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Title:

Predicting outcome in acute interstitial nephritis: a case series examining the importance of histological parameters

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ABSTRACT

Background: The clinical significance of common histological parameters in acute interstitial nephritis (AIN) is uncertain. We aimed to evaluate the utility of histology in predicting clinical outcomes in patients with AIN.

Methods and results: Adult renal biopsies yielding a diagnosis of AIN between 2000 and 2015 were re-examined. Patients were divided into groups based on: 1) the percentage of non-fibrotic cortex containing inflammation (NFI-score), (NFI-1=0-24%, NFI-2=25-74%, NFI-3=75-100%), and 2) the percentage of cortex containing tubular atrophy (TA score), (TA1=0-9%, TA2=10-24%, TA3=25-100%). The primary outcome was a composite of ≥50% reduction in serum creatinine (sCr) or an eGFR >60 ml/min/1.73m^2 1-year post-biopsy. From a total of 2817 native renal biopsies, there were 120 patients with AIN and adequate data for analysis. Of these, 66 (56%) achieved the primary outcome. On univariable logistic regression, NFI-3 was associated with a 16 times increased likelihood of achieving the primary outcome compared to NFI-1 (OR 16 (95% CI 5.2-50)). In contrast, TA3 were associated with a 90% reduced likelihood of achieving the primary outcome compared to TA1 (OR=0.10 (95%CI 0.0-0.3)). Maximal clinical utility was achieved by combining TA and NFI into a single prognostic 'TANFI' score, which had an independent predictive effect on the primary outcome in a multivariable regression model consisting of age, sex, baseline sCr and identified drug cause.

Conclusions: In patients with biopsy-proven AIN, a lower percentage of cortical tubular atrophy and, paradoxically, a higher percentage of inflammation in non-fibrosed cortex were associated with an increased likelihood of a positive clinical outcome.

Key words: Acute Interstitial Nephritis; Renal histology; Acute kidney injury; Corticosteroids

Introduction

Acute interstitial nephritis (AIN) is a recognised cause of acute kidney injury (AKI), and the incidence of AIN is increasing worldwide.(1–3) Definitive diagnosis requires renal biopsy. AIN is the resulting diagnosis in 1-3% of all renal biopsies and 6.5-35% of biopsies performed in the context of AKI.(4,5) Despite this, there is a lack of consensus regarding precise histological diagnostic criteria and the clinical significance of common histological findings is uncertain.

Two recent studies define AIN as “the presence of prominent interstitial inflammation in the non-fibrotic cortex and tubulitis”.(3,6) Other definitions share similar themes but vary on whether the interstitium containing the infiltrate can be fibrotic or not,(7) and on the presence of co-existent tubulitis and/or oedema (4,5,8,9). Beyond diagnosis, the role of histology in prognostication in AIN is also unclear. Several small studies have explored the relationship between histological findings and clinical outcomes in AIN with conflicting results.(10–13) These 4 studies each included 30 patients or fewer and were performed over 20 years ago, over which time the landscape of AIN has changed.(3) More recent studies have largely focused on the potential influence of corticosteroid treatment in the management of AIN.(14) However, Muriithi *et al* found that increased tubular atrophy correlated with poor response to corticosteroids.(3) While in a multi-centre retrospective study of steroid-treated AIN, the presence of >50% interstitial fibrosis associated with a reduced likelihood of renal recovery.(15)

Beyond tubular atrophy and interstitial fibrosis, the prognostic significance of other histological variables in AIN, in particular interstitial inflammation, has not been assessed. Furthermore, given the wide variation in clinical outcomes in patients with AIN (ranging from complete recovery of renal function to established renal failure), a prognostic score to guide management decisions and patient expectations is desirable.

The aim of the present study is to evaluate the utility of histology in predicting clinical outcomes in a cohort of patients with AIN.

Materials and methods

Patient population

All adult patients with a first native renal biopsy diagnostic of AIN between 2000 and 2015 in the Glasgow Renal & Transplant Unit were identified. This unit serves a defined population of 1.5 million, with the predominant ethnic group being white. Clinical data were collected retrospectively using the West of Scotland Electronic Patient Record. Baseline data at time of biopsy, including patient demographics, serum creatinine (sCr), urine protein:creatinine ratio (uPCR), serum albumin, serum eosinophil count, medications and biopsy report were collected. Clinical correspondence was reviewed to identify the aetiology of AIN where known. Outcome data including sCr and need for renal replacement therapy were collected at 12 +/- 3 months from time of biopsy. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation.(16) The primary outcome was a composite of ≥50% reduction in sCr or an eGFR of >60 ml/min/1.73m^2 at 12 months from the time of biopsy. Instances of 50% reduction in sCr due to dialysis were discounted. The secondary outcome was an eGFR <30 ml/min/1.73m^2 at 12 months from biopsy. Caldicott Guardian Approval was granted for this project.

Renal histology

Kidney biopsies were processed as standard, with hematoxylin and eosin, periodic acid–Schiff, Masson trichrome, and Jones methenamine silver stains used for light microscopy. Additional sections were routinely examined using immunofluorescence and electron microscopy. Histological diagnosis of AIN was based on the presence of an interstitial inflammatory cell infiltrate. All biopsies were reviewed by a single pathologist without knowledge of the clinical outcome and scored for the percentage of total and non-fibrosed

cortex containing inflammation, the percentage of cortex with tubular atrophy, and the presence or absence of, granulomas, prominent eosinophils, prominent neutrophils and oedema. The presence and severity of tubulitis and the density of the infiltrate (mild, moderate, severe) was recorded. The number of glomeruli, the number of sclerosed glomeruli and the severity of arteriosclerosis was also recorded. Guidance for histological analysis is included in supplementary material (S1).

Statistical analysis

Descriptive statistics are reported as mean and standard deviation or median and interquartile range for normally distributed and skewed variables, respectively. Continuous histological variables (such as the percentage of non-fibrosed cortex containing inflammation (NFI), and the percentage of tubular atrophy (TA) were divided into groups based on the spread of data. Kruskal-Wallis tests were used to compare median sCr between groups at baseline and 1 year. Binary logistic regression was applied to assess the ability of individual histological and baseline features to predict the clinical outcomes. Histological variables were entered into a multivariable logistic regression model and then excluded in a stepwise manner until only histological variables that retained independent statistical significance ($p<0.05$) remained. Age, sex, baseline sCr and the presence or absence of an identified drug cause were included in the model on clinical justification. Overall model fit was assessed using Nagelkerke's R^2 and C-statistic, with an increase in both values suggesting a better model fit. Correlation between histological variables was assessed using Spearman-Rank correlation. Pearson Chi squared tests or Mann Whitney U-test were used as appropriate to compare results depending on corticosteroid therapy. All analyses were performed using SPSS 25.0 (IBM, NY) and a conventional significance level of <0.05 was used. Figures were generated using Microsoft Excel® 2011.

Results

Patient population

A total of 2817 native renal biopsies were performed within our centre between 2000 and 2015, of which 147 (5.2%) were first native renal biopsies yielding a diagnosis of AIN. Of these patients, 27 were excluded (12 patients died within the first year of follow-up, 7 had faded pathology slides, 4 had insufficient clinical data available and 4 had dual renal pathology) leaving 120 patients for analysis. 97 (81%) patients received corticosteroids for the treatment of AIN. Table 1 shows baseline features.

Outcomes

A total of 66 (55%) patients achieved the primary outcome of a composite of ≥50% reduction in sCr or an eGFR >60 ml/min/1.73m^2 at 1 year. Three of these patients had required dialysis at time of diagnosis.

A total of 36 (30%) patients achieved the secondary outcome of an eGFR <30ml/min/1.73m^2 at 1 year. Only 1 of these patients had an eGFR greater than 30ml/min/1.73m^2 at time of biopsy. The percentage change in sCr in these patients ranged from a 79% improvement to a 38% deterioration. 5 of these patients went on to require long-term renal replacement therapy (ranging from between 5 months and 4 years post-biopsy).

Drug cause

75 (63%) patients had a suspected drug cause identified (see supplementary material for details (S2)). There was no difference in composite primary outcome between those who did and did not have a drug cause identified (p=0.30).

Histological variables of interest

1) Inflammation in non-fibrotic cortex (NFI)

Patients were divided into 3 groups based on NFI 0-24%, 25-74%, and 75-100% and awarded a score of NFI-1, NFI-2, and NFI-3, respectively. As NFI-score increased from 1 to

3, sCr at baseline increased (table 2, Kruskal-Wallis H 17.1, p<0.001), while sCr at 1 year decreased (table 2, Kruskal-Wallis H 26.4, p<0.001). On univariable logistic regression, an NFI-score of 3 was associated with a 16 times increased likelihood of achieving the primary composite outcome compared to a score of 1 (OR 16.2 (95% CI 5.2-50.1, p<0.001)). There was no difference between an NFI-score score of 1 compared to an NFI-score of 2 in terms of primary outcome (OR 2.6 95% CI 0.96-6.5, p=0.051).

2) Tubular atrophy (TA)

Patients were divided into 3 groups based on TA of 0-9%, 10-24% and 25-100% to correspond to score of TA1, TA2, and TA3, respectively. As TA score increased from 1 to 3, there was no difference in sCr at biopsy (table 2, Kruskal-Wallis H 0.79, p=0.68), but sCr at 1 year increased (table 2, Kruskal-Wallis 33.3, p<0.001). On univariable logistic regression, TA2 and TA3 were associated with 75% and 90% reduced likelihood of achieving the primary outcome compared to TA1 (OR 0.25 (95% CI 0.08-0.75), p=0.013 for TA2; OR=0.10 (95%CI 0.03-0.33) p<0.001 for TA3).

3) Combination of TA and NFI

NFI=1-3 and TA=1-3 had a moderate negative correlation with a coefficient of -0.38, p<0.001). Figure 1 shows the proportion of patients who achieved the primary outcome, stratified by TA-score and NFI-score. Patients with TA3 and NFI-1 were least likely to achieve a favourable renal outcome. A prognostic index score (TANFI) was created by combining TA score with inverse NFI score (i.e. NFI-3 = 1 point, NFI-1 = 3 points) awarding each patient a score of between 2 and 6. As TANFI score increased, the proportion of patients achieving a favourable renal outcome decreased (figure 2; also supplementary material S3). On univariable logistic regression, a TANFI score of 4, 5 or 6 associated with a 92-99% reduced likelihood of the primary outcome compared to a TANFI score of 2 (OR for TANFI-4 = 0.08 (95% CI 0.01-0.63), OR for TANFI-5 = 0.03 (95% CI 0.004-0.29), OR for TANFI-6 = 0.01 (95% CI 0.001-0.12). The TANFI score accounted for 35% of the variability

in primary outcome (Nagelkerke's R2 = 0.35) with a C-statistic of 0.79. When the pre-specified clinical variables of age, sex, baseline sCr and identified drug cause were accounted for, TANFI score had an independent predictive effect on the primary outcome on multivariable logistic regression (table 3). The multivariable model accounted for 47% of the variation in primary outcome (Nagelkerke R^2 0.47), with a C-statistic of 0.86.

When the same variables were entered into a model to predict the secondary outcome, TANFI score independently associated with the secondary outcome (table 3). The direction of effect was inverted in comparison to the primary composite outcome, such that higher TANFI scores associated with a greater likelihood of the secondary outcome. Overall the model accounted for 41% of the variation in secondary outcome (Nagelkerke R^2 0.41) with a C-statistic of 0.84.

4) Other histological findings

Additional histological features including the percentage of inflammation in total cortex (ti), the presence of tubulitis, oedema, granulomas, eosinophils, neutrophils, proportion of sclerosed glomeruli and severity of arteriosclerosis were reported (table 1). Ti score, the severity of tubulitis, the presence of oedema, and the proportion of glomeruli that were sclerosed were all significantly associated with primary outcome on univariable analysis. The presence of granulomas, prominent eosinophils, prominent neutrophils and arteriosclerosis was not associated with outcome on univariable analysis, nor was the density of the infiltrate. No additional histological variable achieved statistical significance in multivariable analysis with TA and NFI (supplementary material S1, S4-S11).

5) Role of corticosteroids

97 (81%) patients received corticosteroids for the treatment of AIN. Median duration of steroid treatment was 5 months. There was no difference in median sCr at baseline or at 1 year between those who did and did not receive corticosteroids (Mann-Whitney U test

p=0.16 and p=0.86, respectively). Of the 97 patients who received corticosteroids, 56 (58%) achieved the primary outcome compared to 10 (43%) of the 23 patients who did not receive corticosteroids (Chi square 1.53, p=0.22). When patients who did not receive corticosteroids were excluded, the histological variables that retained statistical significance in the multivariable model did not change (data not shown). Similarly, when corticosteroid therapy was added into the multivariable regression model for TANFI, the overall model fit did not change (Nagelkerke's R^2 0.47).

Discussion

In this cohort of patients with biopsy-proven AIN, of whom the majority received corticosteroid therapy, histological findings at diagnosis associated with renal outcomes at 1 year. We confirmed previous findings that suggest the likelihood of a favourable renal outcome decreases as the degree of tubular atrophy increases. Conversely, we found a positive association between the extent of non-fibrotic cortex containing inflammation and the likelihood of a favourable renal outcome. Within this cohort, a prognostic score based on the severity of tubular atrophy and non-fibrosed inflammation was able to significantly predict renal outcome in patients with AIN, even when baseline clinical features are accounted for.

Tubular atrophy and interstitial fibrosis (which is highly correlated to tubular atrophy (R=0.98)(17)) are markers of chronic tubulointerstitial damage with recognized prognostic implications in a diverse range of conditions.(18–20) Increasing severity of tubular atrophy and interstitial fibrosis have been shown to associate with poor renal outcomes in AIN.(3,15) Our finding that TA-score is a predictor of poor renal recovery is consistent with these studies. However, our finding that even low levels of tubular atrophy (i.e. affecting 10% of the renal cortex) associates with a worse renal outcome is novel.

The finding that more inflammation in non-atrophic cortex is associated with better outcome is novel in the setting of AIN, but it is consistent with The Boston Kidney Biopsy Cohort study in which inflammation in non-fibrotic cortex was associated with a 50% reduced likelihood of progressive renal disease in a cohort of 676 patients with a diverse range of aetiologies (14 of whom had AIN).(19) In our cohort, increasing NFI-score associated with higher sCr at biopsy (and therefore perhaps mathematically an increased chance of it reducing by 50%) and was negatively correlated with tubular atrophy. However, the correlation between NFI-score and TA-score was not prohibitive for analysis and even when both tubular atrophy and baseline sCr were accounted for in the multi-variable model, NFI-score was still independently associated with the primary outcome. The predictive capabilities of NFI-score are likely explained by the fact it represents salvageable renal cortex affected by a disease process, for which the treatment is highly effective. Furthermore, the more aggressive phenotype (manifest as higher sCr at biopsy) could prompt a more urgent renal biopsy and potentially earlier treatment. Interstitial inflammation in total renal cortex, both fibrotic and non-fibrotic was also associated with better outcome (supplementary material S4). Interstitial inflammation in AIN thus appears to be highly reversible, regardless of its severity, in patients treated with corticosteroid.

In our cohort, 94% received treatment, with 81% prescribed corticosteroids and an additional 12.5% having a suspected drug cause withdrawn. It was therefore not possible to assess the impact of NFI-score on untreated patients, nor was it possible to demonstrate a lower limit of NFI-score that is clinically significant (i.e. a diagnostic threshold for AIN). We presume that increasing NFI-score above such a threshold in an untreated patient (e.g. contraindication to immunosuppression or inability to stop causative drug) would have a negative effect on outcome. We believe consensus on more precise histological diagnostic criteria for AIN, incorporating such a threshold, would greatly aid diagnosis, management and research in AIN.

The clinical utility of scoring tubular atrophy and inflammation in non-fibrotic cortex is enhanced when used in combination. The TANFI score is a simple cumulative metric that combines NFI and TA which, if validated in an independent cohort, can provide valuable information for patients and clinicians regarding prognosis in AIN. Of note, a TANFI score of 4 consists of a heterogenous group, with some patients at opposite ends of severity in terms of fibrosis/inflammation. Further studies are required to examine the potential differential association with outcome that may be seen in the component groups of patients with TANFI = 4 (i.e. does 1+3 associate differently with outcome compared to a score of 3+1) but the subgroups in the present cohort were too small to assess this reliably (Figure 1; supplementary material S3). The TANFI score accounted for 35% of the variability in primary outcome within this cohort. For comparison, the Oxford Classification of IgA nephropathy (MEST-C score), which is an established histological prognostic score, accounts for 19% of the variability in outcome in patients with IgA nephropathy.(21)

There is no trial evidence to support the management of AIN. Nevertheless, steroid therapy is accepted practice in biopsy-proven AIN, in which sCr is not spontaneously improving. We believe that a randomized trial of high-dose versus low dose corticosteroid in patients with biopsy-proven AIN would be clinically useful and ethically justifiable as the first step in improving the treatment of AIN. Duration of treatment should also be examined. Beyond that, the greatest equipoise lies in cases of drug-induced AIN in which withdrawing the causative drug may be sufficient and a trial of early, versus delayed, corticosteroid therapy would be enlightening. Stratification by histological findings will be essential to translate meaningful results into clinical practice.

This study has limitations. It is a single centre, retrospective analysis. We are reassured that our population appears representative of other published series of AIN: 5.2% of native renal biopsies in our centre revealed AIN, compared to 5.0% (3) and 5.9%(22) in other published series, while 61% had a drug cause, compared to 70% in previous reports.(3,4) A single

pathologist re-scored the histological variables of interest. We have previously reported inter-observer reproducibility for TA and total inflammation in a cohort of patients with IgA nephropathy as being 'good' (ICC 0.88; $P = 0.001$) and 'excellent' (ICC 0.91; $P < 0.001$), respectively.(23) Furthermore, the reproducibility of these measures is widely established in transplant biopsies.(24) Nevertheless these results require validation in other cohorts prior to their introduction into clinical practice. We believe the inclusion of diverse AIN aetiologies is justified and improves the generalizability of the results. We did not observe a difference in outcome between patients with and without an identified drug cause.

In patients with biopsy-proven AIN, of whom the majority received steroids, a higher percentage of inflammation in non-fibrosed cortex was associated with an increased likelihood of a positive clinical outcome. This implies inflammation in AIN is reversible, regardless of extent. Consistent with previous findings, increasing percentage of cortical tubular atrophy was associated with worse outcome. In combination, NFI and TA have the potential to give useful prognostic information in treated patients with AIN.

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Authors' Contributions: All authors have reviewed and contributed to this manuscript. DK, EMQ, AR and EC conceived the idea for the study. DK scored the histological parameters. AR, EC, and KG performed the data collection and analysis. AR wrote the manuscript. JC, JF, CG, BM, PM contributed the analysis plan and critically reviewed the manuscript.

Tables & Figures

Table 1. Baseline features.

Baseline data					
		ALL	Corticosteroids	No corticosteroids	p-value
N=		120	97 (81%)	23 (19%)	
Female (n, %)		73 (61%)	62 (64%)	11 (48%)	0.15
Age, years (mean, (SD))		58.2 (16.3)	58.0 (15.9)	58.9 (18.8)	0.45
Serum Creatinine at biopsy, umol/l (median, (IQR))		287 (199, 470)	307 (210, 492)	244 (189, 390)	0.16
Urine protein:creatinine ratio, mg/mmol (median, (IQR))		59 (23, 108)	67 (25, 109)	42 (14, 58)	0.17
Serum albumin, g/L (mean (SD))		35 (6)	36 (8)	34 (6)	0.23
Serum eosinophil count, $x10^9$/L (median, IQR		0.37 (0.29, 0.50)	0.38 (0.3, 0.52)	0.29 (0.20, 0.50)	0.10
Aetiology of TIN					
Drug cause identified (n, %)		75 (63%)	59 (61%)	16 (70%)	0.43
Sarcoid		8 (7%)	8	0	
TINU		8 (7%)	5	3	
Sjogrens		4 (4%)	4	0	
Tuberculosis (TB)		2 (2%)	2**	0	
No cause identified		26 (22%)	22	4	
More than one aetiology*		2 (2%)	0	2	
Histology findings					
NFI, (median, (IQR))		50% (20, 90)	60% (20, 90)	50% (10, 70)	0.19
TA, (median, (IQR))		15% (8,30)	10% (5, 20)	15% (10, 30)	0.81
TI, (median, (IQR))		80% (50, 90)	90% (50, 90)	70% (30, 80)	0.05
Tubulitis, (n, (%))	0	12 (10%)	9	3	0.38
	1	32 (27%)	23	9	
	2	51 (43%)	44	7	
	3	25 (21%)	21	4	
Granulomas (n, (%))		31 (26%)	26	5	0.61
Eosinophils (n, (%))		18 (15%)	16	2	0.34
Neutrophils (n, (%))		20 (17%)	19	1	0.08
Oedema (n, (%))		61 (51%)	54	7	0.03
Glomeruli (median, IQR)		12 (7, 20)	12 (7, 20)	12 (8, 15)	
Percentage of glomeruli sclerosed (median, IQR)		5 (0, 19)	7 (0, 25)	5 (0, 17)	
Arteriosclerosis (n, %)	0	23 (19%)	7	16	
	1	39 (33%)	7	32	
	2	43 (36%)	8	35	

	3	8 (7%)	0	8	

**1 patient had active tuberculosis, sjogrens syndrome and a possible drug cause (rifampicin). Another patient had TINU with a co-existent possible drug cause (rosiglitazone). **Both patients with active TB received anti-TB therapy in addition to corticosteroids.*

P-values were generated using Pearson chi-squared test and Mann Whitney U-test for categorical and continuous variables, respectively, both comparing corticosteroids versus no corticosteroids.

Abbreviations: SD = standard deviation. IQR – interquartile range. TINU – tubulointerstitial nephritis and uveitis, NFI – percentage of non-fibrosed cortex containing inflammation. TI – percentage of total cortex (fibrosed and non-fibrosed) containing inflammation. TA – tubular atrophy.

Table 2. Tables showing serum creatinine (median, intra-quartile range (IQR)) at baseline and 1 year depending on groups of NFI-score (A), and TA-score (B), where 'NFI' is the percentage of non-fibrotic cortex containing inflammation; and 'TA' is the percentage of cortex containing tubular atrophy.

A) NFI-score

	NFI-1 (0-24%)	NFI-2 (25-74%)	NFI-3 (75-100%)
N=	37	41	42
sCr at baseline, umol/l (median, IQR)	228 (174, 322)	273 (210,460)	385 (257, 560)
sCr at 1 year umol/l (median, IQR)	163 (138, 220)	150 (118, 198)	109 (92, 128)

B) TA-score

	TA1 (0-9%)	TA2 (10-24%)	TA3 (25-100%)
N=	30	49	41
sCr at baseline, umol/l (median, IQR)	343 (194, 473)	273 (197,460)	282 (210, 460)
sCr at 1 year umol/l (median, IQR)	106 (88, 135)	120 (107, 150)	170 (149, 220)

Abbreviations: NFI = percentage of non-fibrotic cortex containing inflammation. sCr = serum creatinine. IQR – interquartile range. TA = the percentage of cortex containing tubular atrophy.

Table 3. Table displaying the odds ratios (OR) for the primary outcome (A) and secondary outcome (B) based multivariable logistic regression models consisting of pre-specified baseline clinical variables and TANFI score. Higher TANFI scores associated with a decreased likelihood of achieving the composite primary outcome (50% reduction in sCr or eGFR >60 ml/min/1.73m^2) and increased likelihood of the secondary outcome (eGFR <30 ml/min/1.73m^2)

a) Multivariable binary logistic regression for composite primary outcome.

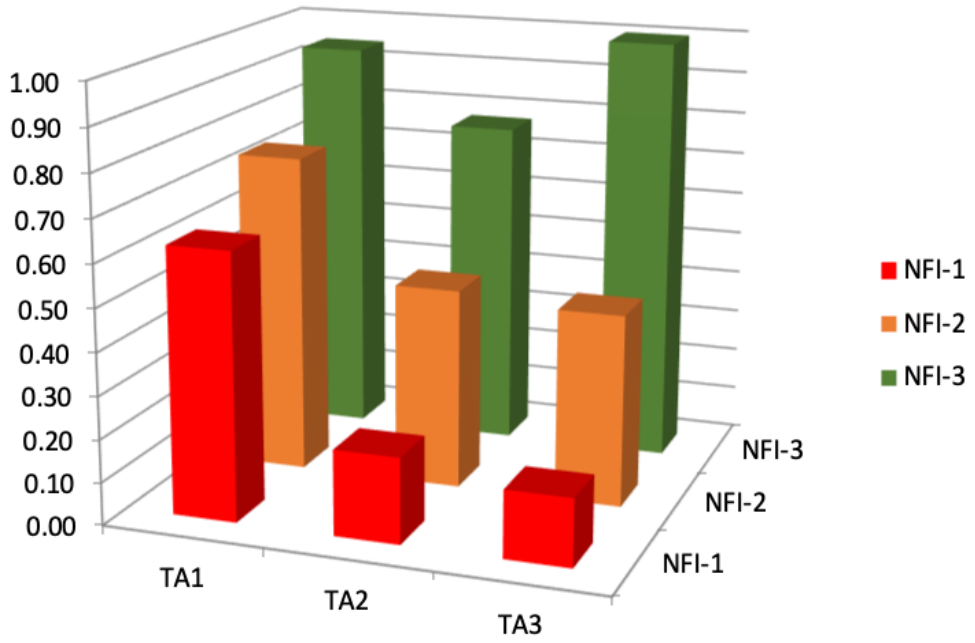
	OR	95% CI of OR	Significance
Male sex	-	-	NS
Age	-	-	NS
Drug cause identified	-	-	NS
sCr at biopsy (per μmol/l)	1.005	1.002-1.008	0.001
TANFI	-	-	-
2	-	-	-
3			NS
4			NS
5	0.05	0.005-0.44	0.008
6	0.01	0.001-0.14	<0.001

b) Multivariable binary logistic regression for secondary outcome.

	OR	95% CI of OR	Significance
Male sex	0.27	0.08-0.86	0.03
Age	-	-	NS
Drug cause identified	-	-	NS
sCr at biopsy (per μmol/l)	1.004	1.001-1.007	0.007
TANFI			
2	-	-	-
3	-	-	NS
4	15.5	1.2-197	0.04
5	78.3	5.8-1042	0.001
6	108.8	7.5-1566	0.001

Abbreviations: sCr = serum creatinine. TANFI = prognostic score ranging from 2-6 based on the combined severity of tubular atrophy (TA) and inflammation in non-fibrosed cortex (NFI).

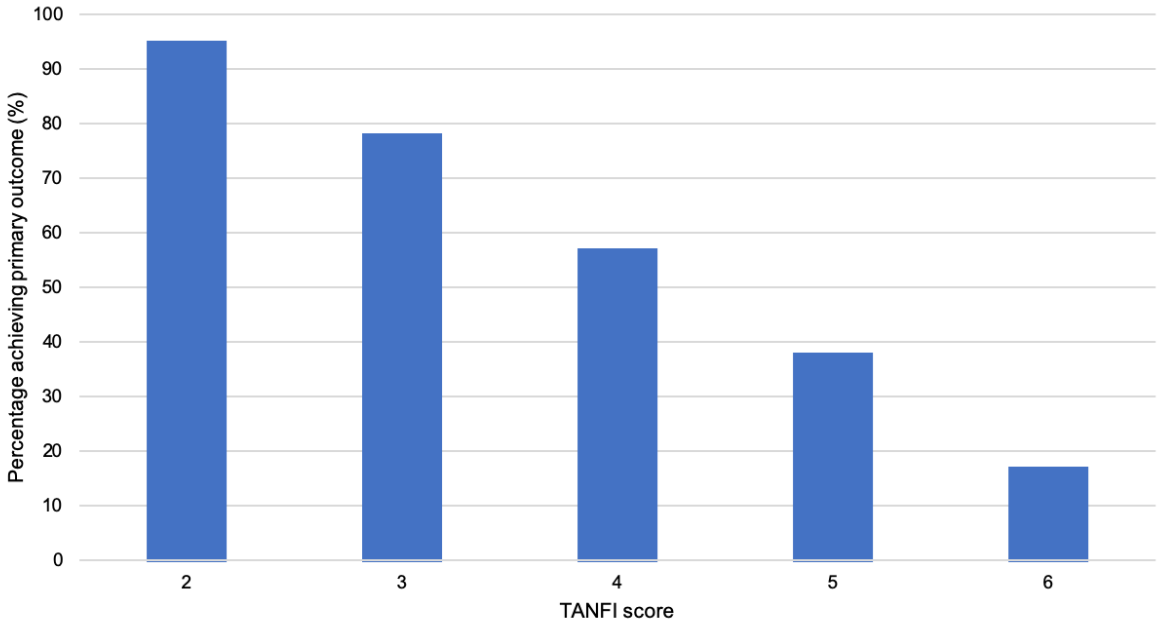
Figure 1. 3-dimensional bar chart showing the proportion of patients achieving the primary composite outcome stratified by tubular atrophy (TA) score and inflammation in non-fibrosed cortex (NFI) score. Patients with high TA-score and low NFI-score were least likely to achieve the primary outcome of a significant improvement in renal function.



	NFI-1	NFI-2	NFI-3
TA1	5/8	3/4	17/18
TA2	2/10	8/17	17/22
TA3	3/19	9/20	2/2

Abbreviations: NFI = inflammation in non-fibrosed cortex. TA = tubular atrophy.

Figure 2. The percentage of patients who achieved the primary composite outcome of a 50% reduction in serum creatinine or an estimated glomerular filtration rate of greater than 60 ml/min/1.73m^2 stratified by TANFI score. The TANFI score is a novel prognostic score ranging from 2-6 based on the combined severity of tubular atrophy (TA) and inflammation in non-fibrosed cortex (NFI).



TANFI score	2	3	4	5	6
Proportion achieving primary outcome	17/18	20/26	15/27	11/30	3/19

Supplementary material

S1) Instructions for histological analysis

Inflammation in interstitium should be detectable at x10 magnification, with confirmation at x20 if required. Inflammation in the immediate subcapsular zone or immediately next to large arteries at the corticomedullary junction was not counted. An area with non-atrophic tubules surrounded by fibrotic interstitium was considered not scarred. An interstitial fibrotic area with no tubules (especially around sclerosed glomeruli) was considered scarred. A tubule was deemed to be atrophic if any of the following features were present: thyroidisation; diameter ≤25% of normal with or without thickened basement membrane; diameter <50% of normal with thickened basement membrane visible at x10 magnification.

Granulomas were well defined (obvious at x10 magnification). Tubulitis was scored as follows: t1=1-4 mononuclear cells per tubular cross section or 10 epithelial cells; t2=5-10 mononuclear cells per tubular cross section/10 epithelial cells; t3= more than 10 mononuclear cells per tubular cross section/10 epithelial cells.

ADDITIONAL RESULTS:

S2) Drug causes: details

In all cases of drug induced AIN, the implicated drug was stopped and 59 (79%) patients also received corticosteroids. Proton pump inhibitors (PPIs) were identified as the sole implicated drug in 31 cases, while non-steroidal anti-inflammatory drugs (NSAIDs) were identified as the sole cause in 11 cases. A further 10 cases had exposure to both PPIs and NSAIDs. In 15 cases antibiotics were implicated, of which 3 were also exposed to PPIs and 2 NSAIDS. In 4 cases mesalazine was identified as the causative drug and in 4 cases other drugs were implicated.

S3) Components of TANFI subgroups

TANFI scores of 3, 4, and 5 consist of a combination of patients with different individual NFI and TA scores. Most notably a TANFI score of 4 consists of a heterogenous group, with some patients at opposite ends of severity in terms of fibrosis/inflammation. The figure below displays the proportion of patients achieving the primary outcome by TANFI score, including their component subgroups.

Supplementary table 1. Proportion of patients achieving the primary outcome by TANFI score sub-divided by their component groups

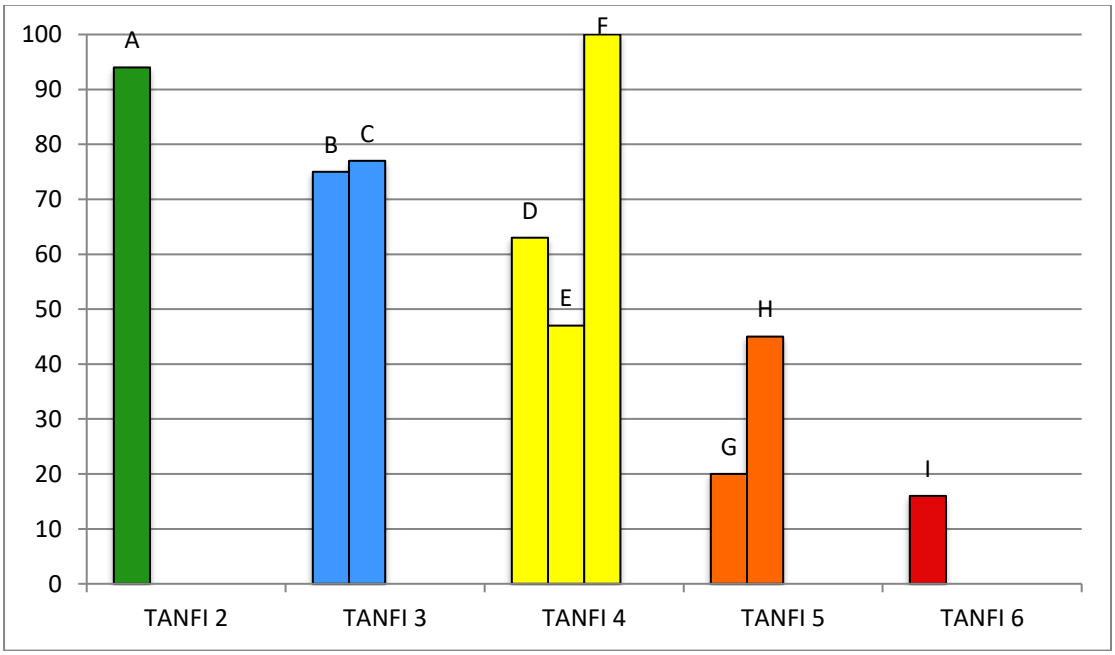


Chart legend:
(A) NFI 3 + TA 1;
(B) NFI 2 + TA 1; (C) NFI 3 + TA 2;
(D) NFI 1 + TA 1; (E) NFI 2 + TA 2; (F) NFI 3 + TA 3;
(G) NFI 1 + TA 2; (H) NFI 2 + TA 3;
(I) NFI 1 + TA 3

NFI and TA associate with the primary outcome in opposite directions. Accordingly the NFI score is inverted when used as part of the TANFI score, such that an NFI score of 3 contributes 1 point to TANFI score, NFI 2 contributes 2 points, and NFI 3 contributes 1 point. In contrast, TA 3 contributes 3 points, TA 2 contributes 2 points and TA 1 contributes 1 point to TANFI score.

Supplementary table 3: Number of patients in each TANFI score component

TANFI score	Components of the score (number of patients)		
2	NFI 3 + TA 1 (n=18)		
3	NFI 2 + TA 1 (n=4)	NFI 3 + TA 2 (n=22)	
4	NFI 1 + TA 1 (n=8)	NFI 2 + TA 2 (n=17)	NFI 3 + TA 3 (n=2)
5	NFI 1 + TA 2 (n=10)	NFI 2 + TA 3 (n=20)	

6	NFI 1 + TA 3 (n=19)		

S4) Inflammation in total cortex (ti)

Patients were divided into 3 groups based on ti of 0-50%, 51- 80% and 81-100% and awarded a score of ti-1, ti-2, and ti-3, respectively. As ti-score increased from 1 to 3, serum creatinine at baseline increased (supplementary table 1, Kruskal-Wallis H 13.6, p=0.001), while serum creatinine at 1 year reduced (supplementary table 1, Kruskal-Wallis H 6.25, p=0.04). On univariable logistic regression a ti score of 3 was associated with a 5 times increase in the likelihood of achieving the primary outcome compared to ti-1 (OR 5.0 (95% CI 2.0-12.5) p=0.001). There was no difference between a ti score of 2 and 1 (OR 1.8 (95% CI 0.67-4.8) p=0.25).

Supplementary material table 2. Table showing serum creatinine (median, intra-quartile range (IQR)) at baseline and 1 year depending on the percentage of total cortex, both scarred and non-scarred, containing inflammation (ti).

	ti-1 (0-50%)	ti-2 (51-80%)	ti-3 (81-100%)
N=	35	31	54
sCr at baseline, umol/l (median, IQR)	190 (171, 381)	273 (213,492)	325 (247, 496)
sCr at 1 year umol/l (median, IQR)	149 (124, 207)	138 (111, 181)	120 (97, 169)

S5) Tubulitis (t)

On univariable logistic regression, the presence of moderate (t-2) and severe (t-3) tubulitis was associated with a 9 and 10 times increase in the likelihood of achieving the primary outcome compared to no tubulitis (t-0), respectively (OR 9.2 (95% CI 1.8-46.5) p=0.007 for t-2; OR 10.6 (95% CI 1.9-60.2) p=0.008 for t-3). There was no difference between mild tubulitis (t-1) and absent tubulitis (t-0) (OR 3.9; 95% CI 0.73-20.6) p=0.11).

S6) Oedema

The presence of oedema was associated with a 2.5 times increase in the likelihood of achieving the primary composite outcome on univariable logistic regression (OR 2.5 (95% CI 1.2-5.2) p=0.015).

S7) Granulomas

Of the 31 patients with granulomas present on biopsy, 13 had a drug cause, 10 no cause identified, 5 sarcoid, 1 TINU, and 1 Sjogren's. A further 1 patient had active TB, Sjogren's syndrome and a possible drug cause. The presence of granulomas was not associated with the primary composite outcome on univariable logistic regression (p=0.10).

S8) Cellular Infiltrate

The presence of prominent eosinophils or neutrophils on biopsy did not predict a drug cause and it had no association with the likelihood of achieving the primary outcome, nor did the density of the inflammatory cell infiltrate.

S9) Sclerosed glomeruli

The median percentage of glomeruli that were sclerosed was 5% (IQR 0-19%). On univariable analysis, for each unit increase in the percentage of sclerosed glomeruli the likelihood of primary outcome reduced by 3% (OR 0.97 (0.95-0.99) p= 0.046)

S10) Arteriosclerosis

Arteriosclerosis was graded 0-3 (table 1) and was not associated with the primary outcome on univariable logistic regression.

S11) Correlation between histological variables

Supplementary material table 2 shows the bi-variate correlation coefficient for each histological parameter. NFI=1-3 and TA=1-3 had a moderate negative correlation with a

coefficient of -0.38, p<0.001).Tubulitis and oedema both had a moderate correlation with NFI=1-3 of 0.52 and 0.58, respectively (p<0.001 for both).

Supplementary table 3. Bivariable Spearman's rank correlation coefficient for histological variables. Non-significant correlations not shown (p<0.001 for all coefficients shown).

	NFI 1-3	TI 1-3	TA 1-3	Tubulitis	Oedema	Arteriosclerosis	Percentage sclerosed Glomeruli
NFI-1-3		.70	-.38	.59	.52	-	-
TI-1-3				.44	.38		
TA-1-3				-.43	-.37	0.27	0.28
Tubulitis					.58		

References

1. Valluri A, Hetherington L, McQuarrie E et al. Acute tubulointerstitial nephritis in Scotland. Qjm 2015; 108: 527–532.
2. Wilson GJ, Kark AL, Francis LP, Hoy W, Healy HG, Mallett AJ. The increasing rates of acute interstitial nephritis in Australia: A single centre case series. BMC Nephrology 2017; 18: 1–8.

3. Muriithi AK, Leung N, Valeri AM et al. Biopsy-proven acute interstitial nephritis, 1993-2011: A case series. American Journal of Kidney Diseases 2014; 64: 558–566.
4. Praga M, González E. Acute interstitial nephritis. Kidney International 2010; 77: 956–961.
5. Raghavan R, Eknoyan G. Acute interstitial nephritis - a reappraisal and update. Clinical nephrology 2014; 82: 149–162.
6. Su T, Gu Y, Sun P et al. Etiology and renal outcomes of acute tubulointerstitial nephritis: A single-center prospective cohort study in China. Nephrology Dialysis Transplantation 2018; 33: 1180–1188.
7. Clarkson MR, Giblin L, O'Connell FP et al. Acute interstitial nephritis: Clinical features and response to corticosteroid therapy. Nephrology Dialysis Transplantation 2004; 19: 2778–2783.
8. Kodner CM, Kudrimoti A. Diagnosis and management of acute interstitial nephritis. American Family Physician 2003; 67: 2527–2534+2539.
9. Michel DM, Kelly CJ. DISEASE OF THE MONTH Acute Interstitial Nephritis. 1998.
10. Laberke HG, Bohle A. Acute interstitial nephritis: correlations between clinical and morphological findings. Clinical nephrology 1980; 14: 263–273.
11. Kida H, Abe T, Tomosugi N, Koshino Y, Yokoyama H, Hattori N. Prediction of the long-term outcome in acute interstitial nephritis. Clinical nephrology 1984; 22: 55–60.
12. Bhaumik SK, Kher V, Arora P et al. Evaluation of clinical and histological prognostic markers in drug-induced acute interstitial nephritis. Renal failure 1996; 18: 97–104.
13. Buysen JG, Houthoff HJ, Krediet RT, Arisz L. Acute interstitial nephritis: a clinical and morphological study in 27 patients. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 1990; 5: 94–99.
14. Moledina DG, Perazella MA. Treatment of Drug-Induced Acute Tubulointerstitial Nephritis. Clinical Journal of the American Society of Nephrology 2018; : CJN.12001018.

15. Fernandez-Juarez G, Perez JV, Caravaca-Fontán F et al. Duration of Treatment with Corticosteroids and Recovery of Kidney Function in Acute Interstitial Nephritis. Clinical journal of the American Society of Nephrology : CJASN 2018; 13: 1851–1858.
16. Levey AS, Stevens LA, Schmid CH et al. A new equation to estimate glomerular filtration rate. Annals of internal medicine 2009; 150: 604–612.
17. Roberts ISD, Cook HT, Troyanov S et al. The Oxford classification of IgA nephropathy: Pathology definitions, correlations, and reproducibility. Kidney International 2009; 76: 546–556.
18. Racusen LC, Solez K, Colvin RB et al. The Banff 97 working classification of renal allograft pathology. Kidney International 1999; 55: 713–723.
19. Srivastava A, Palsson R, Kaze AD et al. The Prognostic Value of Histopathologic Lesions in Native Kidney Biopsy Specimens: Results from the Boston Kidney Biopsy Cohort Study. Journal of the American Society of Nephrology 2018; : ASN.2017121260.
20. Cattran DC, Coppo R, Cook HT et al. The Oxford classification of IgA nephropathy: Rationale, clinicopathological correlations, and classification. Kidney International 2009; 76: 534–545.
21. Barbour SJ, Espino-Hernandez G, Reich HN et al. The MEST score provides earlier risk prediction in lgA nephropathy. Kidney International 2016; 89: 167–175.
22. Prendecki M, Tanna A, Salama AD et al. Long-term outcome in biopsy-proven acute interstitial nephritis treated with steroids.
23. Rankin AJ, Kipgen D, Geddes CC et al. Assessment of active tubulointerstitial nephritis in non-scarred renal cortex improves prediction of renal outcomes in patients with IgA nephropathy. Clinical Kidney Journal 2018;
24. Gough J, Rush D, Jeffery J et al. Reproducibility of the Banff schema in reporting protocol biopsies of stable renal allografts. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2002; 17: 1081–1084.