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Aristolochic acid nephropathy: variation in presentation and prognosis

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Abstract

Background. Aristolochic acid nephropathy (AAN) is a worldwide problem and one of the common causes of chronic kidney disease (CKD) in China.

Methods. Three hundred patients diagnosed as AAN from 1997 to 2006 were enrolled. Medical histories of Chinese herb ingestion, clinical–pathological features and risk factors for renal failure were recorded. Patients were followed up for 2–156 months. Factors involved in the prognosis of AAN were investigated.

Results. The 300 patients with AAN manifested three clinical subtypes, including acute kidney injury (acute AAN) in 13 patients, abrupt tubular dysfunction with normal serum creatinine (Scr) levels in seven cases and chronic tubulointerstitial nephropathy with decreased estimated glomerular filtration rate (eGFR) (chronic AAN) in 280 cases. The acute AAN cases had the highest aristolochic acid (AA)-I intake per day and developed progressive kidney failure during 1–7 years follow-up. The tubular dysfunction AAN patients had the lowest cumulative AA-I intake and were able to keep normal Scr levels during 2–8 years follow-up. The chronic AAN patients took the lowest AA-I dose per day but with the longest period and the highest cumulative dosage and exhibited a very large range of eGFR changing

rate (from −21.6 to 5.2, median −3.5 mL/min/year). The cumulative AA-I intake ($r = 0.330$, $P = 0.045$) and the time course from the termination of AA medication to the start of follow-up ($r = -0.430$, $P = 0.009$) were found to be independent factors correlated with the decrease rate of eGFR in the chronic AAN patients. AA and the metabolites could be detected in a high frequency in patients who had stopped herbal medication for 1 year, which indicates a rather long washout time for these chemicals.

Conclusions. AAN has variant phenotypes with distinct prognosis, which is determined by the variable AA medications. With better understanding of toxic and environmental causes for kidney injury, there would be a better chance to uncover the causal factors of cases of 'CKD without known causes' which is crucial for improving the disease outcomes.

Keywords: aristolochic acid; aristolochic acid nephropathy; chronic kidney disease; prognosis

Introduction

Aristolochic acid nephropathy (AAN) has been a recognized cause of progressive chronic kidney disease (CKD) worldwide for ~20 years [1–12]. The typical feature of AAN was described as extensive interstitial fibrosis and severe loss of the renal tubules along with a rapid progression to end-stage renal disease [1, 5–9]. The 2-year renal survival rate has been reported to be only 17%, which is much worse than the outcome for other tubulointerstitial nephropathies (74%) [13].

After being warned of the prolonged nephrotoxic reactions which occur with the use of aristolochic acid (AA)-containing medications, nephrologists in China started to collect medication histories from all of their CKD patients as a routine clinical practice, especially from those presenting with chronic tubulointerstitial nephropathy of unknown cause. More and more CKD cases were consequently found to be associated with AA-containing medication in the form of eight different kinds of herbs. Over the years, thousands of AAN patients have been diagnosed and followed long-term which allows us to provide a comprehensive view of this form of tubulointerstitial disease.

Through a prospective observational study involving 300 patients with AAN for a period between 2 and 156 months (median 60 months), this paper describes a variety of clinical phenotypes of AAN, along with their distinct prognosis and associated risk factors for progression of disease.

Materials and methods

Patients

Three hundred patients (104 males and 196 females) were enrolled in this study. These individuals (aged 54.2 ± 11.4 years) were diagnosed with AAN in our hospital from 1 January 1997 to 31 December 2006.

Criteria for diagnosis

AAN was diagnosed according to (i) the presence of a definite history of taking AA-containing medications prior to disease onset; (ii) the presence of obvious tubular dysfunction, with or without diminished GFR; (iii) the absence of recent or long-term ingestion of antibiotics, nonsteroidal anti-inflammatory drugs, diuretics or Chinese traditional medicines containing minerals or metals and (iv) the absence of evidence of other glomerular or tubulointerstitial diseases caused by infectious or immune diseases. Among these patients, 57 cases were pathologically confirmed as tubulointerstitial nephropathy by renal biopsy. The cumulative dosage of AA-containing herbal medication was recorded. Since AA-I is known to be the main pathogenic ingredient of AA-containing herbs, the dosage of AA-I was calculated to make the further analysis. The content of AA-I in various herbal medications (pills or decoctions) was measured by the method of high-performance liquid chromatography (HPLC) [14], and the cumulative dosage of AA-I was calculated accordingly.

Evaluation of clinical parameters

Medical charts and prescriptions were carefully reviewed for each enrolled patient, as were their histories of Chinese herb ingestion, with special attention paid to the ingredients, dosage, timing in relation to first-occurring symptoms and the time course of renal dysfunction. The risk factors for renal dysfunction were recorded, including the cumulative intake of AA-I, the presence/absence of hypertension, diabetes and cardiac vascular diseases, smoking, obesity and family history of kidney diseases. Routine laboratory data were collected at the first visit, including levels of haemoglobulin (Hb) and urinalysis. Renal proximal tubule dysfunction was assessed by the presence of renal glycosuria, as well as by elevated levels of urinary *N*-acetyl-β-D-glucosaminidase and low molecular weight urinary proteins, including γ-transglutaminase and α1-microglobulin. Renal distal tubule dysfunction was identified by decreased urine osmolality and abnormal acidification tests. Glomerular function was evaluated in terms of serum creatinine (Scr) levels, and estimated GFR (eGFR) was calculated with a modified MDRD fomula appropriate for Chinese [15]. Kidney size was measured by B-ultrasound. Kidney atrophy was defined as a longitude of <10 cm.

The plasma from 46 chronic AAN patients were randomly selected and the concentrations of AA-I and AA-II and aristolactam I and II (AL-I and AL-II) were tested by the method of HPLC.

Histological examinations

Kidney biopsy sections were processed for light microscopy (hematoxylin-eosin, periodic acid-schiff and Masson's trichrome stain), electron microscopy and immunofluorescence staining. Each section included >10 glomeruli.

Treatment and follow-up

Cases with acute kidney injury (AKI) and/or abrupt tubular dysfunction were treated initially with 0.75 mg/kg/day prednisone for 1 month; the dose was then tapered gradually to 0.1 mg/kg/day prednisone over 2 weeks. Chronic patients were given standard treatments for CKD. Patients were scheduled to attend follow-up visits every 1–3 months. Patients who had CKD Stages 3–5 were recommended standard low protein diet and monitored by a training specialist. At each appointment, details of all symptoms and/or serious events (such as infection and cardiovascular abnormalities) were recorded. Every 3 months, patients were assessed for Hb, urinalysis, serum electrolytes, acid–base balance disturbances and Scr level.

These 300 AAN patients were followed up for 2–156 months (a median of 60 months) by the end of 2009. Two hundred and eighteen patients were followed up for >1 year. During the follow-up, 95 and 97% of these patients reached the targets of blood pressure (stabilized to <130/80 mmHg) and Hb (>110 g/L), respectively. Metabolic acidosis and electrolyte disturbance were well controlled to normal status in all patients.

Statistical analysis

All analyses and calculations were performed with the SPSS statistical package, version 13.0. Data are presented as the mean ± SD or median (range) for continuous variables and as proportions for categorical variables. Proportions and mean values were examined using χ^2 statistics for categorical variables and Mann–Whitney test for continuous values. The relationships between the eGFR decrease rate and clinical and medication data were assessed by Spearman test and Multivariable Lineal Regression analysis. Differences with P-values <0.05 were considered significant.

Results

Clinical features and prognosis of different subtypes of AAN

The prevalence of hypertension was 59.3%, with MAP at 96.4 ± 8.9 mmHg. Four percent of the patients had diabetes. Body mass index of the whole group was 23.3 ± 2.5 kg/m^2 and 3.3% of the patients met the diagnosis of obesity. Seventy-four patients (24.7%) were self-reported smokers. Two patients were of mother and daughter relationship. Both of them took AA-containing herbs and were both diagnosed as AAN. None of the other patients had a family history of kidney diseases.

The 300 AAN patients manifested three different clinical subtypes. The clinical features are listed in Tables 1 and 2.

Thirteen patients developed AKI within 3 months after termination of the herbs and were diagnosed as acute AAN. Twelve (92.3%) exhibited non-oliguric AKI. Polyuria and/or nocturia were quite common (six cases, 46.2%). Gastrointestinal abnormalities were the major complaints the first time the acutely afflicted cases went to the physician. The Scr level had increased to 304.1 ± 196.8 μmol/L (eGFR 24.4 ± 15.6 mL/min) accompanied by obvious tubular dysfunction by the time they were referred to our hospital. Hypertension was detected in nine cases (69.2%) and six cases (46.2%) exhibited mild to moderate anemia. The changes in Scr in these cases during the follow-up course are depicted in Figure 1. Within 1 month of renal biopsy, patients exhibited various degrees of progression, with Scr levels recovering to the normal range in two cases (15.4%), returning to <25% of the basal level in one case (7.7%) and with no recovery in the other 10 cases (83.3%). Ten cases

Table 1. Complaints leading to Consultation with Phyisican—Onset of AAN

	Acute AAN ($n = 13$)	Tubular dysfunction AAN ($n = 7$)	Chronic AAN ($n = 280$)	Total ($n = 300$)
Gender (male/female)	4/9	5/2	95/185	
Age (years)	43.2 ± 11.7*	39.6 ± 10.2*	55.1 ± 10.9	
GI[a] abnormality, n (%)	9 (69.2)	3 (42.9)	50 (17.9)	62 (20.7)
Fatigue, *n* (%)	3 (23.1)	2 (28.6)	43 (15.4)	48 (16.0)
Polyuria or nocturia, *n* (%)	6 (46.2)	3 (42.9)	24 (8.6)	33 (11.0)
Oliguria or anuria, *n* (%)	1 (7.7)	0 (0)	0 (0)	1 (0.3)
Abnormal urinalysis[b], *n* (%)	1 (7.7)	1 (14.3)	18 (6.4)	20 (6.7)
Increased Scr level[c], *n* (%)	0 (0)	0 (0)	146 (52.1)	146 (48.7)
Kidney atrophy dectected by B ultrasound[d], *n* (%)	0 (0)	0 (0)	9 (3.2)	9 (3.0)
Gross hematuria confirmed as TCC, *n* (%)	0 (0)	0 (0)	9 (3.2)	9 (3.0)

[a]Gastrointestinal abnormality.
[b]Including proteinuria, leucocyturia or hematuria by routine physical examination.
[c]Scr > 133 μmol/L by routine physical examination.
[d]Longitude <10 cm.

Table 2. Laboratory analyses of patients with AAN[a]

Parameters	Acute AAN ($n = 13$)	Tubular dysfunction AAN ($n = 7$)	Chronic AAN ($n = 280$)
Hypertension (≥140/90 mmHg), *n* (%)	9 (69.23)	0 (0)	169 (60.36)
Hemoglobulin (g/L)[b]	101.7 ± 19.1*	123.7 ± 18.7	107.4 ± 21.6*
Urinary protein (g/day)[c]	0.50 ± 0.14	1.09 ± 1.30	0.75 ± 0.49
Glucosiuria, *n* (%)	10 (76.9)	6 (85.7)	151 (53.9)
Increased urinary NAG[d], *n* (%)	13 (100)	7 (100)	19 (6.8)
Increased LMW Urinary protein[e], *n* (%)	13 (100)	7 (100)	279 (99.6)
Fanconi's syndrome, *n* (%)	2 (15.4)	5 (71.4)	0
Diabetes insipidus, *n* (%)	5 (38.5)	3 (42.9)	0
Decreased urinary osmolarity[f], *n* (%)	13 (100)	6 (85.7)	217 (77.5)
RTA[g], *n* (%)	6 (46.2)	4 (57.1)	199 (71.1)
Scr (μmol/L)	304.1 ± 196.8*·**	110.9 ± 27.6**	221.4 ± 107.0
Kidney atrophy, *n* (%)	0 (0)	1 (14.3)	184 (65.7)

[a]NAG, *N*-acetyl-β-D-glucosaminidase
[b]Normal range: 12–17 g/dL for males; 11–16 g/dL for females.
[c]Normal range: 0–0.16 g/day.
[d]Urinary NAG>21U/L (normal range: 0–21 U/L).
[e]LMW Urinary protein (Urinary γ-GT and/or Urinary β2-microglobulin) at a level of 2-fold higher than normal range.
[f]Urinary osmolarity < 500 Osm/kg.
[g]RTA: abnormal urinary acidification test accompanied by decreased level of CO_2CP.
*P < 0.005 versus tubular dysfunction AAN, **P < 0.001 versus chronic AAN.

(including one of the above recovered cases) were followed up for 1–7 years (mean of 4 years). Five (50%) had progressed to CKD Stage 5 by the end of follow-up. The other five remained in CKD Stage 4.

Seven cases manifested abrupt tubular dysfunction with normal Scr levels within 3 months after terminating the herbs and were diagnosed as tubular dysfunction AAN. Among these, five cases developed Fanconi's syndrome, three cases manifested diabetes insipidus and four cases displayed renal tubular acidosis. The eGFR was slightly decreased (67.9 ± 19.3 mL/min). No anemia or hypertension was observed. Five patients were followed up for 2–8 years (mean of 5 years) as shown in Figure 2. All of them remained at a normal Scr level (94.0 ± 16.7 μmol/L) with eGFR 80.5 ± 9.4 mL/min. The laboratory examinations revealed much alleviated but still existing tubular dysfunction.

The other 280 cases, presenting with chronic tubulointerstitial nephropathy with decreased eGFR at different degrees, were diagnosed as chronic AAN. Among them, 173 cases (61.8%) had not exhibited any overt symptoms until urinalysis or blood or ultrasound examination revealed abnormal phenomena. The Scr level was 221.4 ± 107.0 μmol/L (eGFR 25.1 ± 15.6 mL/min) at the first visit, with six cases (2.1%) at CKD Stage 2, 107 cases (38.2%) Stage 3, 88 cases (31.4%) Stage 4 and 79 cases (28.2%) Stage 5. Kidney atrophy (left side 9.7 ± 0.9 cm and right side 9.3 ± 1.0 cm), hypertension and anemia were common in this group. It is noteworthy that in nine patients of this group, gross hematuria was the first complaint which prompted them to consult a doctor and urinary transitional cell carcinoma was confirmed. Two hundred and three chronic AAN patients were followed up for >1 year (1–13 years, median 5 years). The median rate of change in eGFR was −3.5 mL/min/year, with a very large range of −21.6 to 5.2 mL/min/year. Among these chronic AAN patients, 44% exhibited rapidly progressive renal dysfunction with an eGFR decreasing rate of faster than 4 mL/min/year. Twenty percent exhibited partial remission, with more or less increment in the eGFR level during the follow-up. The others exhibited rather slowly progressing CRF as most of them had a decreasing eGFR rate of <2 mL/min/year.

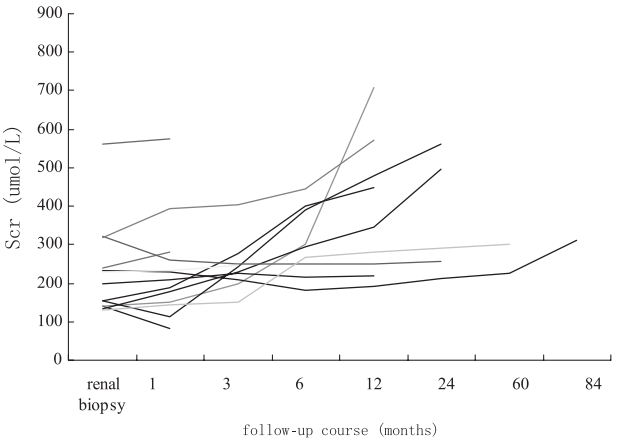

Fig. 1. Changes of Scr in acute AAN patients during follow-up course. The changes in Scr levels of the 13 acute AAN patients are depicted in Figure 1. Within 1 month of renal biopsy, 2 cases exhibited Scr levels recovering to the normal range, 1 case showed a decrement of Scr to <25% of the basal level and the other 10 cases did not exhibit any recovery in renal function. During the long-term follow-up course (1–7 years, mean 2 years), 10 of 10 patients exhibited progressive chronic renal failure. Among them, five had progressed to CKD Stage 5.

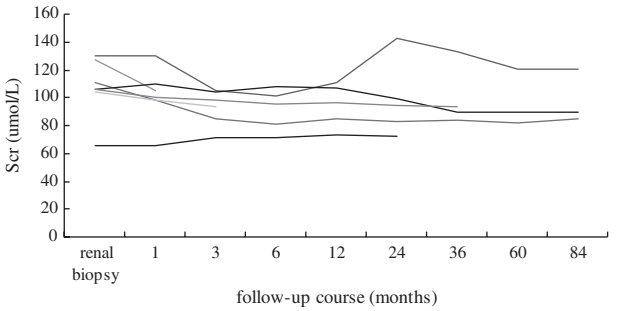

Fig. 2. Changes of Scr in tubular dysfunction AAN patients during follow-up course. The changes in the Scr level of the seven tubular dysfuntion AAN patients are depicted in Figure 2. Five patients were followed for 2–8 years. All of them remained normal in terms of Scr levels.

Pathological features of different subtypes of AAN

Immunofluorescent staining of IgG, IgM, IgA, C_3 and C_1q was negative in all the renal biopsy specimens from the AAN patients. In the acute AAN cases, light microscopy revealed focal tubular epithelial cell vacuolization or granulation, collapse and exfoliation (Figure 3A). Regeneration was rarely found and there was exposure of broad areas of denuded tubular basement membrane. The renal interstitium displayed diffuse edema, with dispersed or local infiltration of lymphocytes and monocytes. Electron microscopy revealed disruption of the tubular brush border and shedding of the microvillus. Swelling of the mitochondria and endoplasmic reticulum was prominent in this setting; mitochondrial cristae were ruptured and the number of lysosomes was remarkably increased. The tubular dysfunction AAN cases had much less degree of tubular necrosis and exfoliation than the acute AAN. In the chronic AAN cases, light microscopy revealed patchy or diffuse tubular epithelial cells atrophy and tubular dilation, interstitial fibrosis and very few mononuclear cells infiltrating within the interstitial compartment (Figure 3B).

Dosage and duration of intake of AA-containing medication in AAN patients

The AA-containing herbs in this study are of five types: Caulis Aristolochiae Manshuriensis (*Guan-Mu-Tong*, contains AA-I ~8.82%) in 221 cases, *Radix Aristolochiae* (*Qing-Mu-Xiang*, contains AA-I ~3.20%) in 50 cases, Radix Aristolochiae Fangchi (*Guang-Fang-Ji*, contains AA-I ~3.10%) in one case, Caulis Aristolochiae Debilis (*Tian-Xian-Teng*, contains AA-I ~0.082%) in one case and Herba Aristolochiae Molissimae (*Xun-Gu-Feng*, AA-I content unknown) in one case. Twenty-seven cases took two of the herbs listed above. These herbs were ingested either directly by herbal mixtures decoction (24 cases, 8%), usually consecutively (1–120 days) in a large intake of the AA-containing herbs or in pill form (262 cases, 87.3%) in a prescribed dosage and in an intermittent manner for a long

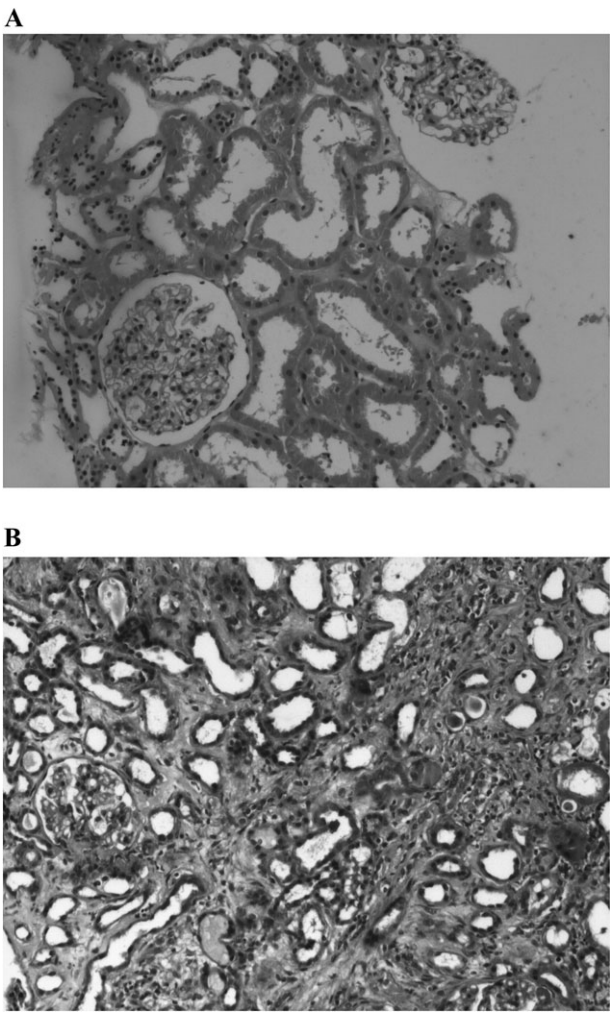

Fig. 3. Pathological features of AAN revealed by light microscopy. (**A**) Acute AAN is characterized by tubular epithelial injury, necrosis, collapse and exfoliation with naked tubular basment membrane. (**B**) Chronic AAN is characterized by patchy or diffuse TEC atrophy and tubular dilatation, interstitial fibrosis and a few mononuclear cells infiltrating within the interstitial compartment.

period (2–41 years, median 7 years). The other 14 patients took both decoction and pills (4.7%).

AA-I intake is the main determinant factor for the different clinical features and prognosis of the AAN

As shown in Table 3, the acute AAN cases exhibited features as the taking of a large amount of cumulative AA-I (0.02–3.96 g, median 1.04 g) within a short period (1–120 days, median 14 days), reflecting the highest AA-I intake per day (0.0063–0.314 g/day, median 0.0366 g/day) among the three groups, which was 20 times that taken by the tubular dysfunction AAN group and 70 times the chronic AAN group ($P < 0.05$, Table 3). The chronic AAN group also had a large cumulative AA-I intake (0.025–16.3 g, median 1.01 g) but in the longest period (6–14965 days, median 1825 days), resulting in the lowest AA-I dose per day of the three groups (0.00001–0.237 g/day, median 0.00005 g/day). The tubular dysfunction AAN cases took a higher AA-I dose per day (0.0037–0.078 g/day, median 0.0187 g/day) than the choric AAN patients ($P < 0.05$) but within a relatively short medication period (8–365 days, median 105 days), resulting in the lowest cumulative AA-I intake (0.2–2.01 g, median 0.52 g) among the three groups ($P < 0.05$).

In the chronic AAN patients, the cumulative AA-I intake ($r = 0.330$, $P = 0.045$) and the time course from the termination of the AA-containing medication to the start of follow-up ($r = -0.430$, $P = 0.009$) were also found out to be independent factors correlated with the decrease rate of eGFR. Other significantly related factors include age ($r = 0.308$, $P = 0.011$), Hb level ($r = -0.314$, $P = 0.001$) and coronary heart disease ($r = 0.495$, $P = 0.000$), tested by multivariable lineal regression analysis. The eGFR decreasing rate was dependent on the length of the period the patient had stopped taking the AA-containing medication: it was 365 days (0, 1825 days) in those patients presenting rapidly progressive renal dysfunction, 745 days (0–2920 days) in patients with slowly decreasing eGFR and 1095 days (180–3830 days) in patients exhibiting partial remission, respectively ($P < 0.05$).

Assay of plasma AAs and ALs in chronic AAN patients

Among the patients who had stopped taking AA-containing medications for no more than 1 year, 14 of 19 cases (73.7%) could be detected with at least one type of AAs or ALs in the plasma. For the patients who had terminated the herbs for >2 years, none of them showed positive detecting results (Table 4).

Discussion

AA is found primarily in the plant *Aristolochia*, including four species covering ~40 kinds of herbs, in which a half is used in herbal medicine. Among the 300 patients diagnosed with AAN in the present study, >90% took a small amount of medication containing AA for a long time, without the advice of medical doctors. The nephrotoxic effects of this herbal medication may come to attention years after cessation of the herbs, resulting in delayed diagnosis and treatment. In China, even though most of the AA-containing herbs have been banned from medical usage by the government since 2003, new cases of AAN keep emerging as herbal drugs are extensively used and easily obtained from rural pharmacies or markets. Also, there are probably other different kinds of herbs which potentially have nephrotoxic effects that have not been declared yet. Therefore, we strongly suggest that more attention should be paid to such medicinal factors in the onset and development of CKD. Also, recent evidence from 'Balkan endemic nephropathy' provides a striking example of toxic renal injury from plants in the local environments [16–19].

AAN patients in this study as well as in general practice in China exhibited quite various clinical features, which might be related to the different patterns of taking the medication. As we depicted in this study, taking a high dose of AA-containing medication frequently induced irreversible non-oliguric acute tubular necrosis and resulted in rapidly

Table 3. Features of AA-containing medications in different groups of AAN

	Acute AAN ($n = 13$)	Tubular dysfunction AAN ($n = 7$)	Chronic AAN ($n = 280$)
Herbs, *n* (%)			
Caulis Aristolochiae Manshuriesis	12 (92.3)	7 (100)	201 (71.8)
Radix Aristolochiae Fangchi	1 (7.7)	0	0
Radix Aristolochiae	0	0	50 (17.9)
Caulis Aristolochiae Debilis	0	0	1 (0.36)
Herba Aristolochiae Molissimae	0	0	1 (0.36)
Others	0	0	27 (10.00)
Method of ingestion			
Decoction, *n* (%)	11 (84.62)	3 (0.43)	10 (3.57)
Patent pill (*n*), *n* (%)	2 (15.39)	4 (0.57)	256 (91.43)
Decoction + patent pill (*n*), *n* (%)	0	0	14 (5)
Duration of medication			
Days (median)	14 (1–120)	105 (8–365)*	1825 (6–14965)
AA-I dose[a] (median)			
Cumulative (g)	1.04 (0.02, 3.96)	0.52 (0.2, 2.01)*	1.01 (0.025, 16.3)
Dose per day (g/day)	0.0366 (0.0063, 0.314)*	0.0187 (0.0037, 0.078)*,#	0.0005 (0.00001, 0.237)
Timecourse to the discovery of AAN (days) (median)			
From the start of medication	40 (1–210)*	45 (10–365)*	2555 (92–15330)
From the end of medication	0 (0–90)*	0 (0–60)*	122 (0–11315)
Still taking the medication at the time when AAN was discovered, *n* (%)	9 (69.2)	6 (85.7)	128 (45.7)

[a]The dosage of AA-I calculated according to HPLC measurement of herbal medications.
*$P < 0.05$ versus chronic AAN, #$P < 0.05$ versus acute AAN.

progressive chronic renal failure. Long-term intake of AA-containing medications, continuously or intermittently at a relatively low dose per day resulted in insidious chronic tubulointerstitial nephropathy, while taking a moderate dose in a shorter period of time induced abrupt tubular dysfunction including Fanconi's syndrome with a comparatively positive outcome, as some Asian nephrologists previously reported [20–22].

The long lasting and progressive renal dysfunction, even after stopping the intake of AA-containing medications, is one of the characteristics of AAN. In the present study, higher cumulative AA-I intake was correlated with worse disease outcome, declaring a dose-dependent renal injury pattern. Our previous pharmacodynamic study revealed that, 40 days after one dose oral administration of AA-I in rats, the kidney had the highest level of AA-I compared with other organs, which indicating an accumulation of the chemical in the kidney [23]. In this study, AAs and the metabolites could be detected in the plasma of patients who had already stopped the AA-containing medications for up to one and a half year, demonstrating a quite long run out time in human beings. Consistent with the assay of plasma AAs, patients who had stopped AA-containing medications within 1 year showed rather fast progressing rate of renal dysfunction. After stopping the medication for two years, the deterioration rate of renal function slowed down, and by 3 years, some patients attained stabilization or even partial remission of renal function. These indicated that the accumulating feature of AAs in the body, especially its concentration in the kidney is one of the main deciders for the consistent rapidly progressive renal dysfunction in AAN patients. Investigating methods that can facilitate the elimination of the chemicals could be valuable for the protection of the kidney.

Table 4. Assay of plasma AAs and ALs in chronic AAN patients

Termination of AA-containing medication[a]	Number	Positive number	Positive rate (%)
≤6 m	15	11	73.3
6–12 m	4	3	75.0
12–18 m	19	9	47.4
18–24 m	0	0	0
>24 m	11	0	0

[a]Time course from the termination of AA-containing medication to the collection of the blood sample.

Except for the accumulating and thereafter the long lasting toxic effect of AA, other pathophysiological factors related to the progression of kidney injury have also been demonstrated. Our previous study revealed that a low expression of epithelial growth factor and vascular endothelial growth factor by the tubular epithelial cells together with impaired peritubular microvasculature resulted in the disability of the epithelium to proliferate in acute AAN, which could also be a major reason for the unpleasant outcome [24].

In the present study, we reported a large group of the different clinical–pathological phenotypes of AAN in Chinese patients, induced by several kinds of AA-containing herbs. The AA medications and the elimination of the chemicals seemed to be major determining factors for the different clinical features and prognosis of the AAN. Since most of the patients were first diagnosed as 'CKD without known causes' for months or even years, it strongly indicates that further examination into the biomarkers of tubular injury, detection of AA and its metabolites, and carefully

screening the medication history are very important to clarify the pathogeny of these patients and further monitor the remnant renal function. With a better understanding of toxic and environmental causes for kidney injury, there is a better chance to uncover the causal factors of cases of CKD without known causes, which is crucial for improving the disease outcomes.

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Conflict of interest statement. None declared.

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