrence. We also described distinguishing clinical characteristics of patients with ICI-AKI, which may help with the diagnosis and possible prevention of ICI-AKI, by reinforcing the strategy of avoidance of AIN-associated drugs in this type of patient. Rechallenge is possible; however, survival outcomes may not depend only on this. Despite promising results, further studies with multicenter collaboration are still warranted to identify and validate the exact combination of biomarkers that are predictive of treatment outcomes and the occurrence of kidney nephrotoxicity.

DISCLOSURES

LK reports grants from Bristol Myers Squibb outside the submitted work. NL has stocks in Checkpoint Therapeutics and is on the advisory board of AbbVie, Takeda, and Aduro. All the other authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

Concept and design: All authors. Acquisition, analysis, or interpretation of data: All authors. Drafting of the manuscript: BI and SMH and LV. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: SMH and LV. Preparation of figures and tables: BI, SMH, MPA, and LV. All authors approved the final version of the manuscript, and all are accountable for all aspects of the submitted work.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Histologic features of biopsied patients in the ICI-AKI and non–ICI-AKI.

Table S2. Results from ANCOVA analysis comparing AKI cause and kidney function and biomarker levels, adjusting for medication usage at ICI initiation and within 14 days before AKI.

Table S3. Kidney function and biomarker levels over time, overall and by cause of AKI.

Table S4. Comparison of characteristics among 16 patients with ICI-AKI who were rechallenged, overall and by AKI status after rechallenge.

Table S5. Comparison of patient characteristics in those with versus without CRP and uRBP/Cr labs.

Figure S1. Boxplot overlaid with jitterplot of log (CRP*uRBP/cr), by cause of AKI.

Figure S2. (A) Longitudinal patient trajectory data of kidney function over follow-up, by AKI cause. (B) Longitudinal patient trajectory data of biomarker labs over follow-up, by AKI cause.

Figure S3. Kaplan-Meier curve of survival time in months among those rechallenged versus not rechallenged.

Figure S4. Flow chart of rechallenge and recurrence of ICI-AKI.

STROBE Statement.

REFERENCES

1. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer*. 2012;12:252–264.
2. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010;363:711–723.
3. Motzer RJ, Tannir NM, McDermott DF, et al. Nivolumab plus ipilimumab versus sunitinib in advanced renal-cell carcinoma. *N Engl J Med*. 2018;378:1277–1290.
4. Hamid O, Robert C, Daud A, et al. Five-year survival outcomes for patients with advanced melanoma treated with pembrolizumab in KEYNOTE-001. *Ann Oncol*. 2019;30:582–588.
5. Postow MA, Sidlow R, Hellmann MD. Immune-related adverse events associated with immune checkpoint blockade. *N Engl J Med*. 2018;378:158–168.
6. Herrmann SM, Perazella MA. Immune checkpoint inhibitors and immune-related adverse renal events. *Kidney Int Rep*. 2020;5:1139–1148.
7. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N Engl J Med*. 2015;373:23–34.
8. Wolchok JD, Chiarion-Sileni V, Gonzalez R, et al. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. *N Engl J Med*. 2017;377:1345–1356.
9. Haanen J, Carbonnel F, Robert C, et al. Management of toxicities from immunotherapy: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2017;28:iv119–iv142.

10. Cortazar FB, Marrone KA, Troxell ML, et al. Clinicopathological features of acute kidney injury associated with immune checkpoint inhibitors. *Kidney Int*. 2016;90:638–647.
11. Shirali AC, Perazella MA, Gettinger S. Association of acute interstitial nephritis with programmed cell death 1 inhibitor therapy in lung cancer patients. *Am J Kidney Dis*. 2016;68:287–291.
12. Mamlouk O, Selamet U, Machado S, et al. Nephrotoxicity of immune checkpoint inhibitors beyond tubulointerstitial nephritis: single-center experience. *J Immunother Cancer*. 2019;7:2.
13. Seethapathy H, Zhao S, Chute DF, et al. The incidence, causes, and risk factors of acute kidney injury in patients receiving immune checkpoint inhibitors. *Clin J Am Soc Nephrol*. 2019;14:1692–1700.
14. Manohar S, Ghamrawi R, Chengappa M, et al. Acute interstitial nephritis and checkpoint inhibitor therapy. *Single Center Experience of Management and Drug Rechallenge*. 2020;1:16–24.
15. Meraz-Muñoz A, Amir E, Ng P, et al. Acute kidney injury associated with immune checkpoint inhibitor therapy: incidence, risk factors and outcomes. *J Immunother Cancer*. 2020;8, e000467.
16. Cortazar FB, Kibbelaar ZA, Glezerman IG, et al. Clinical features and outcomes of immune checkpoint inhibitor-associated AKI: a multicenter study. *J Am Soc Nephrol*. 2020;31:435–446.
17. Kitchlu A, McArthur E, Amir E, et al. Acute kidney injury in patients receiving systemic treatment for cancer: a population-based cohort study. *J Natl Cancer Inst*. 2019;111:727–736.
18. Thompson JA, Schneider BJ, Brahmer J, et al. Management of immunotherapy-related toxicities, version 1.2019. *J Natl Compr Canc Netw*. 2019;17:255–289.
19. Khwaja A. KDIGO clinical practice guidelines for acute kidney injury. *Nephron Clin Pract*. 2012;120:c179–c184.
20. Nakamura Y. Biomarkers for immune checkpoint inhibitor-mediated tumor response and adverse events. *Front Med (Lausanne)*. 2019;6:119.
21. von Itzstein MS, Khan S, Gerber DE. Investigational biomarkers for checkpoint inhibitor immune-related adverse event prediction and diagnosis. *Clin Chem*. 2020;66:779–793.
22. Abolhassani A-R, Schuler G, Kirchberger MC, Heinzerling L. C-reactive protein as an early marker of immune-related adverse events. *J Cancer Res Clin Oncol*. 2019;145:2625–2631.
23. Kirsztajn GM, Nishida SK, Silva MS, et al. Urinary retinol-binding protein as a prognostic marker in glomerulopathies. *Nephron*. 2002;90:424–431.
24. Domingos MAM, Moreira SR, Gomez L, et al. Urinary retinol-binding protein: relationship to renal function and cardiovascular risk factors in chronic kidney disease. *PLoS One*. 2016;11, e0162782.
25. Norden AG, Lapsley M, Unwin RJ. Urine retinol-binding protein 4: a functional biomarker of the proximal renal tubule. *Adv Clin Chem*. 2014;63:85–122.
26. Manohar S, Albright RC Jr. Interstitial nephritis in immune checkpoint inhibitor therapy. *Kidney Int*. 2019;96:252.
27. Oleas D, Bolufer M, Agraz I, et al. Acute interstitial nephritis associated with immune checkpoint inhibitors: a single-centre experience. *Clin Kidney J*. 2020:sfaa008.
28. Muriithi AK, Leung N, Valeri AM, et al. Biopsy-proven acute interstitial nephritis, 1993–2011: a case series. *Am J Kidney Dis*. 2014;64:558–566.
29. Izzedine H, Mathian A, Champiat S, et al. Renal toxicities associated with pembrolizumab. *Clin Kidney J*. 2019;12:81–88.