A LOW COST PORTABLE SMARTPHONE DIGITAL MICROSCOPE TO ACCURATELY PREDICT KIDNEY BIOPSY ADEQUACY

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INTRODUCTION

On kidney biopsies, at least 8-10 glomeruli are conventionally required to reach a final diagnosis (1). When the tissue is scarce, the correct allocation of the sample for light microscopy, immunofluorescence and electronic microscopy can be troublesome. Without intraoperative assessment, the risk of an inadequate sample increases to nearly 4 times (2). New portable smartphone microscopes represent promising tools, albeit still requiring validation (3).

METHODS

We conducted a prospective cohort study on kidney biopsies performed in our Department in the University Hospital of Parma between June 2020 and April 2021. We compared the number of visible glomeruli by a digital microscope (Bodelin ProScope, **Figure 1**) with the total number by LM, IF and EM. All biopsies were ultrasound-guided. Pearson's correlation analysis was used to explore the relationship between the two counts. ROC curve analysis was performed to evaluate the ability of our tool to predict specimen adequacy (a total sum ≥10 glomeruli observed by LM, IF and EM).

RESULTS

We analysed 83 specimens of both allograft (73%) and native kidney biopsies (27%) [**Table 1**]. The mean glomerular yield was 25. A final diagnosis was reached in all cases. There was a strong positive linear correlation between the number of glomeruli assessed at the bedside and the sum of glomeruli observed by LM, IF and EM (Pearson's coefficient r=0.825, p <0.001). The area under the ROC curve was 1.0, indicating high accuracy of the method. The most sensitive and specific threshold of the Bodelin Proscope count to predict specimen adequacy was of ≥9 glomeruli, with both a sensitivity and specificity of 100%.

CONCLUSIONS

The Bodelin ProScope grants a low-cost tool to intraoperatively assess kidney biopsy adequacy with high grade confidence. Larger scale studies in which the number of biopsy cores is guided by the threshold we identified are warranted, potentially leading to a decrease in the number of passages and therefore bleeding risk.

Table 1. Biopsy details

Number of biopsies	83
Biopsy type, n (%)	
Native kidney biopsy	22 (27)
Transplant biopsy	61 (73)
Total number of cores, n	129
Number of cores per patient, n (SD)	1.5 (0.5)
Final pathology results	
Glomeruli obtained per patient, n (SD)	25 (11.5)
Able to make diagnosis, n (%)	83 (100)

Figure 1. Picture of a kidney biopsy core taken with the Bodelin Proscope. Glomeruli are identified as small red circles in clear contrast with the rest of the cortical parenchyma.

(1)Kidney Disease: Improving Global Outcomes (KDIGO) Glomerular Diseases Work Group. KDIGO 2021 Clinical Practice Guideline for the Management of Glomerular Diseases. Kidney Int. 2021 Oct;100(4S):S1-S276. doi: 10.1016/j.kint.2021.05.021. PMID: 34556256.
(2)Syed Gilani, MD, Hong Qu, MD, Daniel Ockner, MD, Role of On-site Microscopic Evaluation of the Kidney Biopsy for the Adequacy and Allocation of the Glomeruli: Comparison of Renal Biopsies With and Without On-site Microscopic Evaluation, American Journal of Clinical Pathology, Volume 138, Issue suppl_1, July 2012, Page A343, https://doi.org/10.1093/ajcp/138.suppl1.319
(3)Singh, Gurmukteshwar1; Massak, Mark2; Czaplicki, Michael2; Young, Evan2; Sharma, Shree3; Chang, Alex1; Bhanushali, Ashok2; Anand, Prince1. Use of a Smartphone Camera at the Bedside to Assess Adequacy of Kidney Biopsies. JASN 32(12):p 3024-3026, December 2021. | DOI: 10.1681/ASN.2021070898

PathoSpotter
Histological Lesion Detection

AUTOMATIC COMPUTATIONAL SEARCH OF HISTOLOGICAL IMAGES OF RENAL LESION USING SEMANTIC ATTRIBUTES

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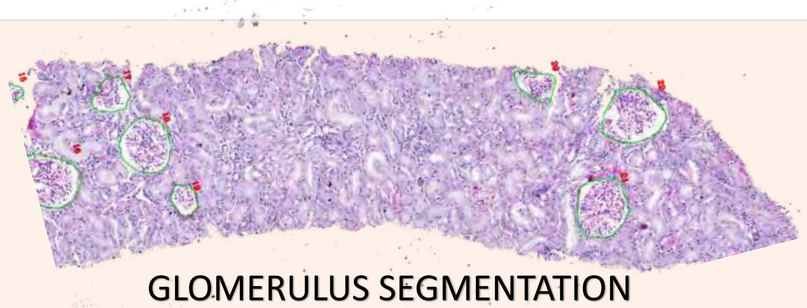
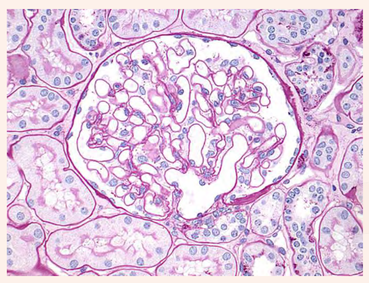
We propose a system that finds biopsy images using semantic attributes despite staining variations. It can identify similarities between images via glomerulus segmentation and lesion classification. Our team's previous work has been used to establish semantic attributes for the new search engine.

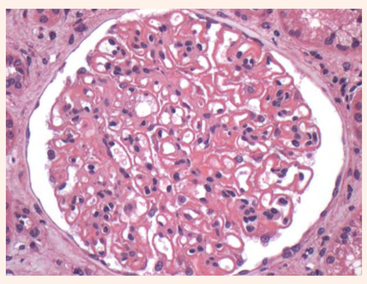


GLOMERULUS SEGMENTATION

The new DS-Fnet achieved a dice score (DSC) of 95.05% in the "HuBMAP - Hacking the Kidney" challenge on Kaggle. On the NEPTUNE and proprietary FIOCRUZ data sets, DS-FNet also obtained a high DSC, while only trained with HE stain images and predicting over images stained with other techniques.
https://doi.org/10.1016/j.compmedimag.2022.102104

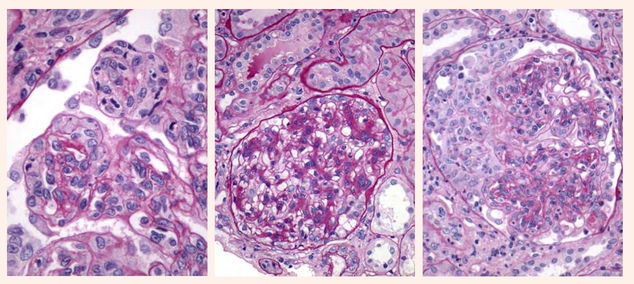


NORMAL GLOMERULUS



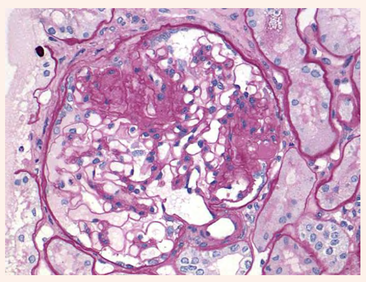
MEMBRANOUS GLOMERULOPATHY

We evaluated four deep-learning architectures (ResNet-18, MobileNet, DenseNet, and Wide-ResNet) using Monte Carlo dropout for uncertainty estimation. Wide-ResNet had the highest accuracy (93.2%). By using uncertainty-based thresholds improved accuracy to 96%.
https://doi.org/10.1080/21681163.2022.2029573



GLOMERULAR HYPERCELLULARITY

CNN and SVM achieved near-perfect classification results for normal/lesioned glomeruli in a proprietary FIOCRUZ dataset. For the multiclassification of hypercellularity sub-lesions (mesangial, endocapillary, and combined), the model achieved an average accuracy of 82%.
https://doi.org/10.1016/j.artmed.2020.101808

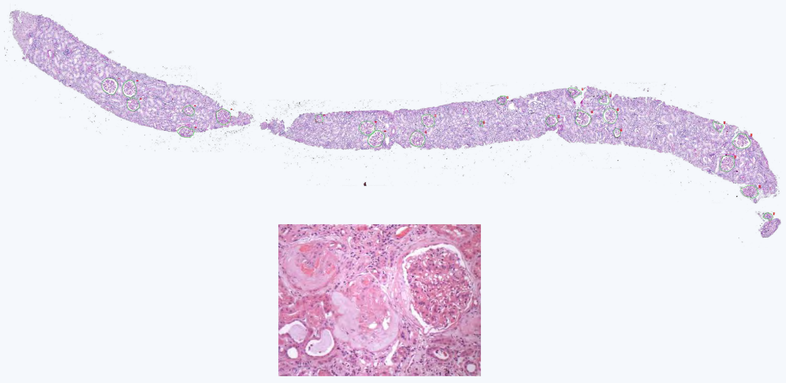


SEGMENTAL GLOMERULAR SCLEROSIS

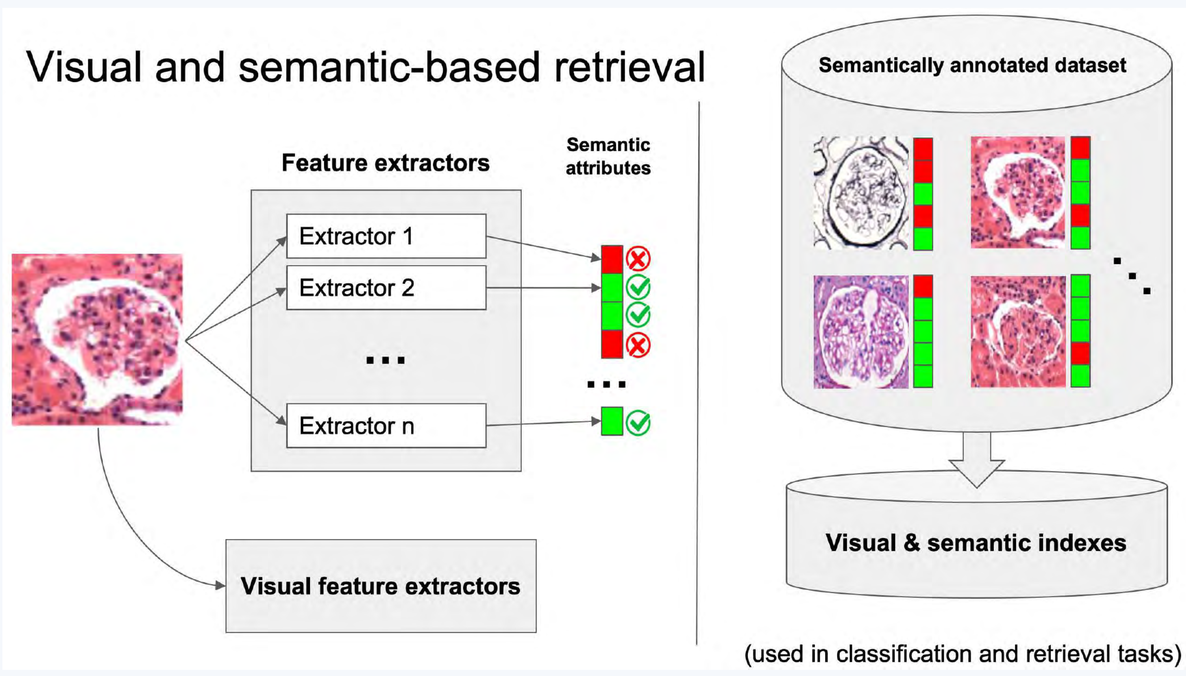
An ensemble approach composed of CNNs (VGG-19, Inception-V3, ResNet-50, DenseNet-201, and EfficientNet-B2) was used to detect glomerulosclerosis with near-perfect performance (accuracy 99.0% and kappa of 98.0%).
https://doi.org/10.1007/s00180-022-01307-3

A new system is being developed to find glomerulus images based on semantic attributes of an input image. Attributes can be categorical or numerical, which are matched with stored data to indicate the presence or absence of a lesion and to analyze the extension of a lesion. Attributes are easily pluggable to the system.

INPUT



PROCESSING



OUTPUTS

- NUMBER AND LOCATION OF GLOMERULI
- CLASSIFICATION AND IDENTIFICATION OF LESIONS
- EXPLANATION OF THE RESULTS
- GALLERY OF RETRIEVED IMAGES

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Automatic segmentation of glomerular substructures by deep learning

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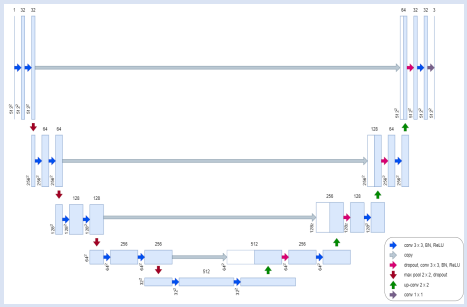
Introduction

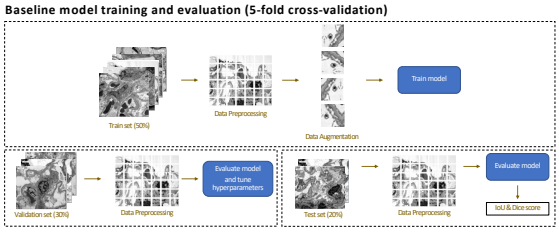
Electron microscopy (EM) complements light microscopy (LM) evaluation of the kidney biopsy. Foot process effacement, as assessed by EM, helps in diagnosing podocytopathies. However, human interpretation of EM images is time-intensive and often subjective.
In this pilot, we investigate how deep learning techniques can help in segmentation of the glomerular basement membrane (GBM) and podocytes in EM images, in order to contribute to reliable and fast assessment of foot process effacement.

Methods

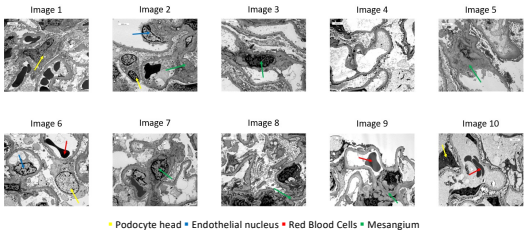
Glomerular basement membranes (GBM) and podocytes of 10 patients, 5 with podocyte disease (minimal change nephropathy) and 5 without glomerular changes, were annotated manually and using a thresholding technique, respectively, in order to provide ground truth. After data preprocessing, including cropping and tiling, a modified U-net model was trained ('baseline model'). The baseline model was compared to a fine-tuned contrastive learning model. The U-Net encoder of the fine-tuned model was pre-trained on 100 additional unlabeled images (SimCLR framework, 'fine-tuned model'). Segmentation performance was measured by IoU score. Models were evaluated on whole images and on diagnostically relevant ROIs (where the regions for diagnostic relevance were indicated by an experienced renal pathologist). Analysis with and without class weights (to correct for overrepresented areas in the images) was performed.



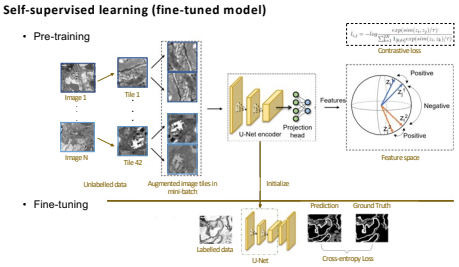
Baseline model training and evaluation (5-fold cross-validation)



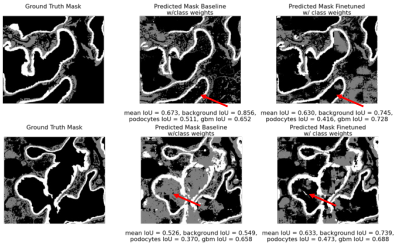
Key image features affecting performance



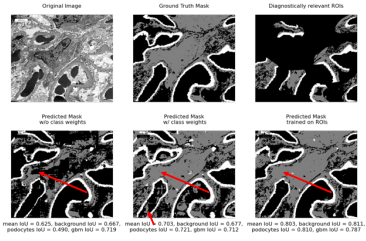
Self-supervised learning (fine-tuned model)



Baseline vs. fine-tuned model



Training on ROIs improves performance



Results

	Mean IoU (%)	Background IoU (%)	Podocytes IoU (%)	GBM IoU (%)
Baseline Model	Evaluated on whole image			
w/o class weights	**56.13±5.6**	**72.59±8.0**	43.67±10.3	52.09±13.9
w/ class weights	56.03±8.7	63.74±11.5	45.44±10.6	**58.93±14.5**
trained on ROIs	55.95±10.8	68.42±10.9	**46.03±13.8**	53.36±13.6
Baseline Model	Evaluated on ROIs			
w/o class weights	66.57±4.5	78.07±5.8	58.03±12.0	63.63±11.9
w/ class weights	**68.18±6.3**	74.34±6.6	59.10±11.8	**71.13±8.9**
trained on ROIs	67.30±9.2	**78.52±6.7**	**59.72±15.6**	63.68±11.4
Finetuned Model	Evaluated on whole image			
w/o class weights	57.22±6.6	**75.35±8.4**	45.31±10.5	50.94±9.6
w/ class weights	**58.71±7.0**	69.87±7.0	**49.16±10.0**	**57.12±13.3**
Finetuned Model	Evaluated on ROIs			
w/o class weights	65.64±7.3	**78.13±7.8**	56.76±13.1	61.99±9.1
w/ class weights	**68.42±5.6**	76.85±5.2	**60.89±11.8**	**67.47±9.3**

In this table the mean cross validation **IoU scores** are illustrated for all tested models.
Segmentation of the **glomerular basement membrane (GBM)** was best achieved by the baseline model and resulted in an IoU score of 0.711+0.089 compared to a IoU score of 0.675 ±0.093 in the fine-tuned model.
Segmentation of the **podocytes** was most successful in the fine-tuned model, with a IoU score of 0.609±0.118 compared to a IoU score of 0.591 ±0.118 in the baseline model.
When we evaluate the models on **ROIs** compared whole image evaluation, we can see a great increase. These higher scores confirm that the models can classify correctly the pixels of these regions.
Overall, segmentation performance of all models was better for GBM than for podocytes.

Conclusion

This study pioneers in segmenting glomerular substructures on EM images by means of a modified U-net architecture. The next step is training and validation in larger datasets. Data annotation remains a challenge. Inclusion of more images and labelling of additional substructures (mesangium, endothelium) is expected to greatly improve the performance of the model.

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End-to-End Glomerulonephritis Diagnosis by Machine Learning on Periodic Acid-Schiff (PAS) Sections with the MILxFormer Architecture

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[7]Sektion Nephrologie, Klinik für Innerere Medizin 1, Universität zu Bonn, Germany; [8] Gerhard-Domagk Institute of Pathology, University Hospital of Münster, Germany; [9] Department of Nephrology, University Hospital of Ulm, Germany; [10]Department of Electrical and Information Engineering (DEI), Polytechnic University of Bari, 70126 Bari, BA, Italy; [11] Nephrology, Dialysis and Transplantation Unit, Department of Emergency and Organ Transplantation, University of Bari Aldo Moro, Bari, Italy; [12] Division of Renal Medicine, "Fatebenefratelli Isola Tiberina – Gemelli Isola", Rome, Italy; [13] Department of Pathology, Johns Hopkins University, Baltimore, USA; [14] Institute of Pathology, University Hospital of Cologne, Cologne, Germany
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Background and Aims:

Machine learning (ML) holds great promise for improving diagnostics, prognostication and theranostics in nephropathology. So far, applications have not gone much further than segmentation of tissue compartments on whole slide images (WSIs) of paraffin sections. As a proof-of-concept study, we describe the development of a diagnostic classifier for glomerulephritis based on expert-annotated or automatically segmented glomerular transections from periodic-acid Schiff (PAS) paraffin sections only.

General Concept: This end-to-end pipeline extracts with the first module (segmentation) glomerular crops from a PAS WSI, generating a "bag of glomerular crops": the second module then uses the Bayesian MILxFormer architecture to predict the glomerulonephritis class.

Method:

A total of n=350 biopsies from 5 institutions with 12 classes of glomerulonephritis IgA nephropathy (IgAN), membranous nephropathy (Membranous), anti-glomerular basement membrane antibody GN (ABMGN), infection-associated GN (IAGN), ANCA-associated GN (ANCA-GN), idiopathic membranoproliferative GN (MPGN), SLE GN class IV (SLE-GN-IV), cryglobulinemic GN (CryoGN), C3 GN (C3-GN), dense deposit disease (DDD), fibrillary GN (FibrillaryGN) and proliferative GN with monoclonal immunoglobulin deposits (PGNMID) were included in the study with their respective PAS sections. Glomerular transections (crops) were expert-annotated by a nephropathologist and automatically segmented with our own transformer-based segmentation model trained on 100 biopsies with thrombotic microangiopathies and a range of vascular, vasculitic and glomerular diseases closely resembling/mimicking thrombotic microangiopathies.

For classification, we divided the cohort into 5 folds for internal cross-validation, performed sample size augmentation with various methods (including shifts in resolution/scale, AutoAugment and others) and trained our proprietary self-attention-based MILx architecture on an EfficientNet backbone with selection of glomerular crop batches by soft Markov chain Monte Carlo sampling in a semi-supervised fashion, with diagnostic class labels for each biopsy.

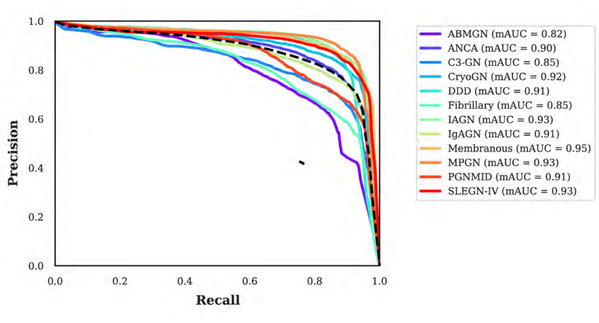
We compared the performance of our proprietary architecture on both expert-annotated and automatically segmented glomerular crops with a recently published benchmark architecture (CLAM) for multiple-instance learning in histopathology.

Results:

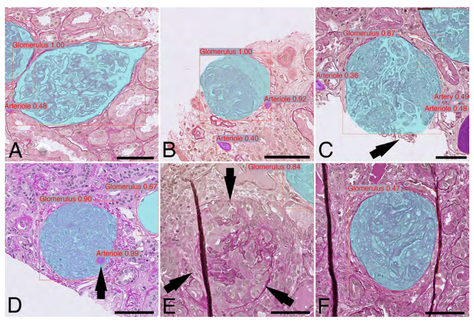
Automatic glomerular segmentation performance was excellent with mean AUC and sensitivity (mean average recall) over all classes at 0.904, with near perfect mean average specificity (0.994), as expected best for Membranous, worst for ABMGN.

Classification performance of MILx with expert-annotated glomerular crops as inputs had a mean balanced accuracy of 0.84, with AUC metrics in descending order of 0.97 for Membranous, 0.89 for ABMGN, 0.88 for IgAN, 0.86 for Fibrillary, 0.83 for MPGN, 0.80 for ANCA-GN, 0.79 for DDD, 0.78 for PGNMID, 0.75 for IAGN, 0.73 for SLE-GN-IV and CryoGN, 0.67 for C3-GN. Performance with MILx was similar for automatically segmented glomerular crops as input

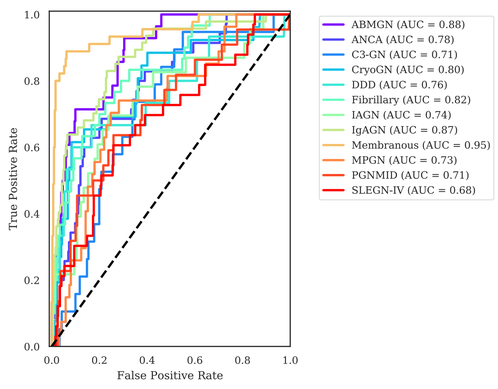
On this dataset, MILx outperformed CLAM with both entire WSIs as well as expert-annotated glomerular crops as inputs (mean balanced accuracy of 0.72) by a significant margin.



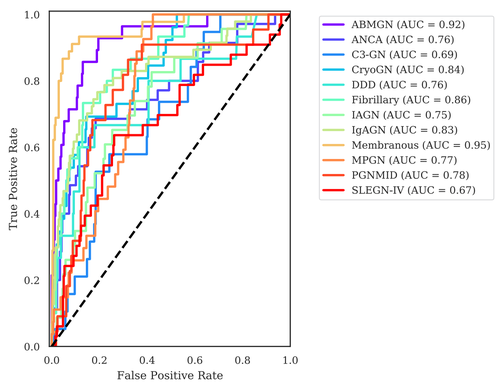
Performance of the glomerular segmentation module on the entire set of 350 PanGN biopsies as precision/recall curves.



Exemplary glomerular predictions (automatic segmentations) and mis-predictions with their certainty generated by the first module of our end-to-end diagnostic pipeline.



Mean receiver operating characteristics (ROC) for the MILxFormer classifier module when applied to expert segmented glomeruli.



Mean receiver operating characteristics (ROC) for the MILxFormer classifier module when applied to automatically segmented glomeruli.

GT Diagnostic Class	N	Pred	PPV	NPV	Sens	Spec	F1 Score	Accuracy	Balanced Accuracy
ABMGN	28	Top-1	46.43	95.34	46.43	95.34	46.43	46.43	70.89
		Top-2	71.43	97.52	71.43	97.52	71.43	71.43	84.47
		Top-3	82.14	98.45	82.14	98.45	82.14	82.14	90.3
ANCA	35	Top-1	30.56	92.36	31.43	92.06	30.99	31.43	61.75
		Top-2	56.76	95.53	60.0	94.92	58.33	60.0	77.46
		Top-3	66.67	96.5	68.57	96.19	67.61	68.57	82.38
C3-GN	20	Top-1	6.25	94.61	5.26	95.47	5.71	5.26	50.37
		Top-2	42.86	96.13	31.58	97.58	36.36	31.58	64.58
		Top-3	61.11	97.59	57.89	97.89	59.46	57.89	77.89
CryoGN	27	Top-1	36.0	94.77	34.62	95.06	35.29	34.62	64.84
		Top-2	51.72	96.57	57.69	95.68	54.55	57.69	76.69
		Top-3	62.07	97.51	69.23	96.6	65.45	69.23	82.92
DDD	15	Top-1	37.5	96.49	20.0	98.51	26.09	20.0	59.25
		Top-2	60.0	97.35	40.0	98.81	48.0	40.0	69.4
		Top-3	66.67	97.36	40.0	99.1	50.0	40.0	69.55
Fibrillary	30	Top-1	33.33	95.36	53.33	90.0	41.03	53.33	71.67
		Top-2	48.78	96.76	66.67	93.44	56.34	66.67	80.05
		Top-3	62.16	97.76	76.67	95.63	68.66	76.67	86.15
IAGN	23	Top-1	23.81	94.53	21.74	95.11	22.73	21.74	58.42
		Top-2	33.33	94.88	26.09	96.33	29.27	26.09	61.21
		Top-3	47.06	95.5	34.78	97.25	40.0	34.78	66.02
IgAGN	47	Top-1	61.54	92.6	51.06	95.05	55.81	51.06	73.06
		Top-2	75.0	95.42	70.21	96.37	72.53	70.21	83.29
		Top-3	79.07	95.77	72.34	97.03	75.56	72.34	84.69
Membranous	45	Top-1	73.58	97.98	86.67	95.41	79.59	86.67	91.04
		Top-2	82.0	98.67	91.11	97.05	86.32	91.11	94.08
		Top-3	82.0	98.67	91.11	97.05	86.32	91.11	94.08
MPGN	27	Top-1	17.65	93.35	22.22	91.33	19.67	22.22	56.78
		Top-2	37.5	95.28	44.44	93.81	40.68	44.44	69.13
		Top-3	51.61	96.55	59.26	95.36	55.17	59.26	77.31
PGNMID	22	Top-1	16.67	94.08	9.09	96.95	11.76	9.09	53.02
		Top-2	55.0	96.67	50.0	97.26	52.38	50.0	73.63
		Top-3	66.67	97.57	63.64	97.87	65.12	63.64	80.75
SLEGN-IV	33	Top-1	23.33	91.87	21.21	92.74	22.22	21.21	56.98
		Top-2	40.74	93.19	33.33	94.95	36.67	33.33	64.14
		Top-3	54.84	94.98	51.52	95.58	53.12	51.52	73.55
All Classes	350	Top-1	38.15	94.37	38.86	94.18	37.95	38.86	66.52
		Top-2	57.31	96.16	57.71	95.99	57.11	57.71	76.85
		Top-3	66.95	96.98	67.14	96.85	66.68	67.14	81.99

Classification performance for the MILxFormer classification module on expert annotated (segmented) glomeruli (all values presented as percentages).

		Prediction Diagnostic Class											
GT Diagnostic Class	Pred	ABMGN	ANCA	C3-GN	Cryo-GN	DDD	Fibrillary	IAGN	IgAGN	Mem-branous	MPGN	PGNMID	SLEGN-IV
ABMGN	Top-1	13	7	0	0	0	2	1	0	1	1	0	3
	Top-2	20	3	0	0	0	1	0	0	1	1	0	2
	Top-3	23	1	0	0	0	0	0	0	1	1	0	2
ANCA	Top-1	4	11	0	0	1	2	3	3	4	3	0	4
	Top-2	1	21	0	0	1	2	3	1	2	3	0	1
	Top-3	1	24	0	0	1	2	3	0	2	2	0	0
C3-GN	Top-1	1	1	1	2	0	4	1	0	1	6	0	2
	Top-2	1	1	6	2	0	2	1	0	1	4	0	1
	Top-3	1	1	11	2	0	0	0	0	1	3	0	0
CryoGN	Top-1	1	1	3	9	1	3	0	1	0	5	1	1
	Top-2	1	0	2	15	1	3	0	1	0	1	1	1
	Top-3	1	0	2	18	0	3	0	1	0	0	0	1
DDD	Top-1	2	0	0	2	3	2	1	1	0	3	0	1
	Top-2	0	0	0	2	6	1	1	1	0	3	0	1
	Top-3	0	0	0	2	6	1	1	1	0	3	0	1
Fibrillary	Top-1	3	1	0	0	0	16	1	1	5	1	1	1
	Top-2	2	1	0	0	0	20	1	1	2	1	1	1
	Top-3	0	1	0	0	0	23	1	1	2	0	1	1
IAGN	Top-1	1	1	1	2	0	2	5	3	0	3	2	3
	Top-2	1	1	1	2	0	2	6	2	0	3	2	3
	Top-3	0	1	1	2	0	2	8	2	0	2	2	3
IgAGN	Top-1	0	9	3	0	0	5	2	24	1	0	0	3
	Top-2	0	5	2	0	0	2	2	33	1	0	0	2
	Top-3	0	5	2	0	0	2	1	34	1	0	0	2
Mem-branous	Top-1	0	0	0	0	0	2	0	3	39	0	1	0
	Top-2	0	0	0	0	0	1	0	2	41	0	1	0
	Top-3	0	0	0	0	0	1	0	2	41	0	1	0
MPGN	Top-1	0	0	3	2	2	2	2	0	0	6	5	5
	Top-2	0	0	1	2	1	2	1	0	0	12	4	4
	Top-3	0	0	1	0	1	1	1	0	0	16	3	4
PGNMID	Top-1	0	1	2	5	0	7	1	0	1	3	2	0
	Top-2	0	1	0	3	0	4	0	0	1	2	11	0
	Top-3	0	1	0	3	0	1	0	0	1	2	14	0
SLEGN-IV	Top-1	3	4	3	3	1	1	4	3	1	3	0	7
	Top-2	2	4	2	3	1	1	3	3	1	2	0	11
	Top-3	2	2	1	2	1	1	2	2	1	2	0	17

Confusion matrix for the MILxFormer classification module when applied to expert segmented glomerular crops. Correct predictions (Pred) are highlighted in green, the most common mis-predictions in red.

Conclusion:

This proof-of-concept-study indicates that nephropathology-specific architectures like our MILx can be trained for complex tasks on relatively small biopsy cohorts. We should be able to deliver an end-to-end-pipeline for this diagnostic and other tasks based on training sets with case-labels provided by trusted institutions with only minimal expert labeling or annotation required.

THE SPECTRUM OF RENAL BIOPSY FINDINGS IN PATIENTS WITH DIABETES MELLITUS

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Introduction

- Diabetic nephropathy (DN) is one of the most common cause of End Stage Renal Disease (ESRD)
- Renal disease is more complex and diverse in Type II diabetics; Undergo renal biopsies if there is an atypical course
- Prevalence of Non-Diabetic renal disease (NDRD) ranges from 27-79% in Type II diabetic patients(1)
- Diagnosing NDRD is important as it leads to a specific change in therapy. However, the utility of pathological diagnosis in predicting the prognosis of Type II diabetics is still questionable

Aim

- To assess the frequency of Diabetic nephropathy (DN), Diabetic Nephropathy with superimposed Non-Diabetic Renal Disease (NDRD) and Non-Diabetic Renal Disease among patients who underwent renal biopsy
- To correlate the various clinical parameters and laboratory data with the subgroups and classes of DN

Methods

- This is a combined retrospective and prospective study for a period of 5 years
- The biopsies were divided into three subgroups: DN, DN with superimposed NDRD and NDRD
- DN was classified into four classes according to Tervaret classification
- Relevant statistical analysis was used. P value less than 0.05 is considered as statistically significant

Table 1. Frequency of DN with superimposed NDRD & NDRD alone

DN with superimposed NDRD	Frequency (n)	Percentage (%)
Membranous nephropathy	2	6.45%
Immune complex mediated Membranoproliferative glomerulonephritis	1	3.22%
Infection related glomerulonephritis	4	12.9%
IgA dominant post infectious glomerulonephritis	2	6.45%
IgA nephropathy	5	16.12%
FSGS	3	9.67%
Collapsing glomerulopathy	2	6.45%
Acute pyelonephritis	1	3.22%
Tubulointerstitial nephritis	11	35.48%
NDRD alone	**Frequency (n)**	**Percentage (%)**
Membranous nephropathy	4	16.7%
Immune complex mediated Membranoproliferative glomerulonephritis	3	12.5%
Infection related glomerulonephritis	3	12.5%
IgA nephropathy	6	25%
FSGS	4	16.7%
C3 glomerulopathy with granulomatous TIN	1	4.2%
Immune complex mediated glomerulonephritis with crescents	1	4.2%
Thrombotic microangiopathy (TMA)	1	4.2%
Class III Lupus nephritis	1	4.2%

Table 2. Correlation between the patients of DN, DN with superimposed NDRD and NDRD with various clinical and laboratory parameters

Parameters		DN alone (n= 53)	DN with superimposed NDRD (n=32)	NDRD alone (n=24)	Overall (n=109)	p value
Age (years)		56.06±8.7	52.8 ±11.98	53.2 ±11.4	54.5±10.3	0.387
Sex	Males	45(84.9%)	25(78.1%)	15(63%)	85 (78%)	0.089
	Females	8(15.1%)	7(21.9%)	9(37.5%)	24 (22%)	
Duration (years)		8.8 ±4.6	8.9±7.35	6.8±4.9	8.40±5.64	0.007
HbA1C(%)		8.2±2	6.94±1.58	7±1.44	7.56 ±1.89	0.057
Hypertension		37(69.8%)	26(81.3%)	19(79.2%)	82(75.2%)	0.437
DR		15(28.3%)	6(18.8%)	4(16.7%)	25(22.9%)	0.711
eGFR (mL/min/1.73m^2)		37.7±30.3	28.6±25.8	58.6±40.2	39.6±30.1	0.002
24 hour urine protein (mg)		3939.04 ± 1325.5	4047.3± 1491.8	2909.4± 1988.5	3744.13 ± 1589.85	0.012
Serum creatinine (mg/dL)		3.71±3.49	4.38±3.48	2.27±2.48	3.59±3.35	0.06
Serum albumin (g/dL)		2.77±1.28	2.69±1.21	3.07±1.02	2.81±1.21	0.466
UPCR		7.85±6.95	8.2±5.89	5.99±6.17	7.55±6.48	0.404
FBS (mg/dL)		148.4±51.8	118.9±34.4	122.2±35.4	133.9±45.8	0.005
Hematuria		18(33.96%)	13(40.63%)	8(33.33%)	39(35.8%)	0.926
Pyuria		12(22.64%)	10(31.25%)	10(41.7%)	32(29.4%)	0.087
Proteinuria: <3500mg/24 hour		17(32.1%)	8(25%)	13(54.2%)	38(34.9%)	0.126
Proteinuria: >3500mg/24 hour		36 (67.9%)	24 (75%)	11 (45.8%)	71(65.1%)	

Table 3. Correlation of the Classes of DN with various clinical and laboratory parameters

Parameters		Class IIA(n=9)	Class IIb (n=3)	Class III (n=25)	Class IV (n=16)	p value
Age(years)		59.67 ± 7.7	49 ± 9.8	56.96 ± 9.1	53.94 ± 7.7	0.1
Sex	Males	9(100%)	3(100%)	19(76%)	14(88%)	0.288
	Females	0	0	6(24%)	2(12.5%)	
Duration (years)		8.22 ± 4.4	2 ± 1.7	9.52 ± 5.3	9.38 ± 3.1	0.057
HbA1C(%)		8.49 ± 1.3	9.2 ± 2.01	8.02 ± 1.92	8.09 ± 2.63	0.78
DR		2(22.2%)	1(33.3%)	9(36%)	3(18.6%)	0.323
Hypertension		6(66.7%)	1(33.3%)	17(68%)	13(81.3%)	0.396
eGFR (mL/min/1.73m^2)		35.3±27.7	83.5± 47.2	46.5±27.3	16.7± 16.1	<0.001
24 hour urine protein (mg/24 hours)		4111± 1441.41	3606.53 ± 856.9	3595.62 ± 1467.51	4440.07 ± 966.66	0.23
Proteinuria		7(77.8%)	2(66.7%)	14(56%)	13(81.3%)	0.23
Serum creatinine (mg/dL)		3.2 ± 2.46	1.34 ± 0.92	2.29 ± 2.01	6.65 ± 4.31	<0.001
Serum albumin (g/dL)		2.52 ± 1.63	4.03 ± 0.58	2.61 ± 1.25	2.91 ± 1.14	0.28
UPCR		6.67 ± 4.45	7.72 ± 5.67	5.94 ± 3.65	11.6 ± 10.5	0.0078
FBS (mg/dL)		146.8±76.7	161.7±38.1	148.8±36.4	146.2±61.9	0.97
Serum cholesterol (mg/dL))		169.3±61.9	234±92.6	196.6±76.7	208.4±53.5	0.439
Hematuria		2(22.22%)	1(33.33%)	10(40%)	5(31.25%)	0.635
Pyuria		0	1(33.33%)	6(24%)	5(31.25%)	0.1

Results

- In this study of 109 Type 2 Diabetic patients, 48.6% had DN alone, 29.4% had DN with NDRD and 22% had NDRD alone
- 52.8% had Diabetes for > 10 years in DN group whereas the duration was < 4 years in NDRD group in 41.7% individuals
- The most common indication for renal biopsy was nephrotic syndrome(35.8%)
- Class III DN(47.2%) was the most common class
- The most common DN with superimposed NDRD and NDRD were tubulointerstitial nephritis(34.4%) and IgA nephropathy(25%) (Table 1)
- Long duration of Diabetes, low eGFR and increased 24-hour urine protein were found to be significant in the DN with superimposed NDRD group (Table 2)
- Low eGFR, increased UPCR and raised serum creatinine were significantly higher in class IV DN as compared to the other class (Table 3)
- IFTA score was significant in DN group as compared to others (p value 0.02) whereas hyalinosis was more commonly seen in mixed group (p value 0.002)
- Higher score of IFTA and globally sclerosed glomeruli were more commonly seen in class IV DN (<0.001 and <0.001)
- 30 patients out of 72(27.5%) were dialysis dependent during follow up
- The mean duration of follow up was 18.8±18.8 months
- The renal outcome between the subgroups and classes of DN were not statistically significant with p value of 0.586 and 0.135 respectively

Discussion & Conclusion

- This was a comprehensive study of renal biopsies in diabetic patients
- Similar to other studies, IgA nephropathy and Tubulointerstitial nephritis were the most common renal disease in NDRD and mixed group respectively(2,3)
- DN with NDRD had a longer duration of diabetes, low eGFR and heavy degree of proteinuria
- Frequently, more than one disease process is discovered in a diabetic renal biopsy. Hence, biopsy is an invaluable tool in detecting non-diabetic renal changes, helps in guiding management decision and assessing the prognosis

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Diagnostic approach to genetic diseases of the nephron

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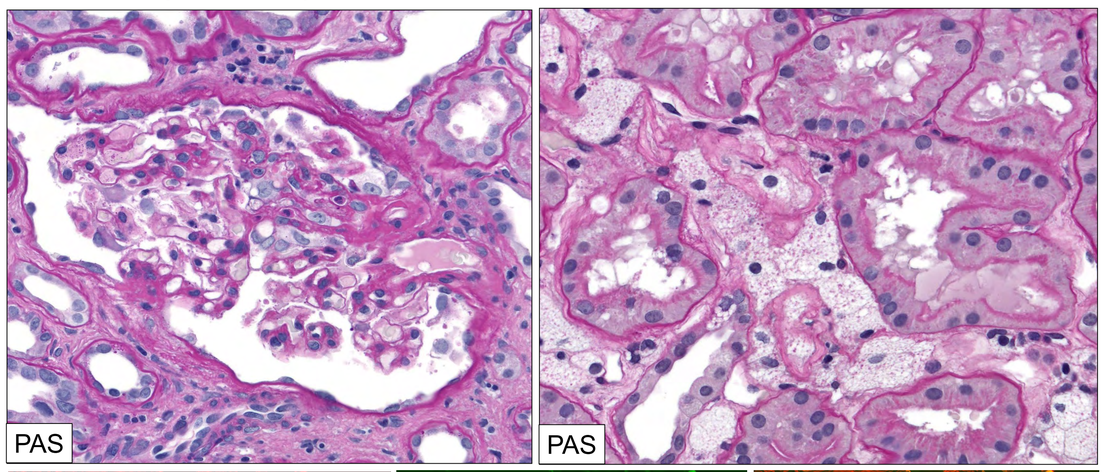
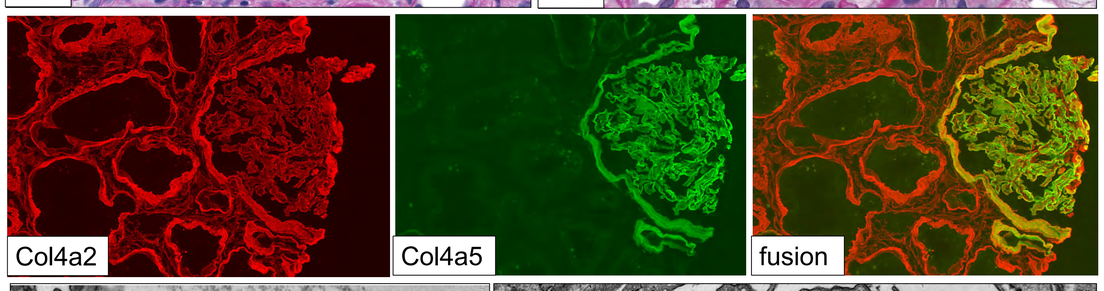
Aim

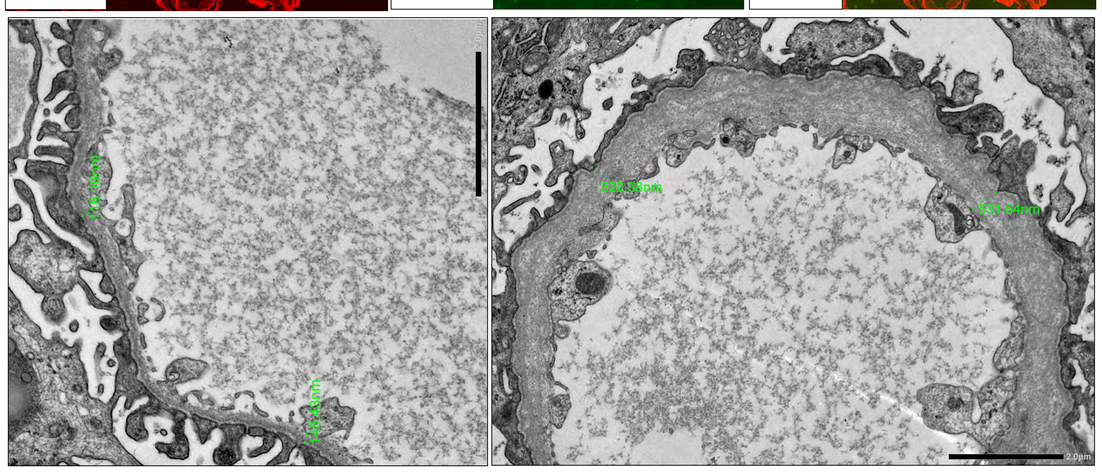
As much as 30% of CKD patients are characterized by a posivite family history. Moreover, in around 15% of ESRD cases, the etiology remains unknown. It is indicated that genetic investigation may identify the cause of ESRD of unknown etiology in a great number of such patients. A subset of glomerular diseases is determined by genetic abnormalities affecting the podocytes or the glomerular basement membrane (GBM). The usual histological pattern is focal segmental glomerulosclerosis (FSGS). In the daily practice, the diagnosis of genetic FSGS is often challenging. We present some general and practical considerations for interpreting renal biopsy findings in genetic diseases of the nephron.

Methods

Five cases are presented from the archives of the Department of Pathology, University of Szeged, evaluated originally via light microscopy (LM), immunofluorescence (IF), and electron microscopy (EM). Based on the clinical data and the biopsy findings, genetic testing of targeted genes was performed, which confirmed genetic kidney disease.

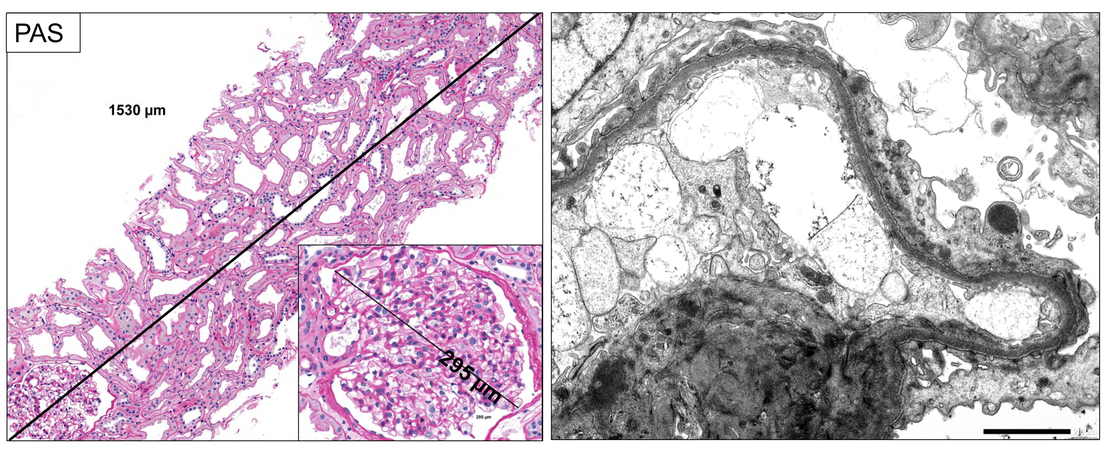






Case 1. 43 year-old male. Chronically declining renal function, serum creatinine 205 μmol/l, eGFR 33 ml/min, proteinuria (2,73 g/d), microhematuria. LM: focal-segmental glomerulosclerosis (6%), foamy cells. IF: preserved, mild Col4a5 positivity. No ICs. EM: Thin basement membranes in 2 glomeruli; typical XLAS lesions in 1. Genetic investigation revealed a 17-bp deletion in *COL4A5*, present in the patient and his daughter.

Diagnosis: adult-onset X-linked Alport nephropathy.



Case 2. 23 year-old female. CKD for 2 years, family history: negative. Serum creatinine 145 μmol/l, eGFR 43 ml/min, proteinuria for 7 years (2,5 g/d). IF: negative.LM: Glomerulomegaly. Perihilar FSGS, probably secondary etiology. Low mean glomerular density (0.68/mm^2) → Oligomeganephronia. EM: Segmental foot process effacement.

Diagnosis: *PAX2* mutation-related oligomeganephronia with adaptive FSGS.

Results

The age of the patients ranged from 7 months to 43 years at biopsy. Each of them was admitted because of heavy proteinuria, with our without hematuria, and impaired kidney function. IF was negative for immune complexes or monoclonal immunoglobulins. The primary diagnosis was FSGS in each case, which was refined following EM analysis. EM revealed either podocytic or GBM abnormalities suspicious for genetic conditions. Genetic investigation identified pathogenic mutations in the genes *ACTN4, COL4A5, PAX2, PDSS2* or *CLCN5*.

Conclusion

Certain features associated with compatible clinical data should raise the possibility of genetically determined renal disease. These include age under 35 years, extrarenal symptoms, positive family history, proteinuria or the nephrotic syndrome without obvious explanation, no response to steroid therapy, negative findings on IF, interstitial foamy cells and medullary nephrocalcinosis by LM. On EM, the extent of podocyte foot process effacement, the absence of slit membranes, dense aggregates in the cytoplasm of podocytes, and GBM abnormalities provide important information for conclusion and targeted genetic testing. We present an algorithmic approach to genetic testing of FSGS.

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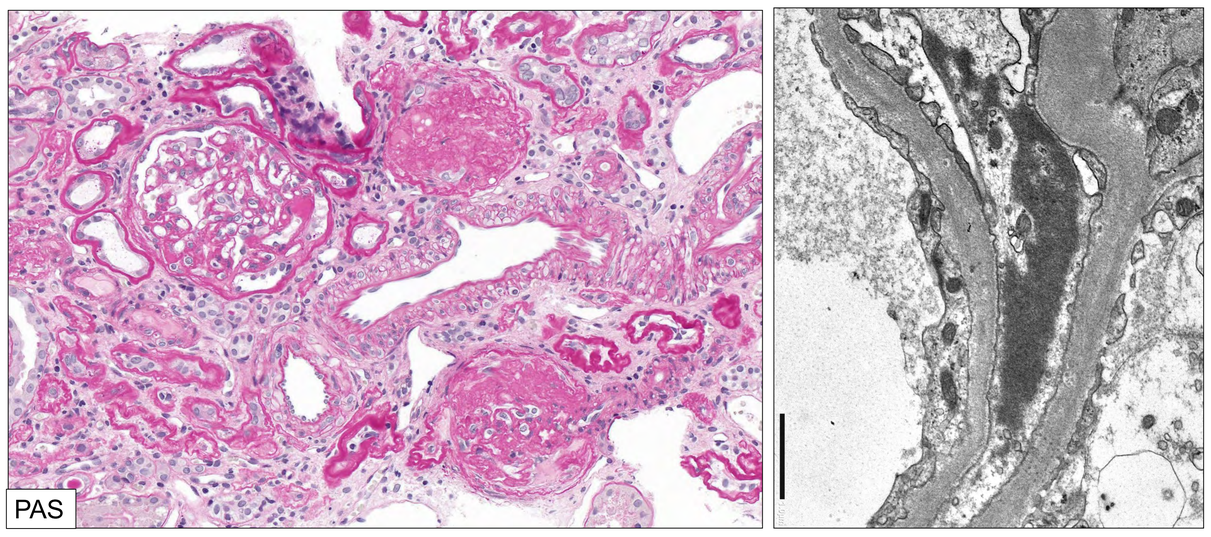
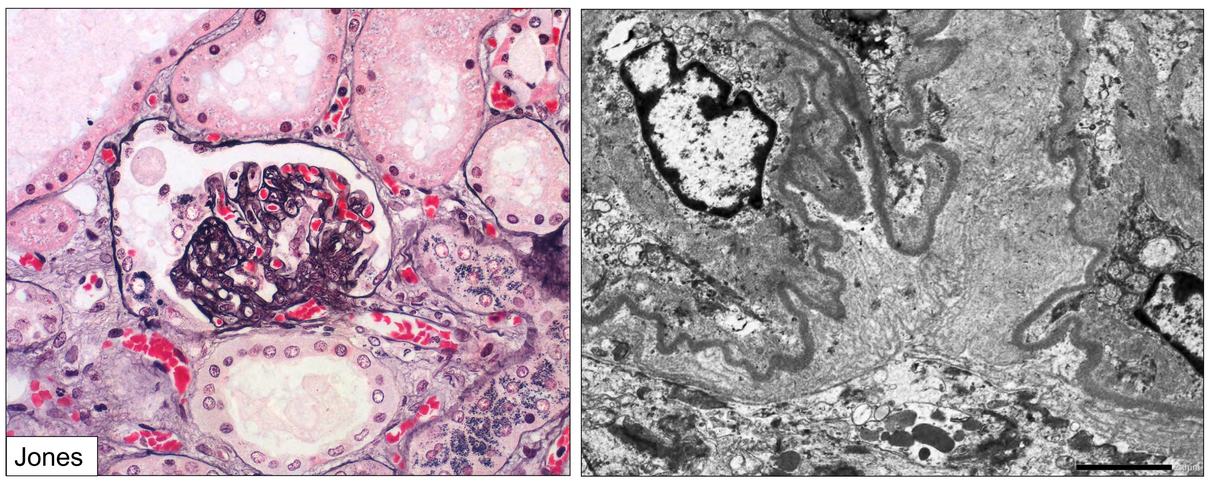


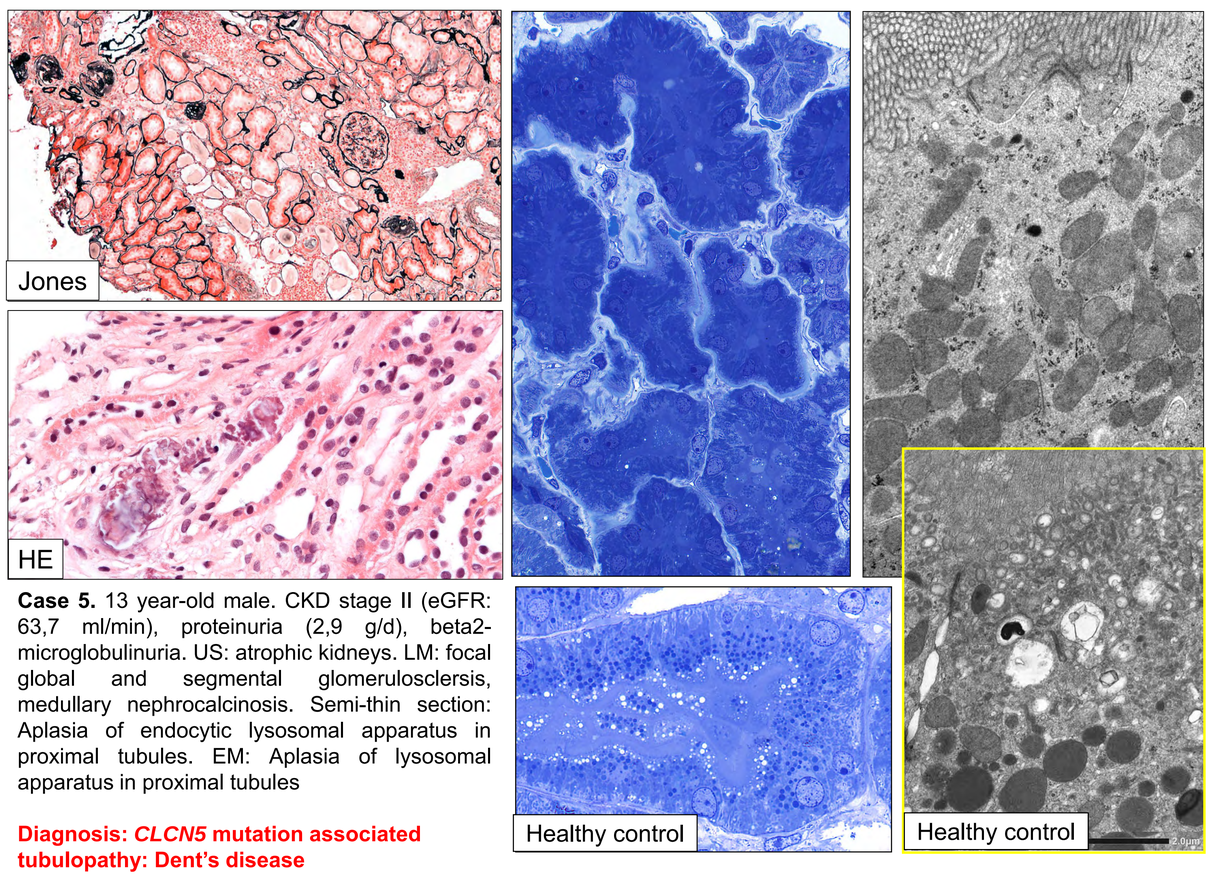
Case 3. 31 year-old male CKD for 7 years Father: ESRD. Serum creatinine 190 μmol/l, eGFR 39 ml/min Proteinuria (3 g/d) Microhematuria LM: Sclerosing glomerulopathy, 61% global sclerosis, 6% segmental sclerosis. IF: Negative. EM Dense aggregates in podocyes.

Diagnosis: *ACTN4* mutation related FSGS



Case 4. 7-month-old male infant, healthy parents. Nephrotic syndrome, encephalomyopathy, hypertrophic cardiomyopathy, deafness, retinitis pigmentosa → suspicious for CoQ_{10} deficiency.
Despite treatment, he passed away in 1 month. LM: mesangial expansion, segmental closure of capillary loops. IF: negative. EM: 70% foot process effacement, basement membrane material in capillary walls, mesangial expansion.

Diagnosis: Diffuse mesangial sclerosis (*PDSS2* mutation-induced CoQ10 deficiency)



Case 5. 13 year-old male. CKD stage II (eGFR: 63,7 ml/min), proteinuria (2,9 g/d), beta2-microglobulinuria. US: atrophic kidneys. LM: focal global and segmental glomerulosclersis, medullary nephrocalcinosis. Semi-thin section: Aplasia of endocytic lysosomal apparatus in proximal tubules. EM: Aplasia of lysosomal apparatus in proximal tubules

Diagnosis: *CLCN5* mutation associated tubulopathy: Dent's disease

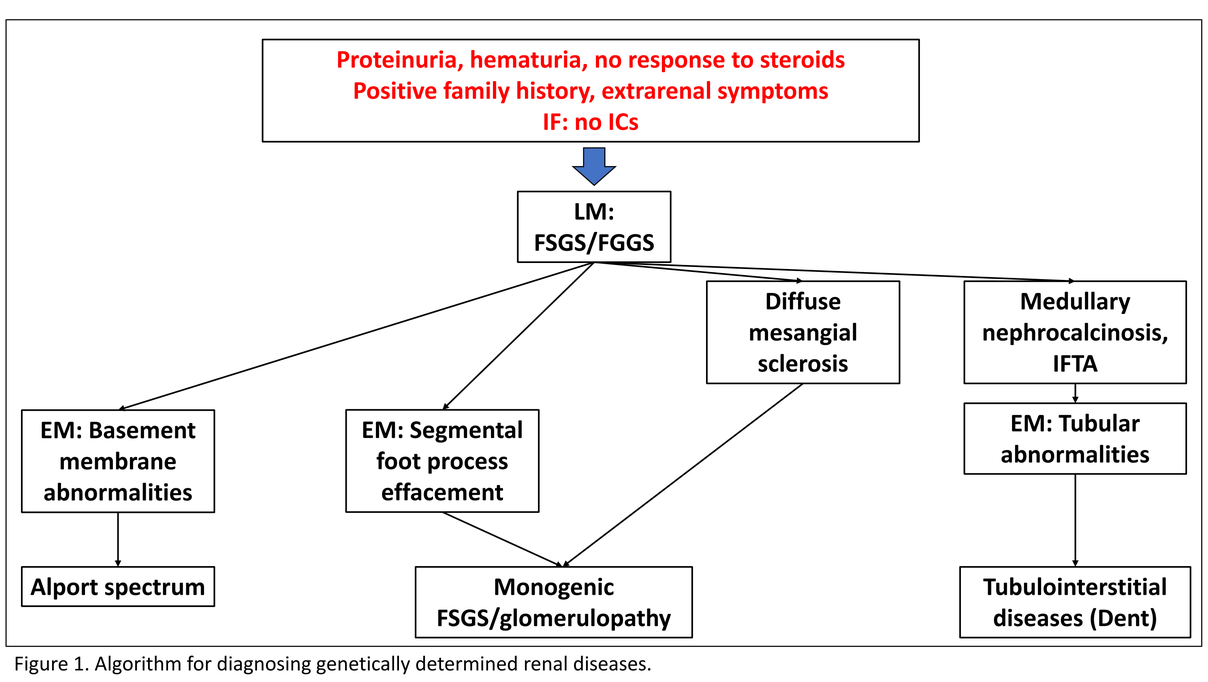


Figure 1. Algorithm for diagnosing genetically determined renal diseases.

WIDESPREAD KALLIKREIN EXPRESSION DURING HUMAN KIDNEY DEVELOPMENT BECOMES RESTRICTED TO TUBULES WITH A REDUCED NUMBER OF PRIMARY CILIA IN POSTNATAL KIDNEYS

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Introduction

The primary cilium is a signalling organelle present in most cell types and it has a crucial role in the regulation of development and homeostasis. Kallikrein is an enzyme that is part of the kallilkrein-kinin system involved in regulation of natriuresis and blood pressure. Our goal was to determine the relationship between primary cilia and kallikrein expression in the developing human mesonephros and metanephros and postnatal kidneys.

Materials and Methods

We used double immunofluorescence to analyze kallikrein and acetylated α-tubulin (primary cilia marker) expression in 5- to 16-week-old human conceptuses and postnatal kidneys. The density of primary cilia was calculated using ImageJ as the ratio of the number of cilia counted and the length of the apical surface of tubules in mm. Statistical analysis was performed in GraphPad Prism.

Results

Our preliminary results show that, during development, diffuse kallikrein expression characterized both podocytes and parietal epithelial cells (PECs) of renal corpuscles, while only PECs retained primary cilia past the embryonic period (Figure 1). In the postnatal kidney, the entire corpuscle became devoid of kallikrein, while only some tubules, morphologically resembling proximal tubules, demonstrated strong apical expression (Figure 1). When comparing postnatal kidney tubules with and without kallikrein expression, a noticeable reduction in the number of primary cilia in kallikrein-positive tubules was observed (Figure 2). Statistical analysis showed a significant difference ($p<0.0001$) in the average density of primary cilia between kallikrein-positive (4.2 cilia/mm) and kallikrein-negative (21 cilia/mm) tubules.

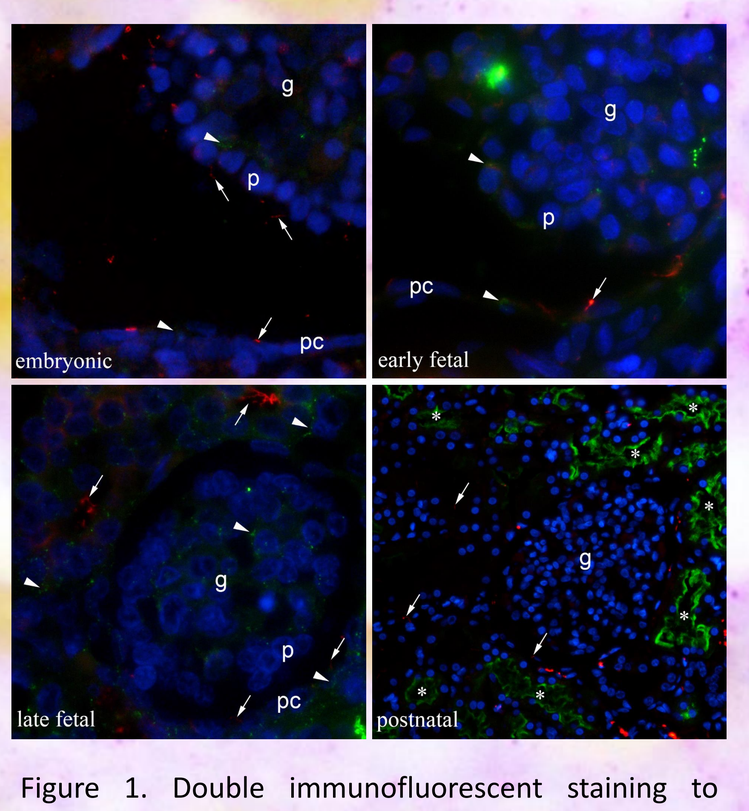

Figure 1. Double immunofluorescent staining to acetylated-α-tubulin and kallikrein in developmental stages of renal corpuscles. g – glomerulus, p – podocytes, pc – parietal epithelial cells, arrows – primary cilia, arrowheads – kallikrein expression, asterisks – kallikrein-positive tubules.

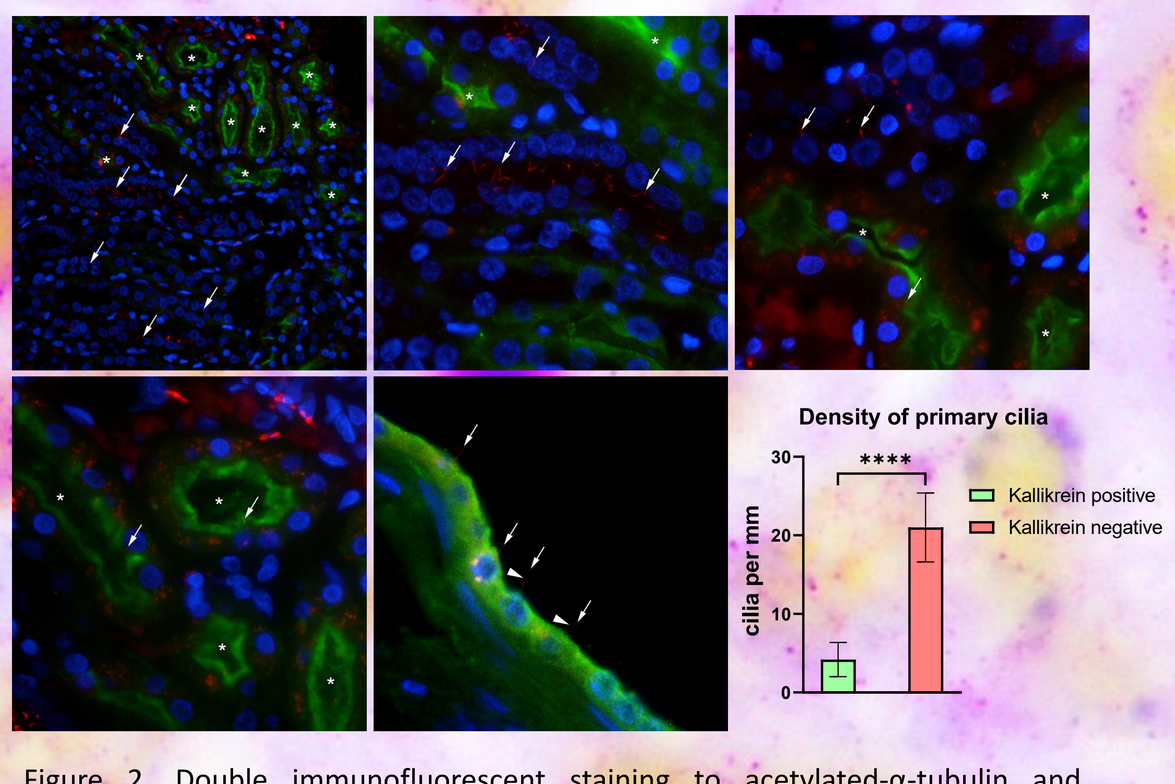

Figure 2. Double immunofluorescent staining to acetylated-α-tubulin and kallikrein in postnatal kidney tubules. arrows – primary cilia, arrowheads – kallikrein expression, asterisks – kallikrein-positive tubules. The graph represents the mean values of primary cilia density; error bars represent the standard deviation; ****$p<0.0001$.

Conclusions

Extensive kallikrein expression present during nephrogenesis becomes limited to certain tubules in the mature kidney and those tubules have comparatively less primary cilia than the rest. This implies an inverse relationship between kallikrein expression and primary cilium signalling.

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Repurposing chemical chaperones to the rescue of mouse models of Alport syndrome

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ABSTRACT

Alport Syndrome (AS) is a severe inherited glomerulopathy caused by mutations in the genes encoding the α-chains of type IV collagen [1,2], the most abundant component of the glomerular basement membrane (GBM). Alport patients lack effective therapies beyond blockade of the renin-angiotensin system.

Recently, we have characterized in detail the kidney phenotype of the knockin mouse [3], carrying the Col4a3-p.Gly1332Glu in homozygosity and of a compound heterozygous mouse model for AS, that carries the Col4a3-p.Gly1332Glu substitution in compound heterozygosity with a Col4a3 knocked-out allele. Both mice recapitulate essential features of AS, including shorten lifespan by 30–35%, increased proteinuria, increased serum urea and creatinine, pathognomonic alternate GBM thinning and thickening, and podocyte foot process effacement [4].

After a long-term treatment, with these two chaperones, we found that the GBM morphology and structure of the 4-PBA treated mice showed a considerable improvement compared with the control (placebo treated) group. Based on EM measurements there is a 43% reduction of lesions and a significant decline of the lesion's severity in the GBM of 4-PBA treated Alport mice. However, the measurements from TUDCA-treated AS mice did not differ from the placebo group. Additionally, the 4-PBA treatment could inhibit proteinuria and hematuria and fibrosis in AS mice when compared with control mice. Probably, the administration of 4-PBA can effectively stabilize the conformation of the mutated Col4a3, improve its folding, alleviating with this way the glomerular filtration barrier in the Alport mice.

METHODS

Treatment with:

Chaperones:

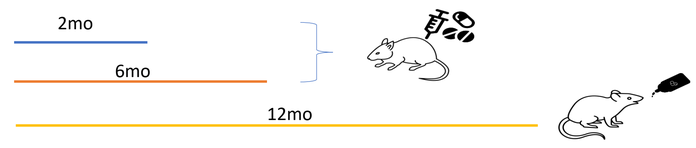
Sodium 4-phenylbutyrate **(4-PBA)**

Sodium tauroursodeoxycholate **(TUDCA)**

Vehicle:

Phosphate buffered saline **(PBS)**

Duration and type of treatment:



Treated mice:

1) Col4a3 knockin (**mut/mut**),
2) Col4a3 compound heterozygous (**mut/-**),
3) Wild type mice

RESULTS

1. Treatment with 4-PBA restores the GBM thickness in AS mice

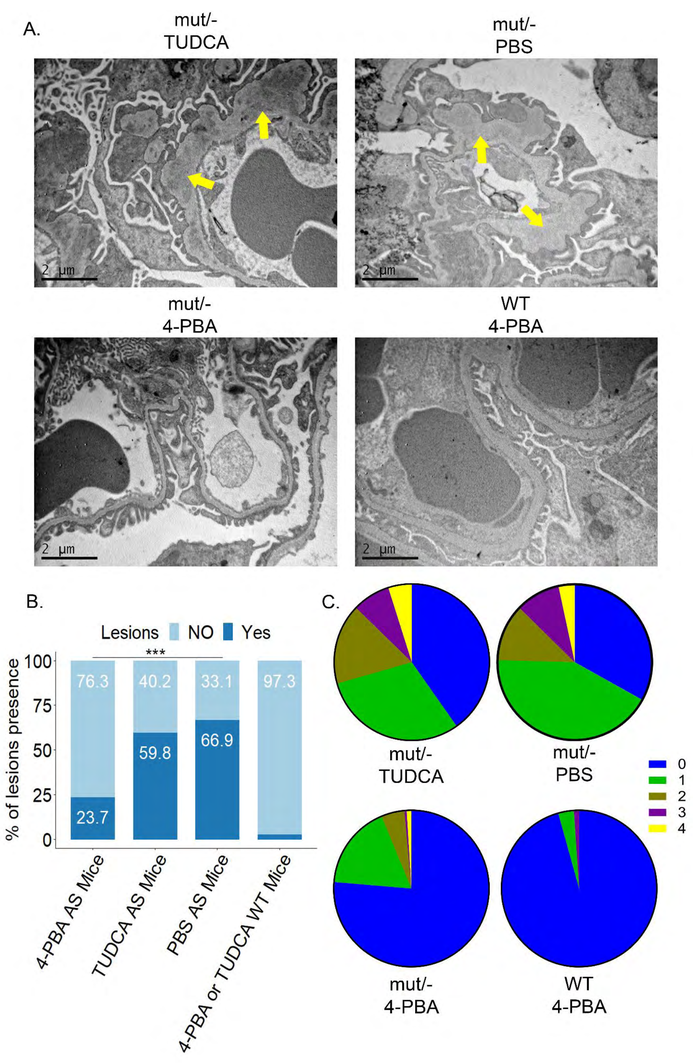


Figure 1: **Administration with 4-PBA can restore the GBM thickness in compound heterozygous Col4a3 mut/− and homozygous Col4a3 mut/mut mice**.
(A) The PBS-treated control group of Col4a3 mut/− mice, demonstrate thin GBMs with areas of severe irregular thickening (yellow arrows), consistent with AS nephritis. The same irregular morphology was noticed in the GBM of the TUDCA treated Col4a3 mut/− mice. However, the 4-PBA treated Col4a3 mut/− mice showed a restored and more regular GBM compared to control groups. Importantly, the treatment of wild type mice with these two chaperones had no adverse effects on the GBM morphology. **(B)** Diagram showing the percentage of lesions (dark blue color) that are present in 539 examined loops in the GBM from all groups. Note the small percentage (23,7%, n=207) of lesions in the 4-PBA treated mutant mice compared to the PBS (66,9%, n=118, p-value <0.0001) and TUDCA (59,8%, n=102, p-value= 0.2719 NS) group. Results were analysed using chi-square with Tukey post-testing.
(C) Diagram showing the degree of lesions severity in the GBM of each treated group. The 4-PBA treated mutant mice had less severe or milder lesions compared to the PBS or TUDCA treated mutant mice. Samples that followed 4-PBA treatment have statistical differences with P<0.001 compared with non-treated samples. Results were analysed using one-way ANOVA with Tukey post-testing.

2. Decreased interstitial fibrosis, segmental and global glomerulosclerosis in the 4-PBA treated AS mice

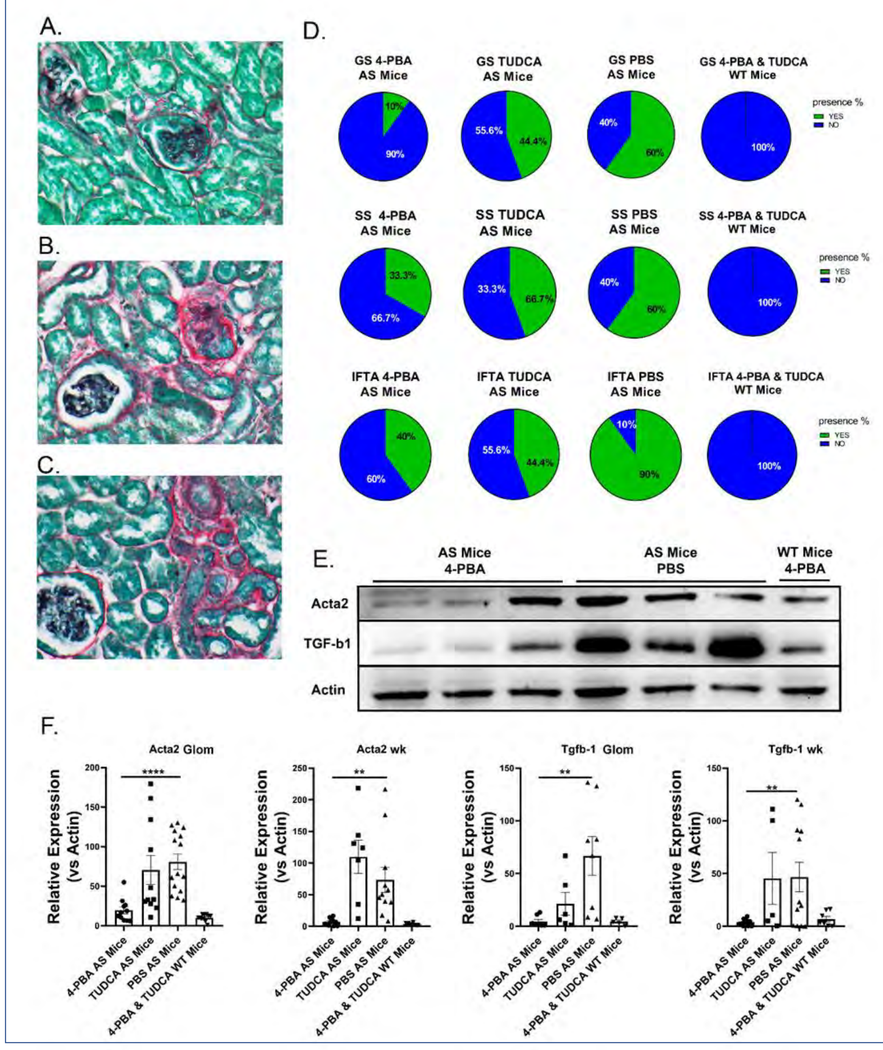


Figure3: **Reduced interstitial fibrosis, segmental and global glomerulosclerosis in the 4-PBA treated AS mice**

(**A-C**) Sirius-red staining on kidney sections of 4-PBA treated mice (A) and PBS-treated mutant mice (B-C) to assess renal fibrosis. (D) Diagram showing the percentage of (green colour) of glomeruli with segmental sclerosis (SS), global glomerulosclerosis (GS) and interstitial fibrosis and tubular atrophy (IFTA) that are present in treated and non-treated mice. (E-F) Western blot analysis of profibrotic markers TGF-β1 and Acta2 displaying decreased levels in glomeruli isolates of 4-PBA treated AS mice. (F) Quantification of representative blots for the expression levels of Acta2 and Tgfb1 in either glomerular lysates (left graph) or whole kidney lysates (right graph) in treated and non-treated AS mice normalized to Actin levels after the long-term treatment. AS mice: Col4a3 mut/mut and Col4a3 mut/− mice; Acta2, alpha-smooth muscle actin.

3. Less albuminuria and hematuria in 4-PBA treated AS mice

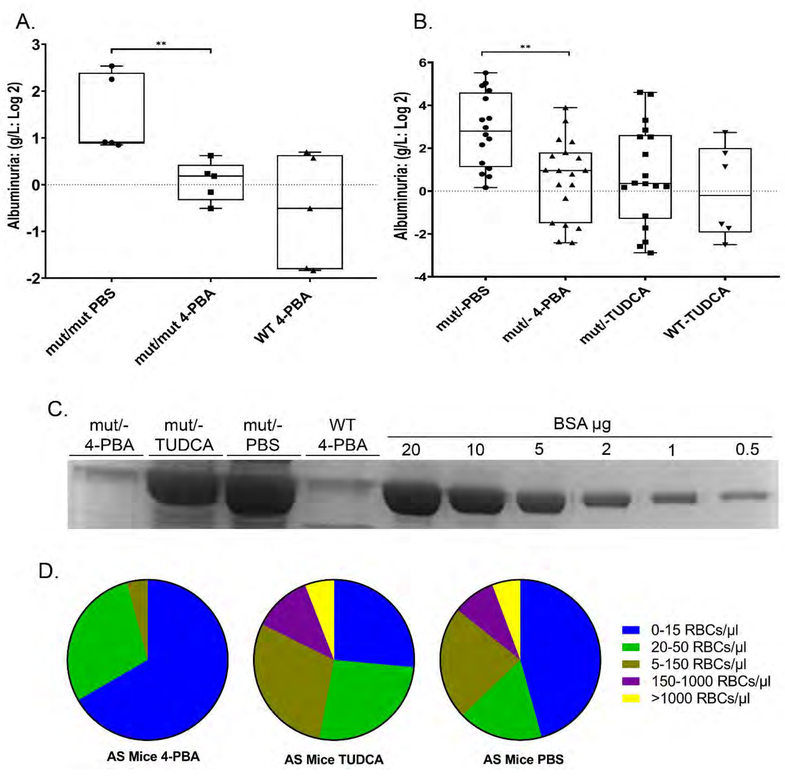


Figure 2: **Reduced albuminuria and hematuria in Col4a3 mutant mice after the long-term treatment with 4-PBA chaperone.** (A,B) Graph of urine albumin concentration (g/L) in chaperone treated and non-treated mut/mut (left graph) and mut/- (right graph) mice after the long-term treatment. Results were analysed using one-way ANOVA with Tukey post-testing. Statistically significant differences (*P*<0.01) were noted in 4-PBA treated mice compared with non-treated samples.
(C) An illustrative example of urine fractionation by SDS-PAGE. 20μL of urine from mut/mut mice, that were treated either with 4-PBA (1st lane) or with PBS (2nd lane), were fractionated together with standard concentrations of Bovine serum albumin on a 7.5% SDS-PAGE and stained with Coomassie blue. D) Hematuria in chaperone treated mutant mice. Diagram represents the percentages of AS Mice that have different levels of hematuria (number of red blood cells per μl of urine) after the long-term treatment compared to control mice.

4. Reduction of Col4a3 in glomeruli of 4-PBA treated AS mice

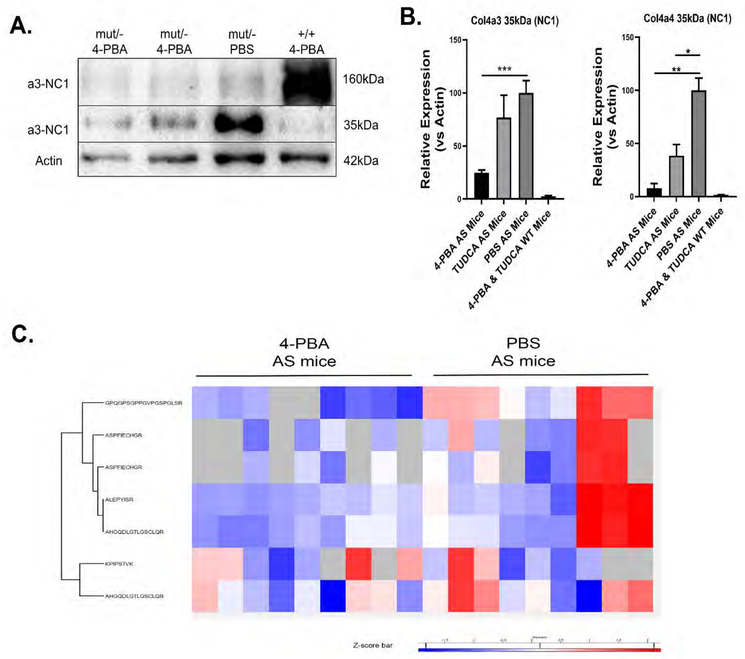


Figure 4: **The 35-kDa Col4a3 fragment is downregulated in glomerular isolates of 4-PBA treated AS mutants (mut/mut and mut/-)**. (**A**) Representative WB to demonstrate Col4a3 expression level change in glomeruli lysates after 4-PBA treatment. A specific antibody that recognizes the NC1 domain of Col4a3 chains was used to identify the α3 expression. (**B**) Quantification of representative blots as shown on the graphs where the expression levels of α3 and α4 chains (in glomerular lysates) were normalized to Actin levels after long-term chaperone treatment. Data are means ± SEM (n≥3). Results were analyzed using one-way ANOVA with Tukey post- testing (*P<0.05, **P<0.01, *** P<0.001). (**C**) Mass spectrometry analysis from glomeruli lysates, validating the reduction of Col4a3 peptides that correspond to the 35kDa fragment next to p.1332, in 4-PBA treated AS mice compared to PBS treated AS mutants. Heatmap illustrating specific Col4a3 peptides downregulated in glomeruli isolates which are reduced after 4-PBA treatment of AS mice. High expression is in red and low expression is in blue.

CONCLUSIONS

- The long-term treatment with **4-PBA** proved beneficial for the two AS mouse models: the Col4a3 knockin and the compound heterozygous mouse, that both carry the ***Col4a3***-p.Gly1332Glu substitution
- The chaperone 4-PBA could effectively restore to a sufficient degree the morphology and thickness of GBM in both mutant mice
- The chaperone 4-PBA could prevent the increase of proteinuria or hematuria and inhibit the emergence of fibrosis

ACKNOWLEDGEMENTS

This work was funded by: the Alport Syndrome Foundation, Inc. (ASF), Pedersen Family and the Kidney Foundation of Canada (KFOC) and by the Cyprus Research and Innovation Foundation programme POST-DOC/0916/0190.

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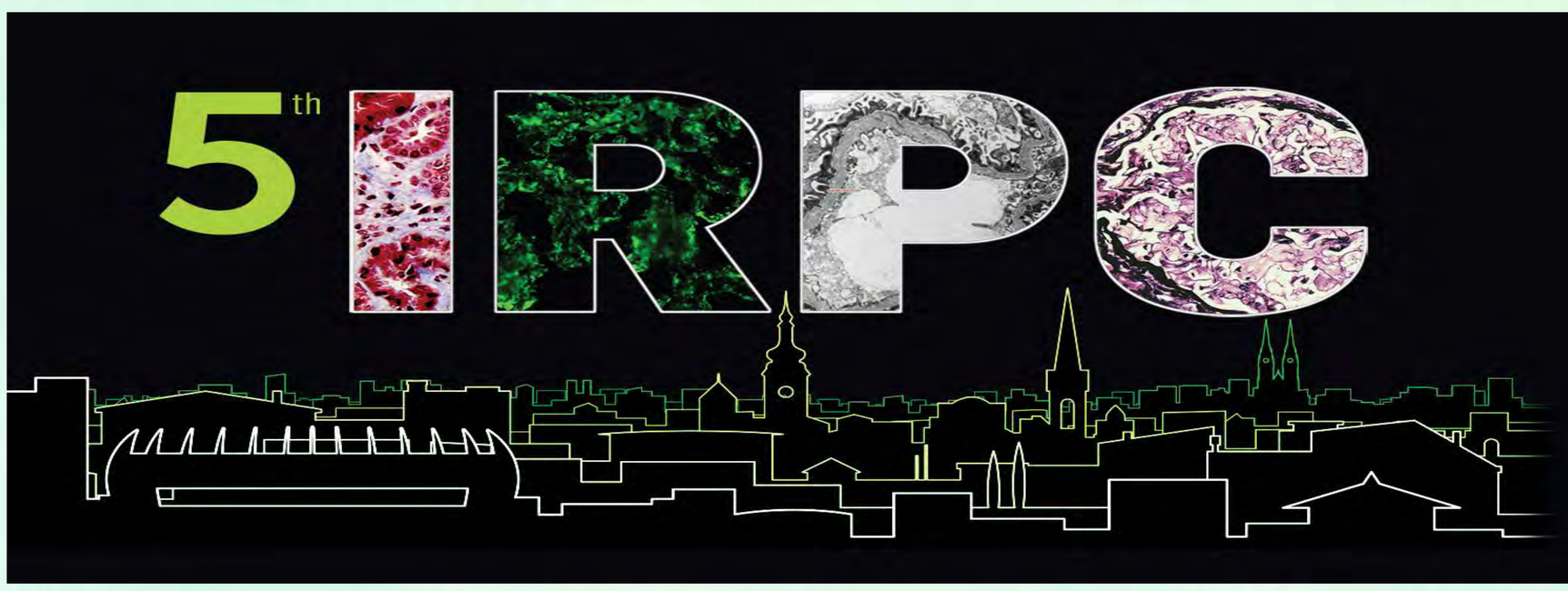
[3] Pieri M, et al. (2014) *J Am Soc Nephrol* [4] Odiatis C, Savva I, et al. (2020) *Matrix Biol Plus*



5th International Renal Pathology Conference
18th – 20th May 2023
Zagreb, Croatia

Genotype and phenotype Alport syndrome differences in the same family

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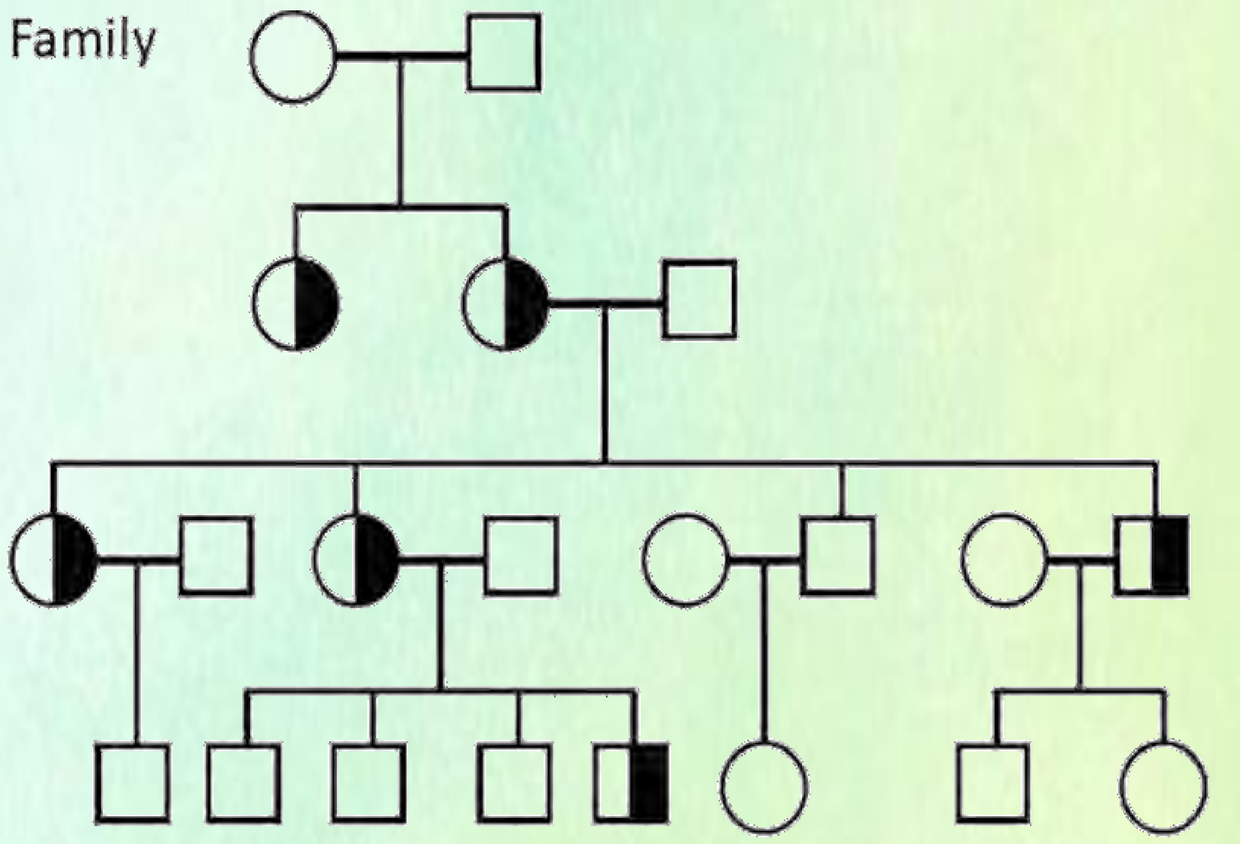
Alport syndrome is a hereditary disorder caused by variants in genes for chains of collagen type IV:COL4A3, COL4A4, and COL4A5.Variants inCOL4A3 andCOL4A4causean autosomal recessive type of disease, while inCOL4A5cause a classic, X-linked disease.

Methods

c.2881+1G>A in COL4A3 gene. The proband presented with end-stage renal disease(ESRD)and had a history of hematuria and renal injury (RI). We have made a family pedigree and gathered all family members for urinalysis and blood sampling for genetic analysis. Genetic testing was performed using NGS (next generation sequencing) for COL4A3, COL4A4 and COL4A5 genes.



Results

All patients tested positive for hematuria and proteinuria. Four patients underwent kidney biopsy. Mother had hematuria and non-nephrotic proteinuria, 2 children had ESRD, 3 had nephrotic proteinuria and 1 son had subnephrotic proteinuria. 3 children had sensorineural hearing loss, 3 were diagnosed with Alport syndrome on kidney biopsy specimen and a pathogenic 'splice site' variant c.2881+1G>A in COL4A3 gene was found in 3 children. Mother hasn't been tested.

Family members with the same variant, despite the same genotype, had different clinical presentation [Table 1.]It is not yet clear what causes phenotype variability. It is possible that some other, not detected variant in COL4 genes which is not pathogenic by itself, but in co-inheritance with the pathogenic variant contributes to more severe phenotype. Possible cause are also variants in other genes connected with the structure and function of the glomerular filtration barrier that were not sequenced by this method. Finally, environmental factors and diseases (smoking, obesity, hypertension) surely play the role in kidney damage and in accordance with that, contribute to severe phenotype and worse prognosis.

		Hematuria +/N	Proteinuria +/g	Creatinine/GFR	NGS	Renal biopsy	Ocular signs	Hearing loss Audiometric check-ups	Blood pressure over 140/90 mmHg
Mother	N ♀	+ 10	+ 0,51gr/24h	74 µmol/L 90 mL/min	No	No	No	No	✓
Child	M ♀	0	0	ESRD/Tx 2010/normal graft	COL4A3 c. 2881+1G> A	x2	No	Yes	✓
Child	P ♀	+ 20	++ 1.46gr/24h 3.5 gr/24h	114 µmol/L 80 mL/min	COL4A3 c. 2881+1G> A	x2	No	Yes	✓
Child	B ♂	+ 12	++ 4.64 gr/24h 8.31 gr/24h	CAPD 2020	COL4A3 c. 2881+1G> A	x3	No	Yes	✓
Child	L ♂	+ 4-6	+ 1.2 gr/24h	76 µmol/L 136.4 mL/min	COL4A3 c. 2881+1G> A	x1	No	0	✓

Table 1 Hematuria +/N (qualitatively/quantitatively), proteinuria +/g (qualitatively/quantitatively) creatinine/GFR (creatinine/glomerular filtration ratio)

Conclusion

Clinical and morphological differences among patients with the same variant point to the need for patients to be monitored more, early detection and prevention of the progression to ESRD

Screening for Fabry Disease Among Patients with Chronic Kidney Disease in Clinical Hospital Center Rijeka

Authors

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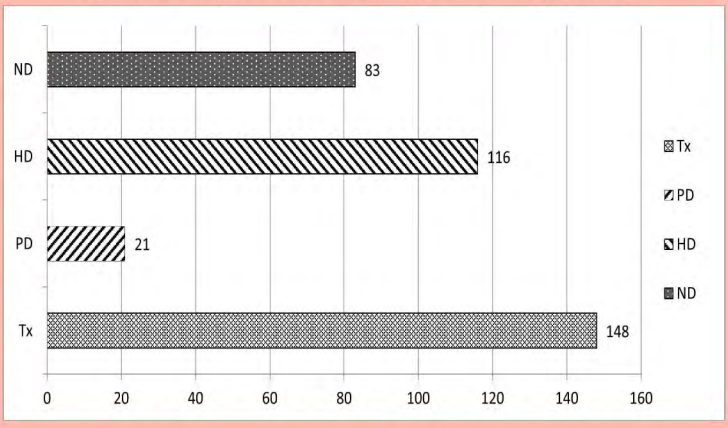
Introduction

Fabry disease (FD) is a rare X-linked lysosomal storage disorder that results from deficiency in α-galactosidase A (α-Gal A) enzyme activity. The hypothesis of our research was that FD could be an unrecognized cause of chronic kidney disease (CKD) in patients with severely decreased kidney function and patients on renal replacement therapy.

Metodology

We have screened 368 CKD patients, from June 21st to September 30th, 2018, with an estimated glomerular filtration rate less than 30 mL/min/1,73m2, including patients on hemodialysis or peritoneal dialysis and patients with a kidney transplant. α-Gal A was determined using dried blood spot tests. We have additionally analyzed globotriaosylsphingosine (lyso-GL-3) concentration in female patients. In patients with enzyme activity less than 1.2 μmol/L/h and/or lyso-GL-3 levels higher than 3.5 ng/mL genetic testing was conducted.

We screened 368 CKD with eGFR less than 30 mL/min/1,73m2, including patients on HD or PD and patients with kidney transplant (Figure 1).



Results

Two male patients were diagnosed with FD. Genetic testing confirmed the pathogenic mutation (c. [540G>C] (p. [L 180F]) in both patients. Laboratory testing showed low α-Gal A activity in both patients, while only the first patient had high concentrations of lyso-GL-3 marker. Laboratory testing also deviated in other patients but without a confirmed genetic mutation. Two male patients had low α-Gal A activity, 1.1 μmol/L/h and 1.0 μmol/L/h respectively. One female patient had borderline enzyme activity of 1.2 μmol/L/h and normal concentration of lyso-GL-3. Five female patients had high concentrations of lyso-GL-3 marker with normal α-Gal A activity. Genetic testing was performed in all eight patients and did not confirm pathogenic mutations for FD.

Conclusion

In this study we confirmed the existence of a pathogenic mutation (c. [540G>C] (p. [L 180F]) responsible for FD in the population of CKD patients in our center. Therefore, given the availability and benefit of early initiation of therapy, it is advisable to consider FD as a cause of CKD.

Keywords: Fabry disease; screening; chronic kidney disease; α-galactosidase A; lyso-GL-3

ZEBRA BODIES ON ELECTRON MYCROSCOPY; A CASE REPORT

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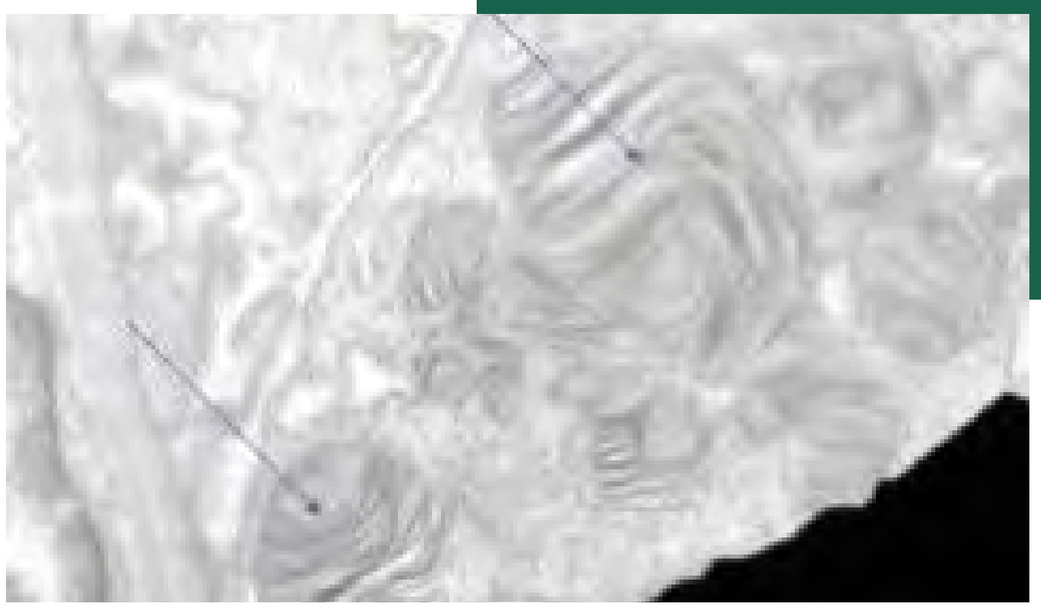
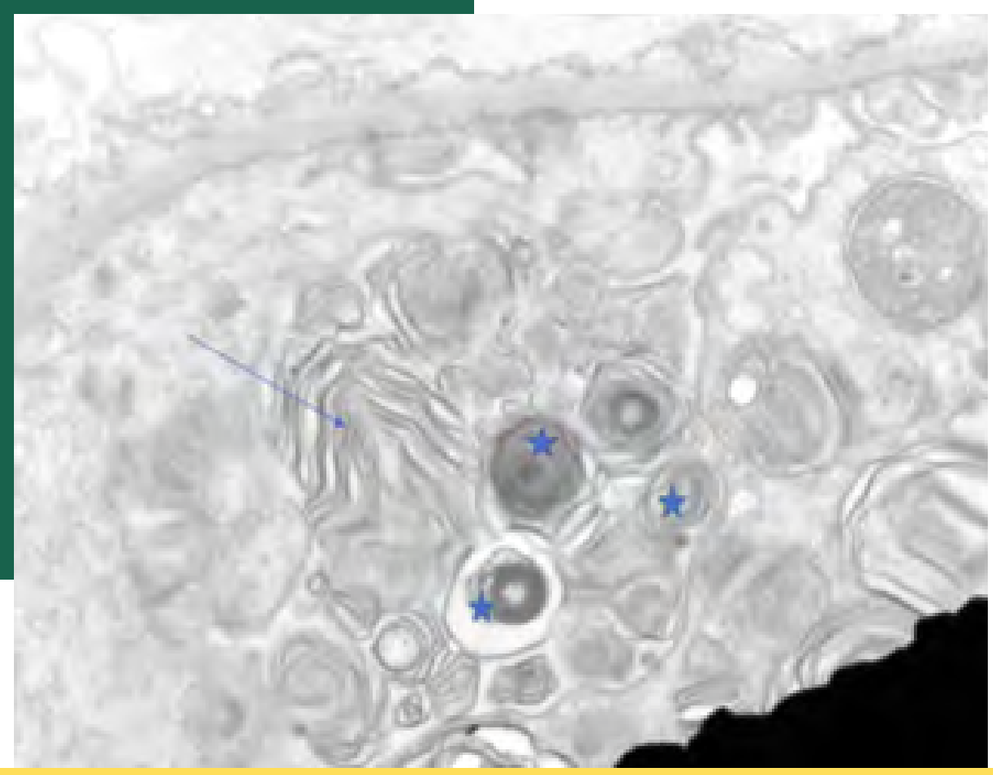


ZEBRA BODIES (ARROWS) ON ELECTRON MICROSCOPY



MYELIN FIGURES (ASTERISK) ON ELECTRON MICROSCOPY

INTRODUCTION: KIDNEY BIOPSY IS A VALUABLE AND OFTEN INDISPENSABLE TOOL GUIDING THE DIAGNOSIS AND OPTIMAL APPROACH TO THE TREATMENT OF INTRINSIC KIDNEY DISEASES.

CASE REPORT: A 61-YEAR OLD MAN WITH SERONEGATIVE RHEUMATOID ARTHRITIS, FREQUENT ATTACKS OF URIC ARTHRITIS AND A FOUR-YEAR DIABETES PRESENTED WITH ACUTE NEPHRITIC SYNDROME. KIDNEY BIOPSY REVEALED IGA NEPHROPATHY OF MINIMAL ACITIVITY AND NO CHRONICITY DESCRIBED BY OXFORD SCORE M0 E0 S0 T0 C0, AND MINIMAL ACUTE TUBULAR INJURY AND FOCAL INTERSTITIAL NEPHRITIS. ELECTRON MICROSCOPY FOUND VERY SCARCE IGA DEPOSITS THAT WERE PROBABLY NON-PATHOGENETIC AND MYELOID OR ZEBRA BODIES IN THE CYTOPLASM OF PODOCYTES, PRIMARILY SUGGESTIVE FOR FABRY DISEASE.

DISCUSSION: MYELOD OR ZEBRA BODIES ARE A HALLMARK OF GLYCOLIPID STORAGE DISORDERS. DEPOSITS OF GLOBOTRIAOSYLCERAMIDE (GB3) AS LAMELLATED MEMBRANE STRUCTURES WITH AN ONION SKIN APPEARANCE MAY BE FOUND IN ALL GLOMERULAR CELL TYPES, MOST PROMINENTLY PODOCYTES, ARTERIOLAR SMOOTH MUSCLE CELLS, AND TUBULES. HOWEVER, LAMELLAR INCLUSIONS HAVE BEEN DESCRIBED IN OTHER CONDITIONS, SUCH AS SILICOSIS, AND WITH THE USE OF CATIONIC AMPHIPHILIC DRUGS SUCH AS ANTIMALARIALS CHLOROQUINE AND HYDROXYCHLOROCHINE, AMPHETAMINE CHLORPHENTERMINE, ANTIHISTAMINE CHLORCYCLIZINE, TRICYCLIC ANTIDEPRESSANTS IMIPRAMINE AND CLOMIPRAMINE, AND ANTIBIOTIC GENTAMYCIN. THE LOCATION OF THE INCLUSIONS IS SOMETIMES HELPFUL IN MAKING THE DISTINCTION BETWEEN THESE DISEASES. LAMELLAR INCLUSIONS ASSOCIATED WITH GENTAMICIN OCCUR IN PROXIMAL TUBULES, WHEREAS, IN FABRY DISEASE, THE INCLUSIONS ARE MOST STRIKING IN PODOCYTES AND DISTAL TUBULES.

MESANGIAL DEPOSITS (ARROWS) ON ELECTRON MICROSCOPY

CONCLUSION: LEUCOCYTE ALPHA-GALACTOSIDASE A (ALPHA-GAL A) ACTIVITY ENZYMATIC ASSAY RULED OUT FABRY DISEASE. SINCE OUR PATIENT HAD BEEN TREATED WITH HYDROXYCHLOROQUINE FOR OVER A DECADE, THE DRUG WAS SUSPENDED. THE PATIENT SIGNIFICANTLY RECOVERED HIS KIDNEY FUNCTION IN THE FOLLOW-UP.

MAUER M, WALLACE E. (2023.). FABRY DISEASE: KIDNEY MANIFESTATIONS. IN A.Q. LAM (ED.), *UP TO DATE*. [CITED 2023 APR 25] AVAILABLE FROM: WWW.UPTODATE.COM

5th IRPC

5th International Renal Pathology Conference

18th – 20th May 2023
Zagreb, Croatia

C3 Glomerulopathy: Patterns of Injury and Response to Treatment

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Objective

To evaluate the clinicopathological features, complement abnormalities, treatment, and outcomes of patients with C3G.

Methods

A retrospective study. C3G was defined as dominant C3 staining on IF with minimal or no IG staining. Patients received low-dose prednisone with MMF, CYP, or ECZ and were followed for 12 months. Renal survival was assessed using Kaplan-Meier estimator.

Results

23 patients with C3G (18 C3GN, 5 DDD) were evaluated at a nephrology center. Nephrotic syndrome was the main clinical presentation, and membranoproliferative glomerulonephritis (65%) was the most frequent pattern of injury (Figure 1). No significant differences were observed in renal function and outcomes between treatment groups at 12-month follow-up. 18% had ESRD or died at the end of follow-up (Table 1).

Conclusion

Immunosuppressive treatments are similarly effective in improving renal function in our population. However, it's crucial to evaluate each C3G patient for complement abnormalities and triggering factors to recommend suitable treatment.

Table 1. Characteristics of C3 Glomerulopathy patients according to treatment group at 12 months follow up

		Treatment Group				
		MMF (n=10)	CYP (n=8)	ECZ (n=5)	Total (n=23)	P Value
Gender, *n* (%)	Age (Mean ± SD)	44,5 ± 19,3	43.4 ± 12,3	25,2 ± 10,1	39.9±16.8	0,06
	Male	6 (60)	4 (50)	2 (40)	12 (52)	0,86
	Female	4 (40)	4 (50)	3 (60)	11 (48)	
Renal Function	Blood Urea Nitrogen (mg/dL)	24,4 ± 16,7	37,7 ± 33,5	15,5 ± 5,8	30,1 ± 17,4	0,55
	Creatinine (mg/dL)	2,13 ± 2,1	3,2 ± 2,1	1,7 ± 1,9	2,42 ± 2,01	0,19
	Proteinuria (g/24 hours)	3,4 ± 1,2	4,4 ± 1,1	2,8 ± 2,3	2,1 ± 6,6	0.23
Complement, *n*	Low C3 <80 mg/dl	4	7	5	16	0,02*
	Low C4 <15 mg/dl	3	6	5	14	0,02*
Outcomes, *n* (%)	ESRD	1 (10)	3 (37)	1 (20)	5 (18)	0,46
	Death	1 (10)	2 (25)	0 (0)	3 (11)	0,57

*MMF: Mofetil Mycophenolate; CYP: Cyclophosphamide, ECZ: Eculizumab. ESRD: End-Stage Renal Disease. *P < 0.05, Fisher exact test.*

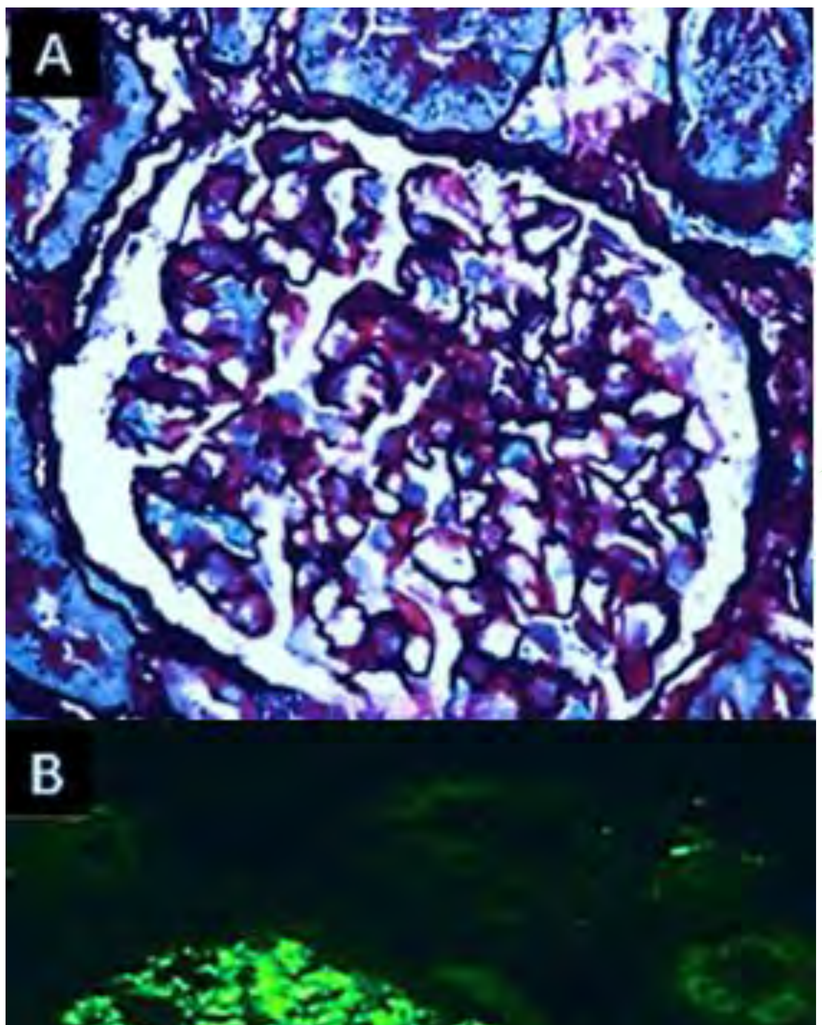

Figure 1: Most common Histopathological pattern in C3 Glomerulopathy

n = 23

(A) Membranoproliferative Glomerulonephritis (65%) – Silver Stain

(B) C3 Glomerulopathy
(with anti-C3 antibody x400)
Diffuse Granular Deposition

SCAN ME

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Prevalence of DNAJB9 positivity in our cohort of patients with fibrillary glomerulonephritis

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Aim

DNAJB9 is a heat-shock protein recently found to be the most abundant antigen in kidney specimens of patients with fibrillary glomerulonephritis (FGN).[1] The presence of DNAJB9 seems to be highly specific and sensitive for FGN.[2] The aim of this study was to investigate clinical and histological features and DNAJB9 positivity of the patients in our cohort of FGN.

Methods

The Registry of kidney biopsies at Department of Renal Pathology and Electron Microscopy, Dubrava University, Zagreb, between 2009 and 2021, was retrospectively reviewed for samples with FGN. Demographic, clinical and pathologic data of FGN patients were analyzed and additional immunohistochemistry (IHC) for DNAJB9 was performed on paraffin-embedded tissue. In order to define patients with FGN, we used following criteria: presence of Congo Red-negative glomerular deposits that were organized into randomly oriented straight fibrils 10-30 nm in diameter without distinct hollow core by EM and stained with antisera to immunoglobulin heavy and light chains by IF.

Results

We identified 17 adults (4 males) with FGN. Table 1 shows relevant demographic and clinical features of our cohort. The most common pattern of glomerular injury based on light microscopy was mesangial GN (13 patients). Light chain restriction was present in four samples of whom two had clear evidence of monoclonal gammopathy (MG) (case 14 and 16) and later were treated due to overt multiple myeloma. IHC for DNAJB9 was positive (Figure 1) in all cases except case 12 and 16. Case 12 did not have light chain restriction as well as evidence of MG or other systemic disease with kidney involvement.

Conclusions

In our cohort of patients with FGN, 88% of kidney samples expressed DNAJB9 positivity by IHC regardless of presence of light chain restriction, MG or systemic diseases with possible kidney involvement.

Table 1 Demographic and clinical characteristics of patients at the time of kidney biopsy.

Case No.	Sex	Age (y)	Year of diagnosis	AH	Proteinuria (g/day)	Full nephrotic syndrome	Microscopic hematuria	eGFR (ml/min/1.73 m^2)	Associated medical conditions
1	M	59	2010	+	3.4	-	+	64	HF/DMT2
2	F	55	2010	+	-	-	+	75	-
3	F	49	2011	+	1.7	-	-	54	SLE
4	F	58	2011	+	0.5	-	+	48	-
5	F	62	2012	+	-	-	+	45	-
6	M	59	2013	+	0.6	-	+	63	LC/DMT2
7	F	54	2013	+	9.8	+	-	64	-
8	F	61	2013	+	3.5	+	+	31	-
9	F	29	2014	-	-	-	+	77	SLE
10	F	54	2014	-	-	-	+	62	-
11	M	60	2014	+	13.7	+	-	36	DMT2
12	F	67	2015	+	1.5	-	+	79	-
13	F	73	2016	+	8.5	+	-	51	DMT2
14	F	77	2017	+	3.5	-	+	13	MG, LC, DMT2
15	F	65	2018	+	1.6	-	+	46	-
16	M	64	2018	+	5.4	+	+	56	MG, DMT2
17	F	63	2018	+	5.8	+	-	49	-
Mean [range]		59 [29-77]			4.6 [0.5-13.7]			54 [13-79]	

AH, arterial hypertension; HF, heart failure; DMT2, type 2 diabetes mellitus; SLE, systemic lupus erythematosus; LC, lung carcinoma; MG, monoclonal gammopathy.

Table 2 Pathohistological features of 17 patients with fibrillary glomerulonephritis.

	Light Microscopy (LM)							IF and IHC				Electron Microscopy	
Case No.	No. of glomeruli	Glomerular pattern by LM	IFTA (%)	GGS (%)	FSGS (%)	ah[a]	AFI[b]	IgG	C3	Light chain	DNAJB 9	Fibril diameter (nm)	Podocyte foot process effacement[c]
1	14	MesGN	20	0	0	2	2	2+	+/-	κ&λ	+	13 ± 3	+
2	30	MesGN	0	20	0	0	0	2+	2+	κ&λ	+	11.5 ± 1.3	-
3	5	MesGN	0	0	0	0	0	+/-	-	κ	+	13 ± 4	++
4	11	N	30	27	18	3	2	+/-	+/-	κ&λ	+	15 ± 2	-
5	17	MesGN	15	29	0	2	1	3+	2+	κ&λ	+	20 ± 4	+[e]
6	13	MesGN[d]	10	8	0	1	0	2+	2+	κ&λ	+	17.6 ± 4	+
7	20	MGN	30	10	20	0	0	2+	+	κ&λ	+	10.2 ± 2	+++
8	18	MesGN[d]	15	11	6	2	2	2+	+	κ&λ	+	16.5 ± 2	+++
9	32	N	0	6	0	0	0	+	+	κ&λ	+	14 ± 2	-[e]
10	23	MesGN	5	0	0	2	1	2+	0	κ&λ	+	14.9 ± 0.9	+
11	19	MesGN	65	32	26	3	3	3+	2+	κ&λ	+	13 ± 4	+
12	11	MesGN	15	11	4	3	2	+	-	κ&λ	-	16.1 ± 2.4	++
13	11	MesGN	10	27	0	3	1	3+	+	κ&λ	+	11 ± 2	+++
14	10	MesGN	50	30	0	3	0	2+	+	κ	+	12 ± 1.1	-
15	8	MesGN	30	25	25	3	0	+	+	κ&λ	+	11 ± 1.1	-
16	24	MesGN	10	25	0	3	0	3+	2+	κ	-	13 ± 2	+++
17	17	DPGN	40	29	18	0	2	+	2+	λ	+	19 ± 2	+++

LM, light microscopy; GGS, global glomerulosclerosis; FSGS, focal segmental glomerulosclerosis; MesGN, mesangial GN; N, normal; MGN, membranous GN; DPGN, diffuse proliferative endocapillary GN.

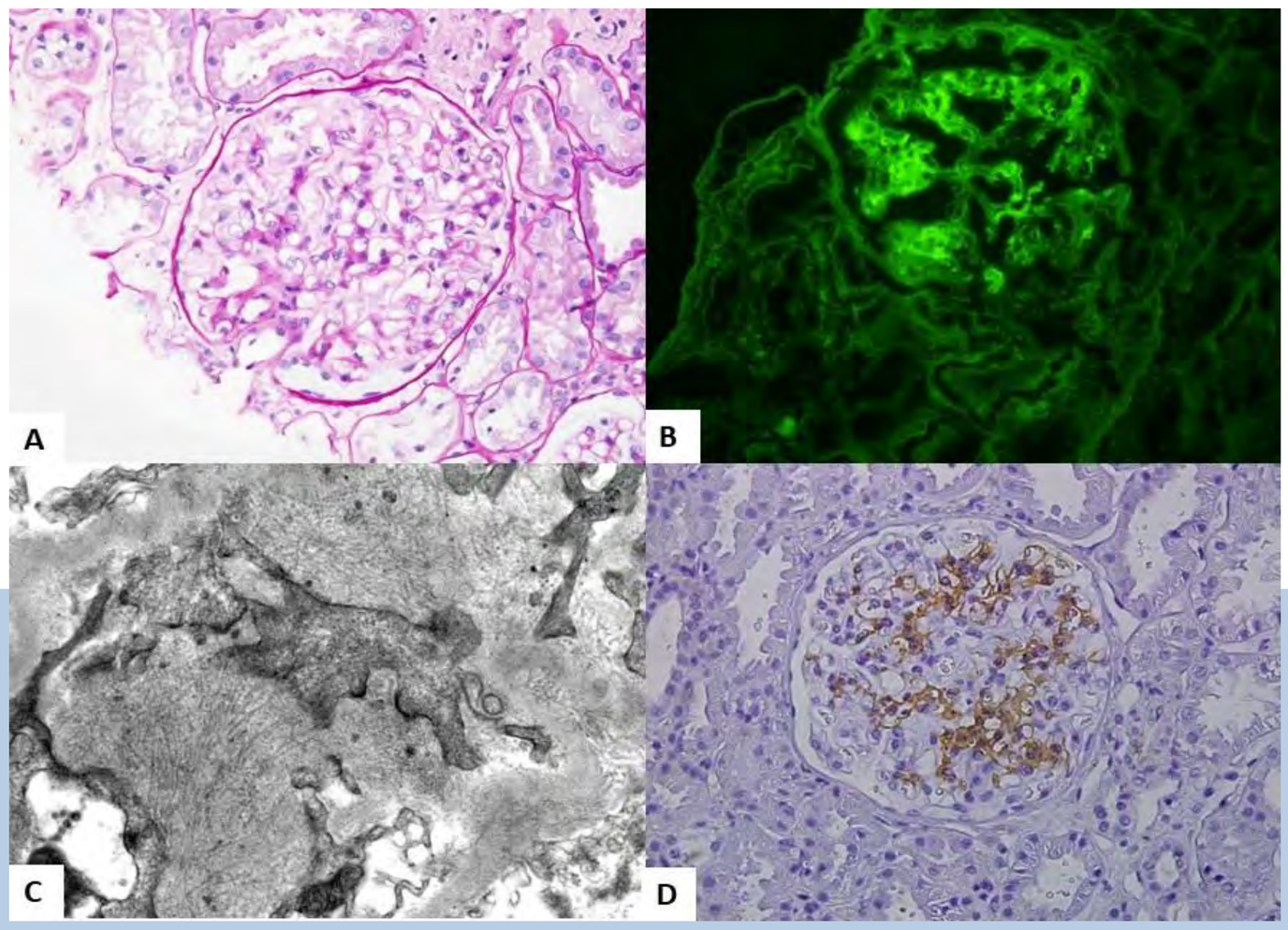

Figure 1 Kidney biopsy findings in case 9. A) Glomerulus with normal morphology. PAS stain, magnification x400. B) Immunofluorescent analysis showing smudgy IgG positivity. Magnification x400. C) Fibrillary deposits in mesangium. TEM x25000. D) Positive DNAJB9 immunohistochemistry stain in glomerulus, x400.

Abbreviations: *[a]Scored as: 0-absent, 1-mild, 2-moderate, 3-severe, [b]Scored as: 0-absent, 1-mild, 2-moderate, 3-severe, [c]Effacement scored as: - (absent), + (focally, in < 50 % of podocyte process surface), ++ (50 - 80 %), +++ (diffuse, > 80 %), [d]Accompanied by mesangial hypercellularity, [e]Additional finding of thin glomerular basement membrane.*

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Progressive renal disease due to crescentic fibrillary glomerulonephritis after AstraZeneca COVID-19 vector vaccine

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Introduction

COVID-19 vaccines are known to cause new or relapsing glomerular diseases due to potent immune dysregulation. **There are cases of crescentic fibrillary glomerulonephritis (FGN) after mRNA Pfizer vaccine**. FGN is a rare glomerular disease found in 0,5-1,4 % of native kidney biopsies. The etiology of FGN is still unknown. Pathognomonic patterns on electronic microscopy are random fibrillary deposits of polyclonal IgG in mesangium and glomerular capillary walls which don´t stain with Congo red. Fibrous crescents are found in 17-50 % of cases. DNA-J heatshock protein family member B9 (DNAJB9) was identified as a highly sensitive and specific biomarker of FGN and is expected to be a new diagnostic tool to replace electron microscopy. FGN presents with hematuria, arterial hypertension, nephrotic proteinuria and renal impairment with rapidly progressive glomerulonephritis and has poor prognosis. There is no established therapy, although treatment with rituximab has been reported to stabilize disease progression in some patients. **We hypothesized that the vectors vaccines could also stimulate immune response that can trigger crescentic FGN**.

Case report

We present 54 –year old male patient whith prediabetes and arterial hypertension as main comorbidities which have been diagnosed during this sequence of events. He was smoker. The family history was negative for any kidney or autoimmune diseases. It is worth mentioning from the epidemiological history that a month before the first report to the emergency department, he was vaccinated with the first dose of AstraZeneca (COVID-19) vaccine.

INITIAL UROLOGY EVALUATION

- On the same day that the patient recieved second dose of AstraZeneca COVID-19 vaccine July 1.2021, he reported to the emergency urological department for sudden pain in the right testicle with frequent urination. In the laboratory findings, the levels of inflammatory parameters were normal with **acute kidney injury** - creatinine level was 173 umol/L and urea 9.9 mmol/L. In the urine test, proteins (2+) and blood (3+) were postive and in sediment there was a mass of erythrocytes, leukocytes and a lot of bacteria. The kidney ultrasound was normal, without residual urine, but **epididymitis and hydrocela of the right testicle** were described. He was treated with ciprofloxacin.

FOLLOW-UPS

- The urine cultures were sterile. Two weeks later July 15,2021 in the follow up examination he presented with high arterial pressure of 191/113 mmHg with edema of the genitals and both lower legs and was given perindopril/indapamid/amlodipine by family doctor. Three weeks later July 22,2021 on the abdominal ultrasound kidneys were thickened parenchyma, obliterated corticomedullary border, hypoechoic structure of parenchyma, 2 cm thick - corresponds to ultrasound signs of **inflammation process (edema) of both kidneys**, ciprofloxacin was continued. Four weeks later July 28,2021 control renal ultrasound examination reveals **dilatation of the left renal canal system** and patient was hospitalized in Urology department. **JJ stent was implanted**.

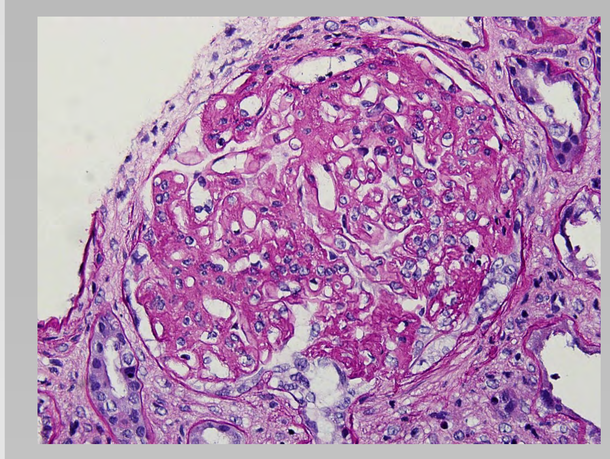
Follow-ups	**Normal range**	**Visit 1 (July 1, 2021)**	**Visit 2 (July 8, 2021)**	**Visit 3 (July 15,2021)**	**Visit 4 (July 22,2021)**	**Visit 5 (July 28,2021)**
creatinine (umol/L)	64-104	173	176	293	445	663
urea (mmol/L)	2,8-8,3	9,9	9	14,5	18,3	21,4
eGFR ml/min/1.73m^2 CKD-EPI		38	37	20	12	4

Table 1: Deterioration of kidney function within five weeks from when he first visited the urologist until hospitalization at Department od nephrologyand a kidney biopsy was performed.
eGFR: estimated glomerular filtration rate.

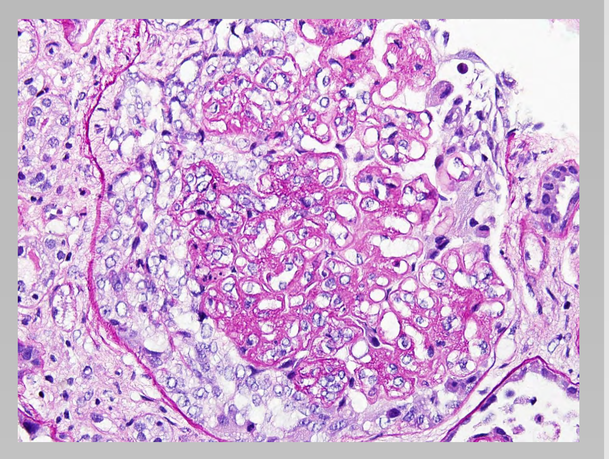
NEPHROLOGY EVALUATION

- After 4 weeks, July 28,2021, he was examined by a nephrologist for the first time and was transferred to the Department of Nephrology for suspected **nephrotic syndrome**. 24h-proteinuria was **17 grams**, initialy he was treated conservatively, with 20% human albumins, Henle loop diuretics, ACE inhibitors, statins, acetylsalicylic acid, corticosteroid boluses. Kidney biopsy was preformed (quoted in text) and because of the further deterioration of renal function he was given the first dose of 1 g **rituximab** (followed by second after two weeks). With a wide diagnostic examination, there were no signs of other specific renal, hematological or autoimmune disease. In the control lab. results there was further worsening of renal function – **urea 28,4 mmol/L, creatinine 866 umol/L and 24h proteinuria – 24 g**. It was agreed to start **renal replacement therapy with hemodialysis** September 2, 2021 via two-luminous tunneled central venous catheter type Hickman in the right internal jugular vein and after with AVF vascular access **without kidney function recovery**.

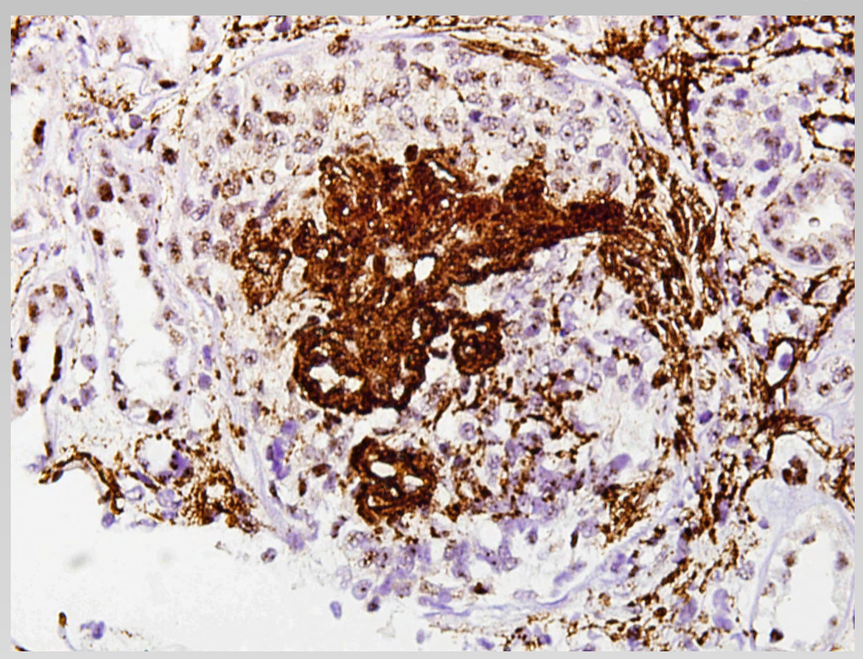
Kidney biopsy: at the level of **light and immunofluorescence microscopy**, the finding corresponds to **fibrillary glomerulonephritis**, most probably **primary** type, with cellular crescents in 24% of glomeruli, segmental scars with adhesions in 12% of glomeruli and 41% of completely connectively altered glomeruli, and interstitial fibrosis and tubular atrophy in 15% of parenchyma.



Glomerular mesangia with hypercellularity and deposition of PAS positive material. PAS stain, x400.

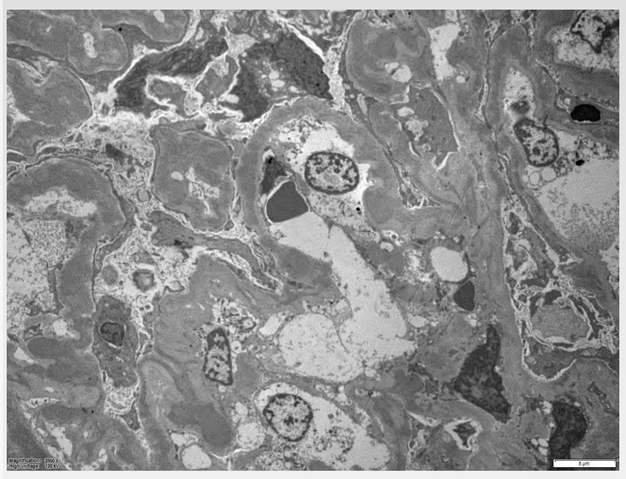


Glomerulus with cellular crescent, remodelling of glomerular basement membrane and widened mesangial.

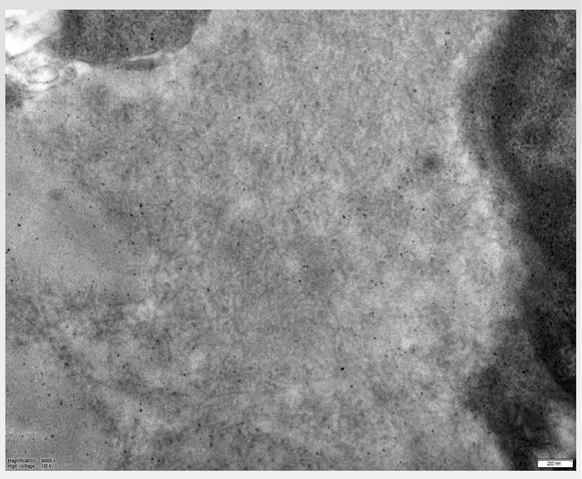


Positive staining for **DNAJB9** in glomerulus. Immunohistochemistry, x400

Electron microscopy



Abundant deposits in mesangial and glomerular basement membrane. TEM, x2500



Fibrillar ultrastructure of deposits. TEM x40000.

CONCLUSION

We present the first case of **crescentic FGN** after COVID-19 AstraZeneca vaccine. A strong temporal association between vaccination, elevated creatinine, and diffuse crescentic fibrillary process was found and confirmed on kidney biopsies. Further investigations will be needed to find the direct role of COVID-19 vaccination in crescentic glomerular diseases.

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Glomerular diameter measurements on light microscopy: A new parameter available to pathologists and its utility in IgA nephropathy.

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INTRODUCTION

- The utility of measuring glomerular diameter has been established in various renal diseases such as diabetes, FSGS etc. [1]
- Reduced nephron number and functional reserve, may cause glomerular enlargement, increasing susceptibility to renal injury.
- The progression of IgA Nephropathy, the most common cause of ESRD worldwide, is currently assessed using the Oxford MEST-C scores. [2]
- Other markers used to assess severity are serum creatinine and 24-hour urinary protein. [2]
- However, there are very few studies in literature assessing the predictive value of glomerular size in IgA Nephropathy (IgAN).

AIM

- To correlate the mean and maximum glomerular diameter (GD) with serum creatinine levels, 24-hour urinary protein and the Oxford MEST-C scores in patients with Ig A Nephropathy.

MATERIALS AND METHODS

- 100 biopsies, with ≥ 8 viable glomeruli, diagnosed as IgAN between 1st April 2022 to 1st January 2023, at Apollo Hospitals, Hyderabad were included.
- The serum creatinine, 24 hour urinary protein and Oxford scores at the time of biopsy were also collected.
- The glomeruli in 20 biopsies of acute tubular injury with no comorbid conditions, chronicity and with ≥ 10 glomeruli were taken as controls.
- The slides were scanned using the Philips Intellisite Pathology Solution- Ultra Fast Scanner.
- The glomeruli were annotated digitally for the measurements

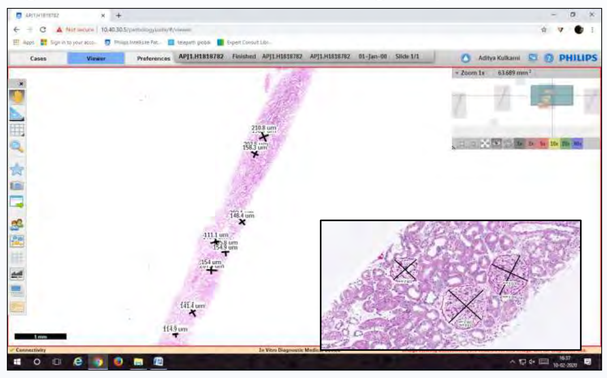

Fig. 1 Whole slide imaging (WSI) of a PAS stained section and digitally annotated glomeruli

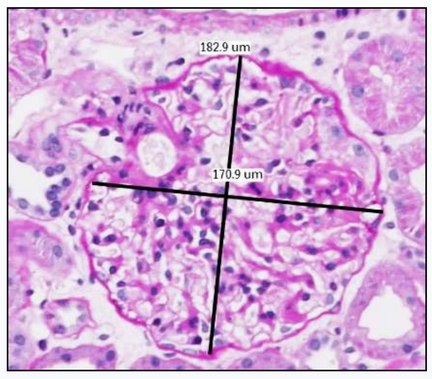

Fig. 2 Measurement of GDs

CALCULATION OF MEAN AND MAX GD

- The maximal diameter (which passes through the geometric centre of the maximal profile of the glomerulus) and the maximal chord perpendicular to it was recorded in each viable glomerulus on a PAS stain as per the guidelines by Tsuboi and Kataoka et al. [1,2]
- Mean GD was calculated as the mean of these measurements.
- Max GD was taken as the mean of the maximally hypertrophied glomerulus. [2]
- Sclerosed or ischemic glomeruli and glomeruli with <40% area visible were excluded. [1]
- The mean and max GD of the control and test biopsies was compared.
- The mean and max GD of the test cases were correlated with serum creatinine, 24 hour urinary protein and Oxford scores.

STATISTICAL ANALYSIS

The data was analyzed using MS Excel (R) office 365, GraphPad prism 8.4.2 and SPSS version 25. Descriptive statistics were presented in the form of proportions/percentages for categorical variables and mean & SD for continuous data variables. Correlation was done using the Spearman Rho/Pearson R correlation. P value of <0.05 was considered significant. A ROC analysis was done to analyze the role of mean and max GD in the assessment of E score.

RESULTS

Table 1. The mean and the max GD were similar for control and test groups

Mean GD	Control	Cases	P Value
No.	20	100	0.174
Mean	156.96	151.83	
Std Dev	15.00	28.70	

Max GD	Control	Case	P Value
No.	20	100	0.5279
Mean	198.79	205.4	
Std Dev	24.89	32.76	

Table 2. Main parameters analysed in test cases

Parameters	Mean (Min-Max)
Age in years	34.67 (11-64)
Male: Female	65:35
Serum Creatinine (mg/dl)	1.82 (0.44-9)
24 hour urinary protein (gm per day)	4.01 (0.13-54.90)
Oxford scores	
• M (0/1)	34/66
• E (0/1)	57/43
• S (0/1)	42/58
• T (0/1/2)	82/15/3
• C (0/1/2)	57/33/10
Number of glomeruli	15.47 (8-36)
Number of sclerosed glomeruli	2.74 (24-0)
Mean glomerular diameter (in µm)	**151.83** (99.9-270.67)
Maximum glomerular diameter (in µm)	**205.40** (140.6-297.8)

Tables 3&4:No significant correlation was found between the mean or max GD and the serum creatinine, 24 hour urinary protein, sclerosed glomeruli or Oxford scores

Mean GD – Corelation univariate analysis					
Parameters	Spearman rho	95% CI	P value	Significant?	n
Creatinine	-0.054	-0.25 to 0.15	0.5967	No	100
24 hour proteinuria	-0.085	-0.28 to 0.11	0.4023	No	100
M	-0.0088	-0.21 to 0.19	0.9309	No	100
E	0.2	-0.0058 to 0.39	0.05	No	100
S	0.19	-0.015 to 0.38	0.0619	No	100
T	-0.11	-0.31 to 0.090	0.258	No	100
C	-0.032	-0.23 to 0.17	0.7548	No	100
No. of sclerosed glomeruli	0.04	-0.16 to 0.23	0.6912	No	100

Max GD – Corelation univariate analysis					
Creatinine	0.079	-0.12 to 0.28	0.4335	No	100
24 hour proteinuria	0.015	-0.18 to 0.21	0.8831	No	100
M	-0.12	-0.32 to 0.083	0.2303	No	100
E	0.17	-0.032 to 0.36	0.0883	No	100
S	0.13	-0.072 to 0.33	0.1906	No	100
T	0.035	-0.17 to 0.24	0.7279	No	100
C	-0.04	-0.24 to 0.16	0.6933	No	100
No. of sclerosed glomeruli	0.091	-0.11 to 0.28	0.3678	No	100

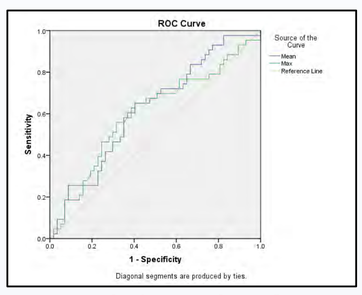

Fig 3. ROC analysis was done for E score as its p value approached significance levels

Tables 5&6: E Score and Mean/Max GD

Parameters for E score	Area under curve	Significant
Mean	0.621	0.038
Max	0.601	0.081

Cut off Value	Sensitivity	Specificity
110	97%	8%
150	61%	62%
180	18%	92%

DISCUSSION

- With the advent of whole slide imaging (WSI), fairly accurate GD measurements are now possible.
- However, age and race-specific normal values need to be established before using them for disease assessment. [3]

	Our study (n=100)	Kataoka et al (n=97) [2]	Tsuboi et al (n=18) [1]
Age (years)	34.67 years (11-64)	34 ± 12.6	30.6 (18-51)
24 hour proteinuria(g/day)	4.01 (0.13-54.90)	0.72 (0–4.20)	1.40 ± 1.3 (0.28–5.6)
Serum Creatinine (mg/dl)	1.82 (0.44-9)	0.90 ± 0.29	0.76 ± 0.16 (0.5–1.0)
No of glomeruli per patient	15.47 (8-36)	13 (5–46)	21.0 ± 10.1 (range 10–42)
Average Max GD (µm)	205.40 (140.6-297.8)	218.3 ± 27	Glomerular density and glomerular volume measured

Parameters	Our study	Kataoka et al [2]	Tsuboi et al [1]
Similar to our study:			
24 hour proteinuria	No correlation	No correlation with Max GD at the time of biopsy. However, significant association seen at a 10 year follow up	No correlation with glomerular density and the mean glomerular volume at presentation.
MEST-C scores	No correlation	No correlation between patients with Max GD <242 µm and >242 µm.	No correlation
No. of sclerosed glomeruli	No correlation	No correlation	No correlation
In contrast to our study:			
Serum creatinine	No correlation with mean or max GD at the time of biopsy	Patients whose Max GD was >242 µm had at least a 1.5-fold increase in their serum creatinine value at the 10-year follow-up examination	Glomerular density at presentation significantly correlated with creatinine clearance but mean glomerular area did not.

CONCLUSION

- In our study, the mean and max GD did not correlate with the serum creatinine, 24 hour urinary protein and the Oxford scores at the time of biopsy.
- At a cut off value of 150 µm for mean GD, there is a 61% sensitivity and 62 % specificity in predicting E1 score.
- Further studies with follow up are needed to determine the utility of GD measurements in IgA nephropathy.

Limitations: 1. Follow up biopsies were not taken to assess the utility of GD in progression of disease.
2. Other renal disorders affecting the glomerular diameter such as obesity and hypertension were not excluded.

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MEST-C SCORE ASSESSED IN IGA NEPHROPATHY AND IN HENOCH SCHOENLEIN PUPURA: IS THERE ANY DIFFERENCE?

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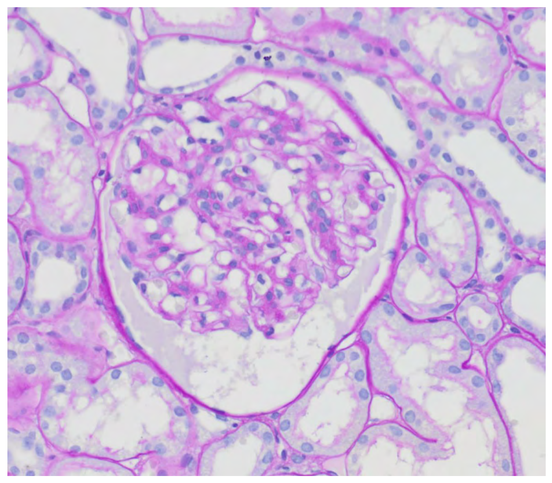
Aim: We conducted a retrospective study, in order to assess potential clinical and morphological differences (assessed by MEST-C score), between IgA nephropathy (IgAN) and Henoch Schoenlein purpura (HSP).

Methods: The study included patients with IgAN (n=67) and HSP (n=28) diagnosed at the Institute of Pathology, Medical Faculty, Belgrade, Serbia. Clinical patient's data (gender, age, hematuria, proteinuria and disease duration before biopsy) and pathohistological MEST-C score were analyzed and compared between IgAN and HSP.

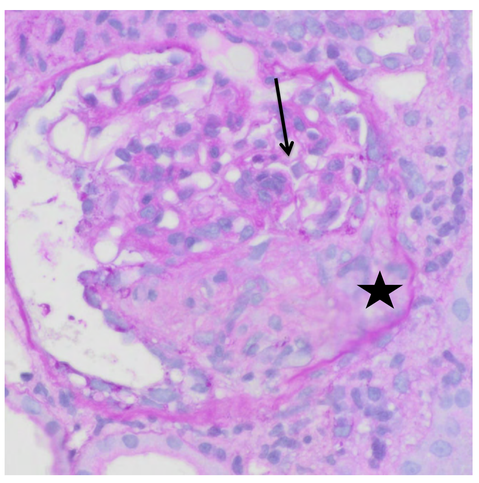
Results: Patients diagnosed with IgAN (33±17.9 years) were significantly older than those with HSP (15±10.3 years), p<0,001. The duration of symptoms and the frequencies of haematuria and proteinuria, were similar in both groups. On the other hand, significantly higher serum creatinine values were observed in HSP (356.5±1071.1 μmol/l) compared to IgAN (117.4±73.6 μmol/l). The majority of patients with IgAN had diffuse mesangial hypercellularity, and only 5 patients had less than 50% of glomeruli involved by this lesion in contrarily in HSP where all patients had diffuse glomerular mesangial hypercellularity. Endocapillary hypercellularity is observed in only 6 patients of IgAN, while significantly greater proportion of HSP revealed endocapillary hypercellularity (13/28). Chronic lesions, such as segmental glomerular sclerosis were detected in more than a half of IgAN cases (36/67), while only a quarter of HSP exhibited these changes (7/28). Nevertheless, tubular atropy and interstitial fibrosis were not observed in HSP, while only 13 patients out of 67 with IgAN had this type of lesion chronicity. Crescents formations were detected in only 3 HSP and in 15 IgAN patients.

***Table 1*. Clinical and pathohistological characteristics of patients with IgA nephropathy and Henoch-Schoenlein purpura**

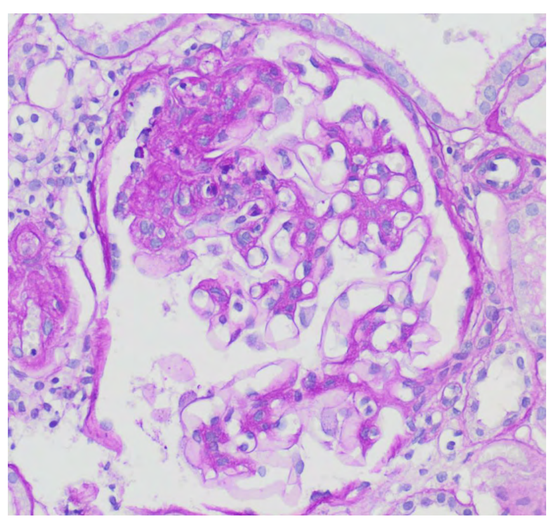
Clinical and pathohistological characteristics		Pathohistological diagnosis		P
		IgA nephropathy	Henoch-Schoenlein purpura	
Gender (%)	Male	45 (76.3%)	14 (23.7%)	p=0.116
	female	22 (61.1%)	14 (38.9%)	
Age (Average age ± SD)		33±17.9	15±10.3	p<0.001
Duration of symptoms		297± 515.36	344±1116.2	p<0.215
Proteinuria (g/24h)	Absent	9 (90.0%)	1 (10.0%)	p=0.987
	Subnephrotic proteinuria	46 (64.8)	25 (35.2%)	
	Nephrotic proteinuria	11 (84.6 %)	2 (15.4%)	
Hematuria	Absent	12 (75.0%)	4 (25.0%)	p=0.646
	Present	54 (69.2%)	24 (30.8%)	
Urea (mmol/l)		6.82±2.85	5.9±2.7	p=0.875
Creatinin (umol/l)		117.37±73.6	356.46±1071.1	p<0.001
Mesangial hypercellularity	<50%	5 (100.0%)	0 (0.0%)	p=0.140
	>50%	62 (68.9%)	28 (31.1%)	
Endocapillary hypercellularity	Absent	58(79.5%)	15(20.5%)	p=0.001
	Present	9(40.9%)	13(59.1%)	
Segmental sclerosis	Absent	31(59.6%)	21(40.4%)	p=0.010
	Present	36(83.7%)	7(16.3%)	
Tubular atrophy and interstitial fibrosis	<25%	51 (64.6%)	28 (35.4%)	p=0.005
	26% - 50%	13 (100%)	0 (0.0%)	
Crescent formation	Absent	52 (67.5%)	25 (32.5%)	p=0.186
	>1 cresent formation	15 (83.3%)	3 (16.7%)	



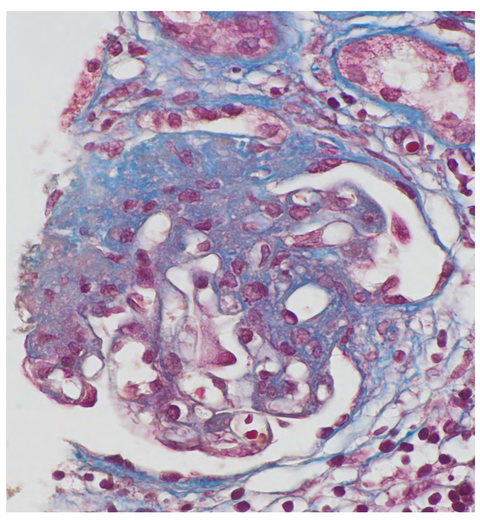
Microscopic image of mesangial hypercellularity, H&E stain



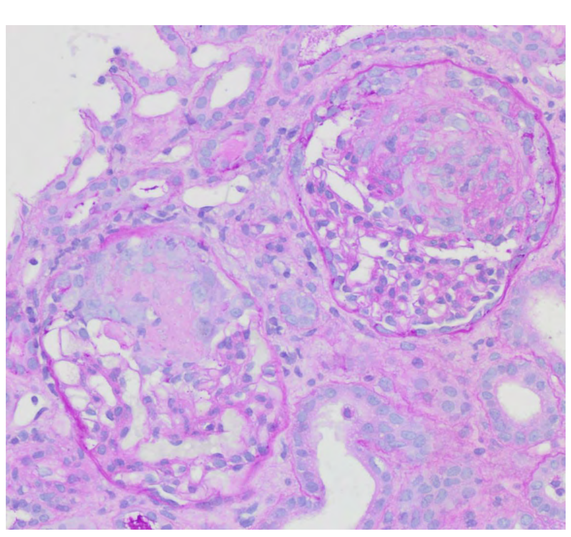
Microscopic image of endocapillary hypercellularity (arrow), and crescent formation (star), H&E stain



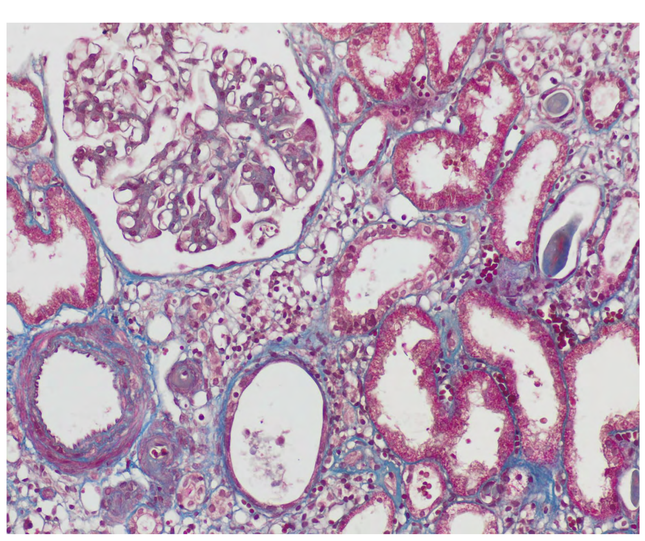
Microscopic image of segmental sclerosis, H&E stain



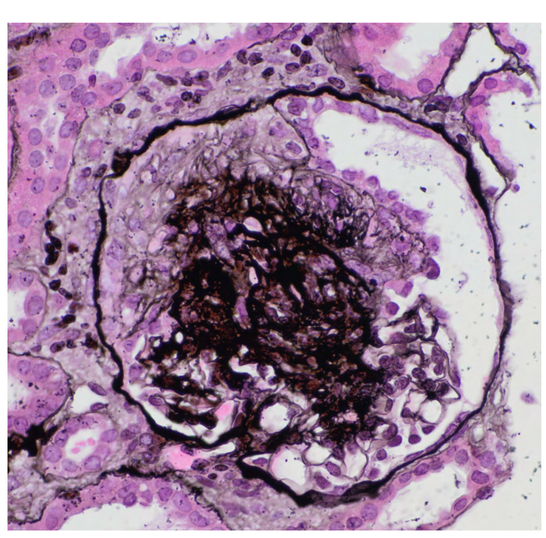
Microscopic image of segmental sclerosis, Masson Trichrome stain



Microscopic image of crescent formation, H&E stain



Microscopic image of hyalinosis of blood vessel, Masson Trichrome stain



Microscopic image of crescent formation, Jones methenamin silver stain

Conclusion:
Active glomerular lesions are characteristics of HSP, while chronic parameters are significantly observed in IgAN patients who are generally older than HSP group.

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Impact of mesangial C3 deposits on renal outcomes in IgA nephropaty

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INTRODUCTION

Activation of alternative complement pathway has recently emerged as important factor in the pathogenesis and clinical expression of IgA nephropathy (IgAN).

High serum IgA:C3 ratio, decreased serum C3 level and intensity of mesangial C3 deposition predict a worse renal outcome.

AIM

This study aimed to investigate clinical, pathological features, and prognosis of adult IgAN patients with mesangial C3 deposition.

METHOD

- retrospective observational single-center study
- patients with kidney biopsy and clinically confirmed primary IgAN from 2011 to 2021
- follow-up of at least 6 months
- C3 deposition was considered significant if the mesangial C3 immunofluorescence intensity was ≥2+
- renal outcome was the composite of a ≥30% decline in eGFR or end-stage renal disease

RESULTS

- *total of 40 pts, 65% male*
- *median follow-up 49 (25 -99) months*
- *65% mesangial C3+*
- *no difference between mesangial C3+ and C3- groups in:*
  1. *age*
  2. *systolic and diastolic blood pressure*
  3. *BMI*
  4. *serum urate*
  5. *IgA prediction tool score*
  6. *eGFR*
  7. *serum levels of C3*

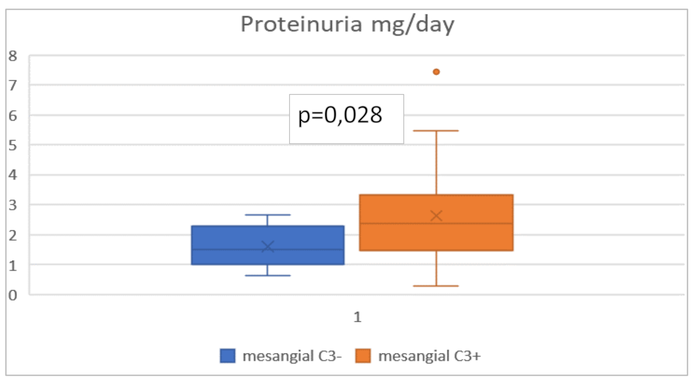

Fig.1 Patients with mesangial C3 deposition had higher initial level of proteinuria

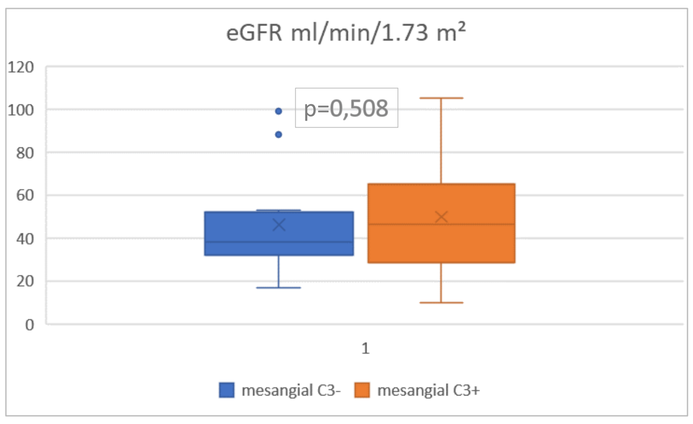

Fig.2 There was no difference in eGFR between groups

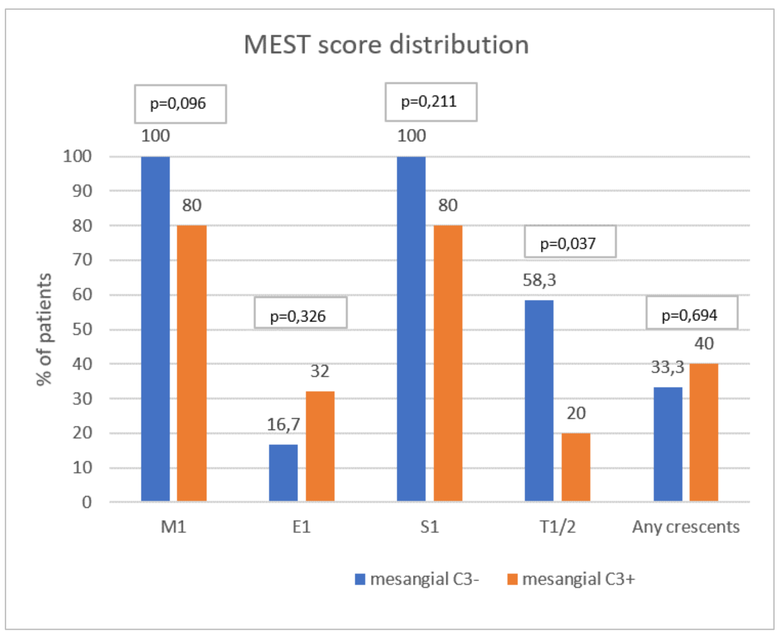

Fig.3 Patients with C3 deposits had higher T score, and no significant difference in M,E,S, and C score

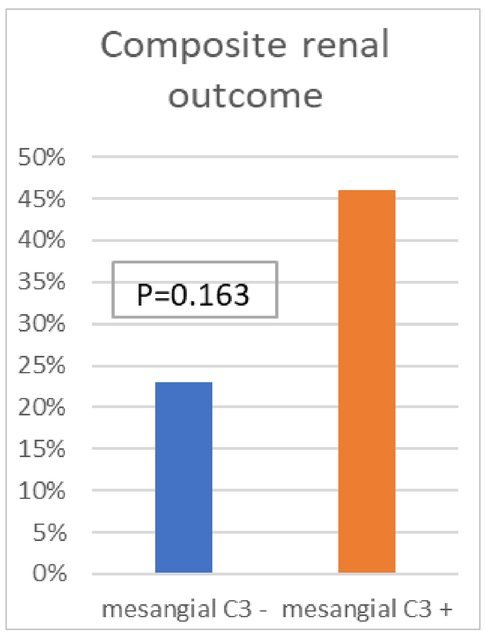

Fig.4 Although more patients with mesangial C3 deposits reached composite renal outcome (≥30% eGFR decline and/or ESRD), this difference was not statistically significant

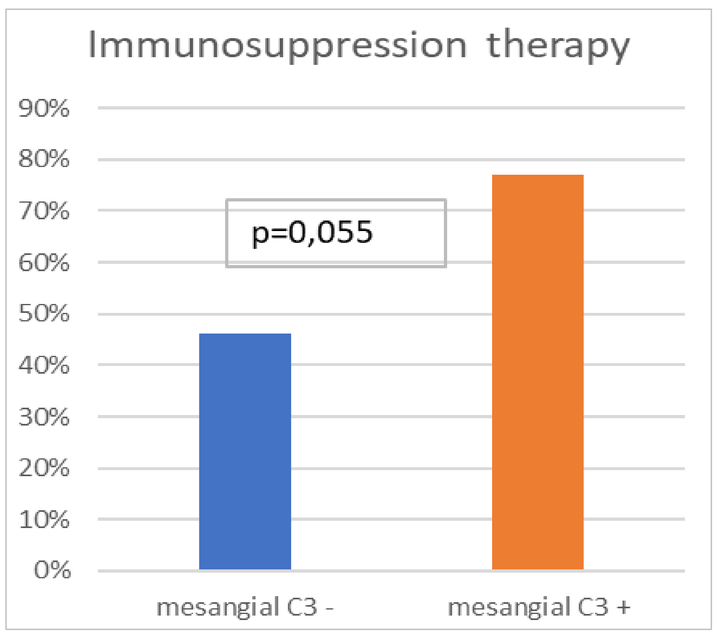

Fig.5 Patients with mesangial C3 deposits were more likely to be treated with immunosuppression therapy, probably due to higher initial proteinuria

This therapeutic intervention might have led to the similar renal survival rates between groups.

CONCLUSIONS

1. IgAN with mesangial C3 deposits clinically presents with higher proteinuria
2. There is a trend toward worse prognosis of IgAN patients with mesangial C3 deposits but larger patient cohort and longer duration of follow up is needed

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CASE REPORT: LUPUS-LIKE IMMUNE COMPLEX GN AFTER COVID-19 INFECTION.

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Introduction.

Acute kidney injury is frequently present in severe acute respiratory syndrome coronavirus 2 infection (coronavirus disease 2019 [COVID-19])! Lupus-like immune complex glomerulonephritis (GN) has been reported as an extremely rare complication of COVID-19 infection with only very few reported cases.[2,3]

Case Presentation.

We present a case report of a 67-year-old Asian male with a biopsy proven immune complex GN after COVID-19 virus infection. The patient had no documented medical history apart from a recent diagnosis of hypertension. He initially presented to the ER in January 2023 with cough, and shortness of breath with worsening pedal edema that had started in August 2022. The patient was diagnosed with COVID-19 infection following a positive SARS-CoV-2 PCR test. Initial lab investigations showed significant proteinuria concerning for nephrotic syndrome, low serum albumin and elevated creatinine with low GFR. The patients edema progressed into anasarca with ascites and a renal biopsy was taken due to worsening of renal function.

Pathologic Findings:

- Examination of H&E, PAS, HPS, Silver and Trichrome stained slides by light microscopy showed one core of renal cortex containing up to 30 glomeruli, three of which were globally sclerosed. The remaining glomeruli showed segmental increase in mesangial matrix and no increase in mesangial cellularity with no segmental scars. The glomerular capillaries were patent with areas of endocapillary hypercellularity and prominent endothelial cells. The glomerular capillary walls were diffusely thickened. The tubulointerstitium showed moderate-severe interstitial fibrosis and tubular atrophy with focal acute tubular injury. The vessels showed mild arteriolopathy and mild arterial sclerosis. (Figure1).
- Immunofluorescence microscopy showed an intense positive granular staining for IgG, IgM, IgA, C3, C1q, Kappa and Lambda with a membranous and mesangiocapillary pattern, "full-house" pattern, which is usually seen in lupus nephritis. (Figure2).
- Two glomeruli were examined ultrastructurally by electron microscopy showed numerous immune-type electron-dense deposits located at different levels of the glomerular basement membranes and to a lesser extent in para-mesangial, mesangial and subendothelial locations forming in several areas what is known as "tram-tracks". (Figure3).

Microscopic findings.

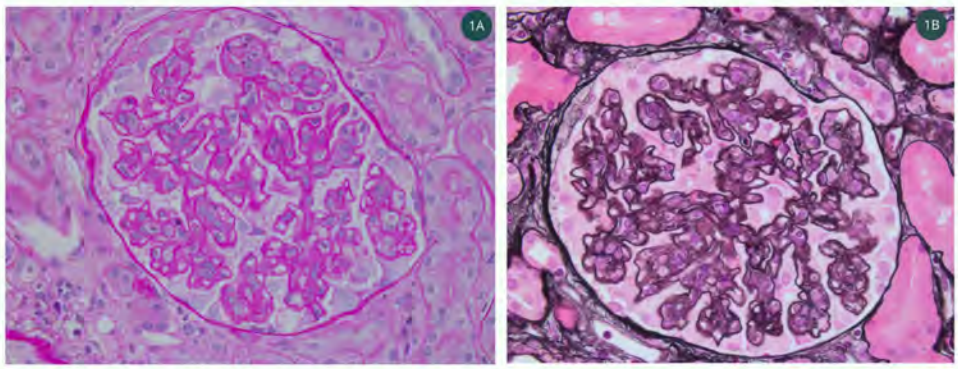

Figure1: Diffuse thickening of glomerular basement membranes with segmental double contours and focal endocapillary hypercellularity. 1A: PAS stain (40X). 1B: Silver stain (40X).

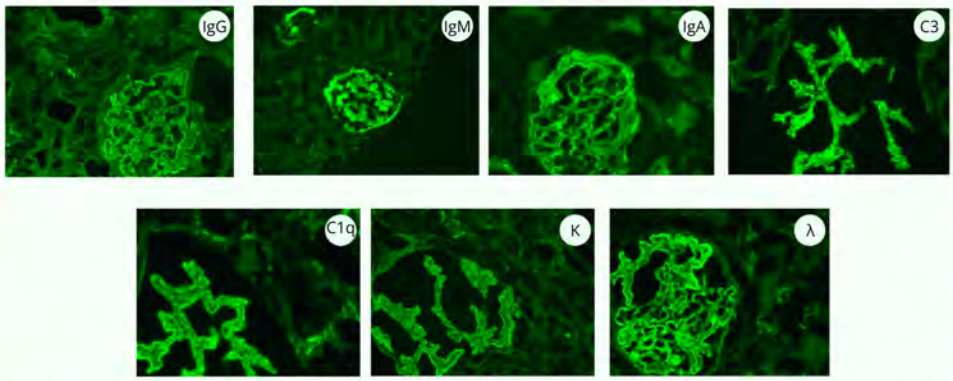

Figure2: There is intense positive granular staining for IgG, IgM, IgA, C3, C1q, Kappa and Lambda with a membranous and mesangiocapillary pattern, Deposits are also seen in peritubular and perivascular locations.

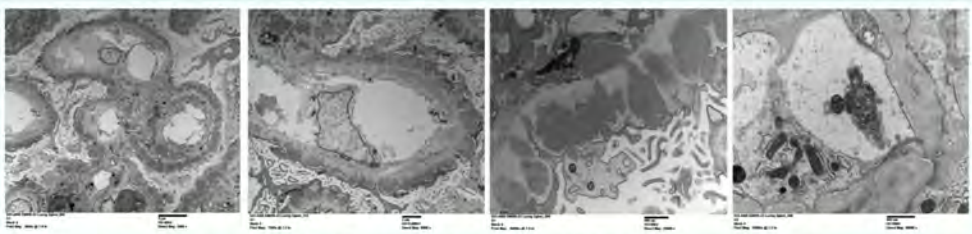

Figure3: Numerous immune-type electron-dense deposits located at different levels of the glomerular basement membranes and to a lesser extent in para-mesangial and subendothelial locations (1A,1B,1C) with areas of extensive remodeling and "tram-track" pattern ( ). Numerous tubuloreticular inclusions are seen in the endothelial cells of the glomerular capillaries (1D). The podocyte foot processes are diffusely effaced with areas of denudation and podocyte injury (1A, 1B, 1C). 1A: TEM, print mag: 3600x @ 7.0 in. 1B: TEM, print mag: 7200x @ 7.0 in. 1C: TEM, print mag: 30000x @ 7.0 in. 1D: TEM, print mag: 35000x @ 7.0 in.

Discussion.

There is limited research on the specific relationship between COVID-19 and SLE-like immune complex glomerulonephritis (ICGN) or flares of lupus nephritis.[2,3] However, there have been some reports suggesting that COVID-19 infection may trigger flares of SLE and lead to new-onset ICGN in some patients. SLE-like ICGN is a rare complication that occurs when immune complexes accumulate in the glomeruli of the kidneys, leading to inflammation and damage. Flares of lupus nephritis may also occur in some patients after COVID-19 infection.

In this patient, a new diagnosis of lupus nephritis, class IV/V, was eventually made after proper lab investigations given the fact that the patient fit the clinical criteria for the diagnosis of SLE, which raises the question weather the patient have had an undiagnosed SLE for a significant period of time prior to this presentation.

Conclusion.

This case report highlights the potential for immune complex GN or flares from previous conditions to occur in association with COVID-19 infection, even in patients without pre-existing renal disease. Clinicians should remain vigilant for the development of renal complications in COVID-19 patients and consider renal biopsy for those with unexplained proteinuria. Further research is needed to better understand the pathogenesis of immune complex GN in COVID-19 patients and develop effective preventive and therapeutic strategies.

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Glomerular C1q positivity is associated with the presence of IgG3 subclass in non-lupus immune-complex mediated glomerulonephritis

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BACKGROUND

Glomerular C1q positivity by immunofluorescence is typically a specific marker of lupus nephritis, but in recent years C1q-positivity has been observed, for example, in some forms of monoclonal gammopathy of renal significance (MGRS). Among IgG subclasses, C1q affinity is higher for IgG3 and IgG1 compared to IgG2 and IgG4[1]. The aim of this study was to assess the relationship between C1q positivity and IgG subclasses in non-lupus immune-complex (IC) mediated glomerular diseases.

METHODS

We retrospectively studied a cohort of 24 patients who underwent kidney biopsy between 2018 and 2022 and had IgG subclasses and C1q immunofluorescence (IF) available for review. The diagnostic conclusions and clinical data were retrieved from medical records. The correlation between C1q IF and the positivity of other reactants on immunofluorescence was assessed with Spearman rho coefficient.

RESULTS

There was a spectrum of glomerular diagnoses among the 24 patients (10 with membranous glomerulopathy, 3 with proliferative glomerulonephritis with monoclonal IgG deposits, 2 with membranoproliferative glomerulonephritis, 2 with IgA-dominant glomerulonephritis, 2 with IC-mediated glomerulonephritis (not otherwise specified), 4 with assorted diagnoses including: fibrillary glomerulonephritis, post-infectious glomerulonephritis, light chain deposition disease and membranous glomerulonephritis associated with IgA glomerulonephritis). Overall, C1q IF was positive (any intensity) in 10 (42%) of the cases. C1q on IF directly correlates with the presence of IgG3 subclass (rho 0.644, p=0.001), while showing inverse correlation with IgG1 and IgG4 subclasses (rho -0.426 and -0.543, p=0.043 and 0.007 respectively) (Figure 1). Principal component analysis suggests that the existence C1q/IgG3/IgM co-deposition constitutes a distinct entity from IgG1/IgG4/IgG2 deposition (Figure 2).

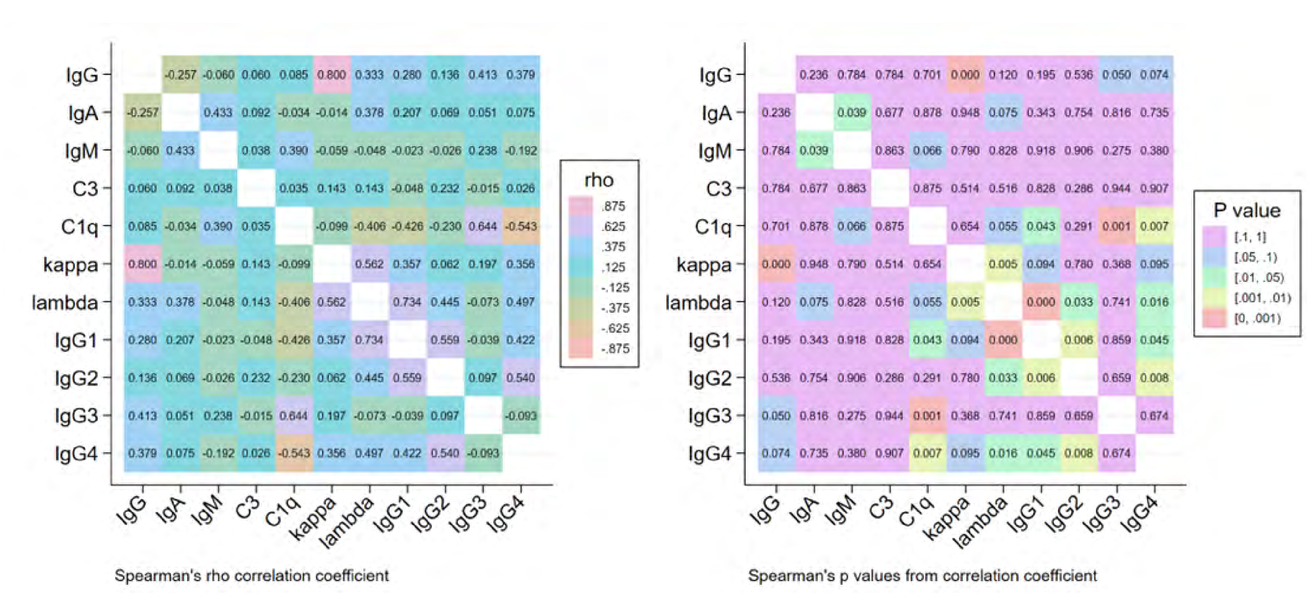

Figure 1. Spearman's rho correlation coefficient between different IF antisera positivity.

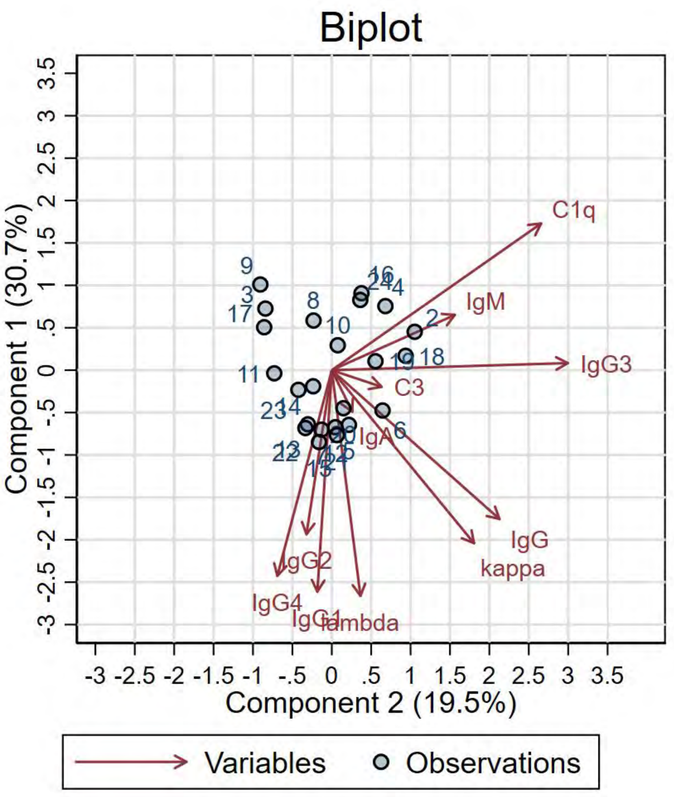

Figure 2. Principal component analysis. Dots represent each patients, arrows show different variables.

CONCLUSIONS

- Outside of lupus nephritis, C1q positivity should raise the possibility of IgG3 subclass presence.
- The clinical significance of IgG3 deposition in different kidney diseases is variable and not completely understood.

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5th IRPC
5th International Renal Pathology Conference
18th – 20th May 2023
Zagreb, Croatia

Mycophenolate mofetil or Cyclophosphamide in Colombian Caribbean patients with lupus nephritis: ¿Which is better?

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Objective

To compare oral MMF with monthly i.v. CyP as induction therapy for active biopsy proven Class III and IV LN

Methods

423 proliferative LN patients were retrospectively studied: MMF (2g/day for 6 months) vs CyP (500mg IV every 15 days for 3 months). ACR criteria assessed therapy response, with disease severity as a secondary endpoint. Renal survival was compared between groups using a Kaplan-Meier estimator.

Results

87% female, mean age 41.1±13.1 years. Proliferative class IV LN (66%). Baseline proteinuria and creatinine differed significantly between MMF and CyP groups ($p<0.05$) (Table 1). MMF showed complete remission in 77% vs 23% for CyP. No-Remission was 80% for MMF and 20% for CyP. Comparable renal survival ($p>0.05$) based on serum creatinine at first presentation (Figure 1).

Conclusion

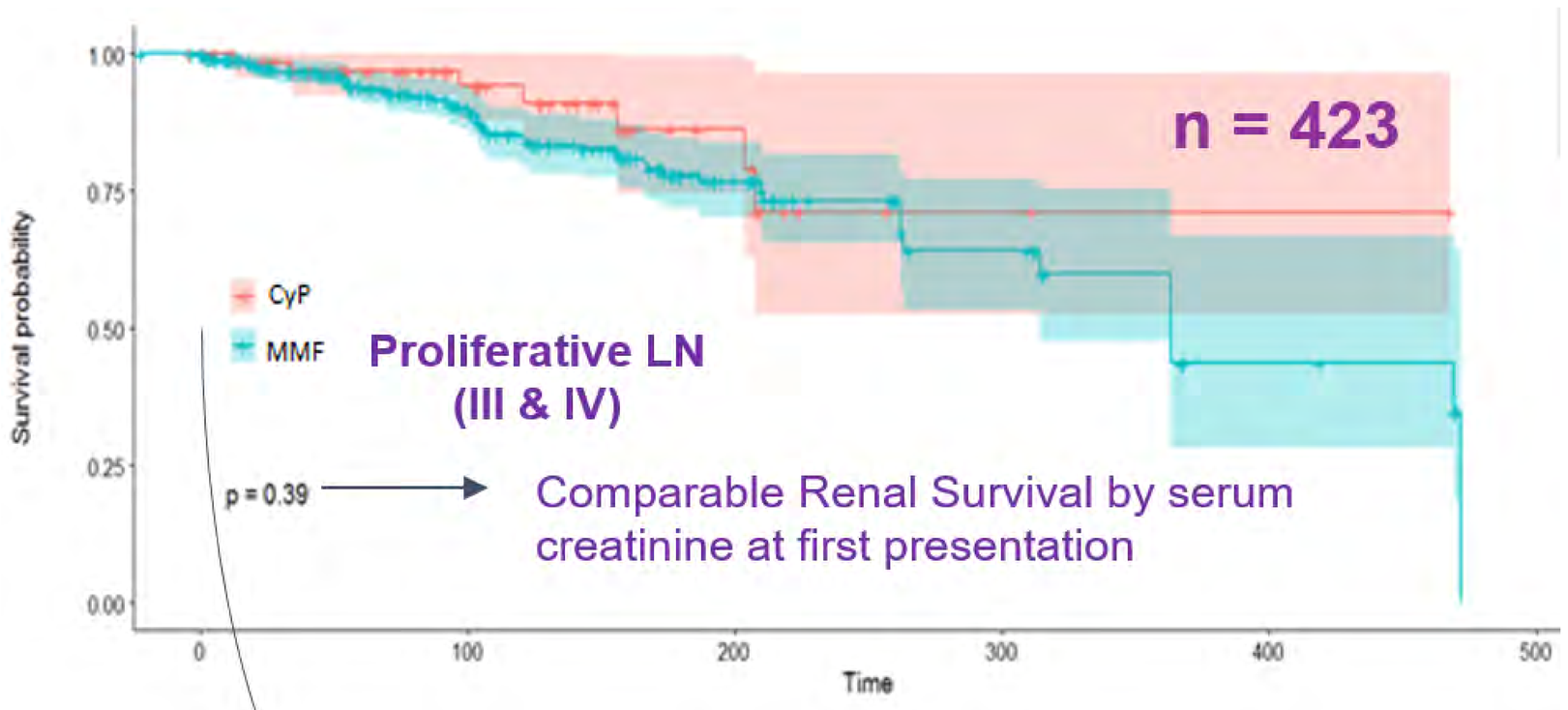
MMF and i.v. CyP showed comparable efficacy in inducing therapy for proliferative LN in our population. i.v. CyP has advantages of ensuring compliance and cost-effectiveness due to spaced doses. Further studies are needed to assess the impact of biologic therapies in this population.

Table 1. Baseline characteristics and treatment response of proliferative Lupus Nephritis Patients

Parameter		Induction Therapy		
		MMF (n=331)	CyP (n=92)	*P value*
Demographic Characteristics	Age, yrs (Mean, range)	37 (18-56)	34 (18-75)	0,6
	Female, (%)	292 (88)	78 (85)	0,09
	Male, (%)	39 (12)	14 (15)	
Disease Severity (Mean, Range)	Proteinuria (g/day)	1,4 (0,1-5,1)	2,1 (0,2-28))	0,001*
	Mean Serum Creatinine (mg/dl)	1,3 (0,4-12,6)	2 (0,5-8,3)	0,02*
	eGFR (ml/min/m2)	65,3 (5,7-75)	43,3 (5,7-64)	0,9
	C3 (mg/dl)	68,7 (17,6-204)	71,4 (21,5-146)	0,6
	C4 (mg/dl)	26 (3-55)	27,1 (8-49,9)	0,5
	Anti-dsDNA (IU/ml)	81,5 (30-129)	86,7 (31-129)	0,1
Treatment Response	Complete Remission, n, (%)	91 (27)	27 (29)	0,3
	Partial Remission, n, (%)	60 (20)	22 (24)	0,8
	No Remission, n, (%)	173 (52)	43 (47)	0,6

*LN: Lupus Nephritis; Yrs.: Years; MMF: Mycophenolate Mofetil; CYP: Cyclophosphamide; C3: C3 Complement; C4: C4 Complement; eGFR: Estimated glomerular filtration rate. *P <0,05. Chi-square test and Mann-Whitney U Test*

Figure 1: Cumulative Kaplan-Meier estimates for renal survival between
Mycophenolate Mofetil (MMF) vs Cyclophosphamide (CyP)



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PATHOLOGICAL DIAGNOSIS OF RENAL AMYLOIDOSIS IN UKRAINE: A SINGLE-CENTRE 10-YEAR DATA

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Introduction and Aim

Accurate typing of amyloid is critical for prognosis, treatment, and proper management of kidney amyloidosis. However, determining the correct type of amyloidosis is sometimes difficult because histopathologic findings may be confused with another related form. In the present study, 10-year pathology data on amyloidosis in adult kidney patients from a single center in Ukraine were analyzed.

Materials and Methods

Histopathologic findings of 231 amyloid-positive biopsies from buccal mucosa, gums, abdominal fat (**Fig. 1**) and kidney tissue (**Fig. 2**) collected between 2013 and 2022 were analyzed by amyloid type. Non-kidney biopsies were studied by bright light (alkaline Congo Red (also polarized microscopy), alkaline Sirius Red) and fluorescence (Thioflavin T, Congo Red), and immunofluorescence (κLC, λLC and AA). Kidney biopsies were assessed by light microscopy (H&E, PAS, PAMS, Trichrome, Picro-Sirius, alkaline Congo Red), immunofluorescence (IgA, IgG, IgM, κLC, λLC, C1q, C3c, fibrinogen, and AA) and electron microscopy (semithin and ultrathin (in part) sections). Data were expressed as means and standard deviations or as median and ranges and compared with ANOVA or Kruskal-Wallis tests. Categorical variables were expressed as proportions and compared using the chi-square (χ^2) test.

Table. The patients' clinical data and histopathological findings according to amyloidosis

	AA AMYLOIDOSIS	AL AMYLOIDOSIS	UNCLASSIFICATED TYPE (NON-AA AMYLOIDOSIS)	p-value
THE TOTAL NUMBER OF PATIENTS WITH BIOPSY-PROVEN AMYLOIDOSIS, n (%)	37 (16.0)	142 (61.5)	52 (22.5)	
KIDNEY BIOPSY, n (%)	18 (21)	50 (60)	16 (19)	
Age, years [median (range)]	45 (17-82)	58 (23-75)	55 (37-72)	**0.01**
Sex, male/female ratio	3.5:1	1:1.5	1.1:1	**0.02**
Hypertension, n (%)	3 (17)	8 (16)	4 (25)	0.71
Renal insufficiency, n (%)	8 (44)	15 (30)	8 (50)	0.27
Proteinuria, g/day [median (range)]	7.6 (3.5-20)	7.1 (1.2-22.4)	6.5 (1.9-23.7)	0.72
Serum Cr, μmol/L [median (range)]	119 (57-769)	98 (56-350)	119 (76-619)	0.08
eGFR, mL/min/1.73 m2 [median (range)]	56.5 (9-116)	60.5 (14-122)	48 (4-160)	0.13
Amyloid distribution, %				
glomerular compartment	100%	100%	100%	-
interstitial compartment	67%	68%	69%	0.99
tubular basement membrane	28%	10%	38%	**0.03**
vascular compartment	94%	86%	69%	0.12
Class (Sen&Sarcic, 2010), %				
I-III	39%	30%	31%	
IV	56%	70%	63%	
V-VI	6%	0	6%	0.44
Grade (Sen&Sarcic, 2010), %				
I	11%	18%	13%	
II	56%	64%	56%	
III	33%	18%	31%	0.64
Glomerular area Congo-positive, % [mean (SD)]	24.6 (15.7)	21.8 (9.6)	29.6 (10.2)	0.11
Cortical interstitial fibrosis area, % (Picro-Sirius method) [mean (SD)]	22.5 (10.1)	19.7 (5.6)	25.0 (8.6)	0,07
PAMS-positive amyloid, %	6%	30%	19%	0.11
Immunofluorescence (scale 0, trace (0.5+), 1-4+, frozen), positive cases, % (mean intensity):				
IgA	72% (1.2)	64% (0.9)	75% (0.9)	0.65
IgG	78% (1.3)	58% (0.9)	81% (1.0)	0.12
IgM	83% (1.2)	70% (1.0)	63% (0.9)	0.41
kappa {AL k}	100% (1.6)	62% (1.4) {100% (3.1) **AL k-12%**}	81% (1.1)	0.17
lambda {AL λ}	100% (2.0)	92% (3.1) {100% (3.6) **AL λ-88%**}	81% (1.2)	0.13
C1q	61% (0.8)	52% (0.8)	56% (0.6)	0.80
C3	67% (1.0)	66% (0.9)	75% (0.8)	0.79
fibrinogen	67% (0.8)	68% (0.7)	75% (0,7)	0.85
NON-KIDNEY BIOPSY, n (%)	19 (12.9)	92 (62.6)	36 (24.5)	
Age, years [median (range)]	48 (26-76)	61 (36-77)	56 (47-81)	**0.02**
Sex, male/female ratio	1:1	1:1	1:2.3	0.11
Amyloid distribution, %				
stromal compartment	79%	91%	81%	0.17
vascular compartment	43%	55%	34%	0.09
Immunofluorescence (scale 0-3+, paraffin), positive cases, % (mean intensity):				
kappa	32% (0.9)	46% (1.5) **{AL k-14%}**	53% (0,9)	0.33
lambda	37% (0.9)	90% (2.7) **{AL λ-86%}**	47% (0,8)	<0.001

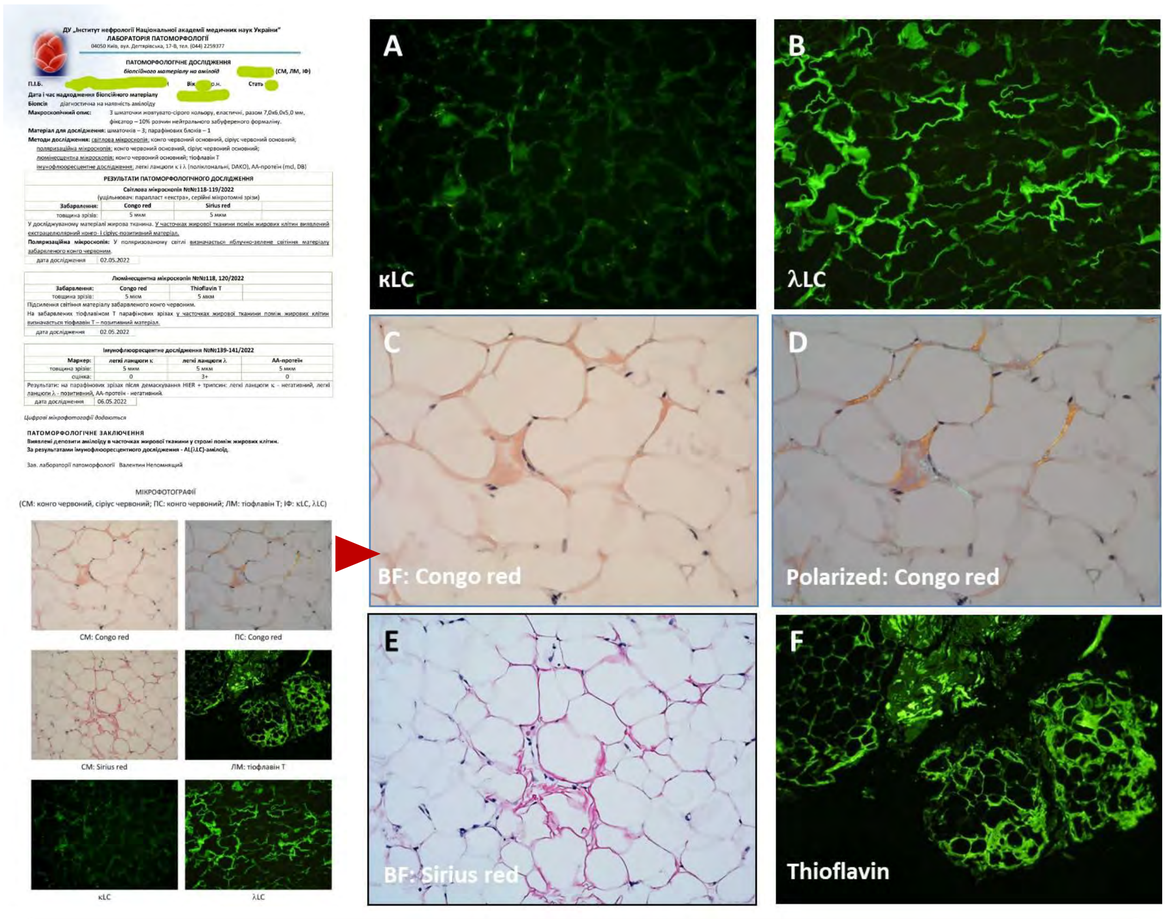


Fig. 1. The abdominal fat biopsy pathology report.
A, B – Immunofluorescence for κ and λ light chains, x200. C, D – Congo Red (bright and polarized field), x400. E – Sirius red, x200 . F – Thioflavin T, x 100.

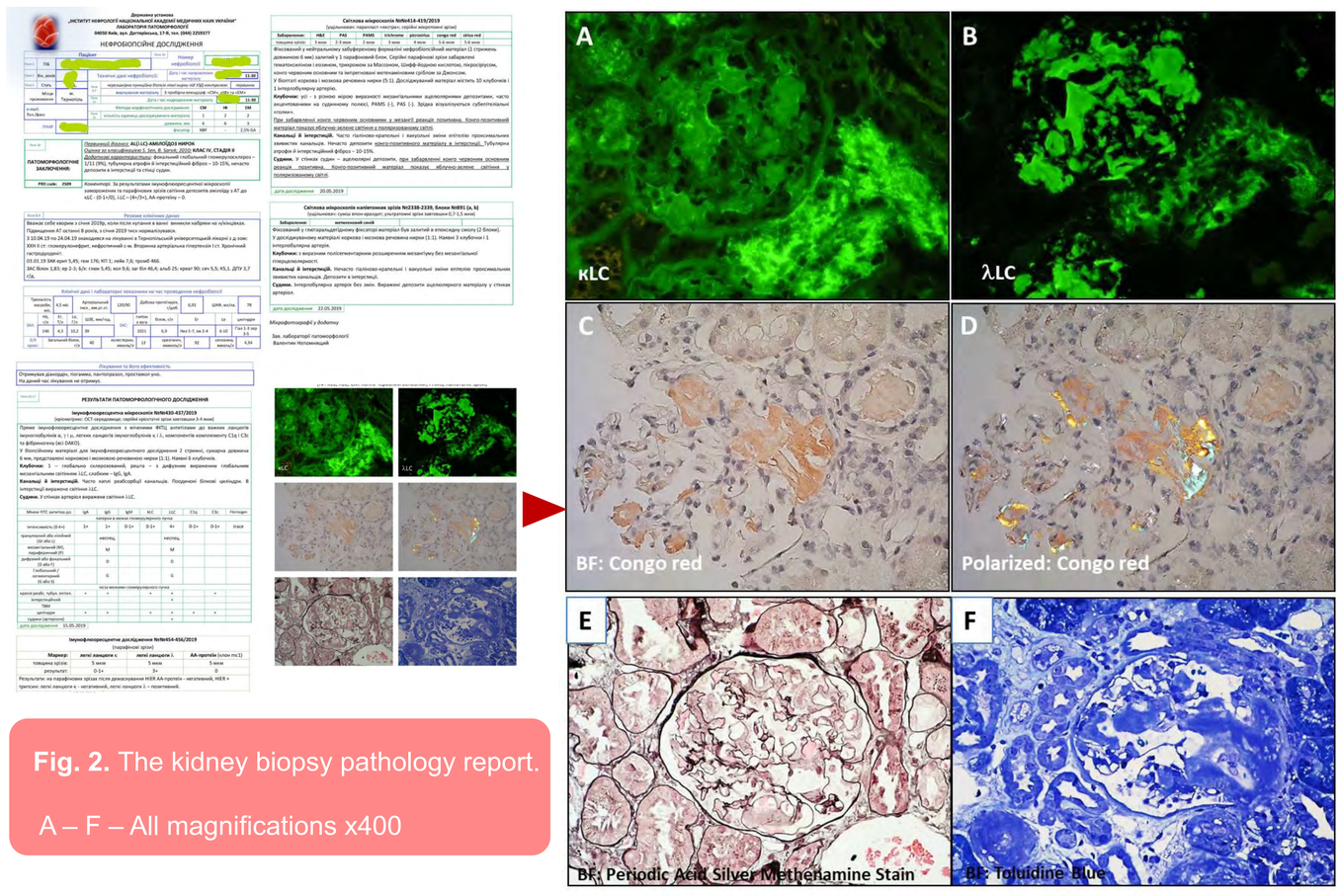


Fig. 2. The kidney biopsy pathology report.

A – F – All magnifications x400

Results

Of the 13477 primary nephrology patients, 1217 (9.1%) were indicated for kidney biopsy, and 424 (3.1%) patients with suspected systemic amyloidosis underwent non-kidney biopsy. Amyloidosis was detected by kidney biopsy in 84 (6.9%)/1217 patients and by non-kidney biopsy in 147 (35%)/424 patients. Among the patients with amyloidosis detected by nephrobiopsy, 79/84 (94%) had nephrotic syndrome and 26 (31%) had kidney failure; in 69/84 (82%) cases, the diagnosis was incidental. The patients' clinical data, typing results, and tissue distribution of amyloid are shown in Table.

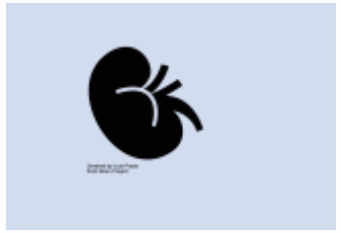
Conclusions

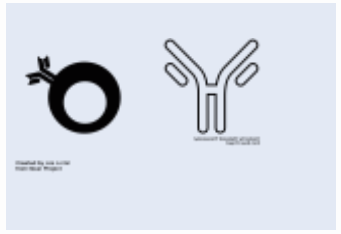
Our 10-year data show a relatively higher prevalence of amyloidosis in Ukrainian kidney patients compared with global data. Although amyloid typing remained unreliable in 22.5% of our patients, AL amyloidosis was the main form of kidney amyloidosis (61.5%). Improvement of amyloidosis diagnosis in Ukraine, especially differentiation between amyloid types, requires the introduction of modern proteomic analysis and genetic testing.

A case of MGRS reported in the Republic of Kosova: challenges in diagnosis, treatment and management

Case report

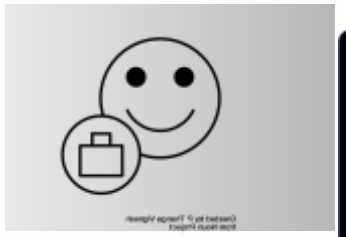
SHOQATA E NEFROLOGËVE TË KOSOVËS
KOSOVA SOCIETY OF NEPHROLOGY





A 62 year old male with proteinuria, hypoalbuminemia, ascites and renal failure.
Secondary glomerular disease was suspected.
SPEP, UPEP, immunofixation assays without any significance.
Bone marrow aspirate: less then 10% plasma cells.
Bone marrow biopsy: on IHC, increased staining for CD 20+ B Cells, CD 138 + cells.

Renal biopsy : features of both Immunotactoid glomerulopathy and Cryoglobulinemic glomerulonephritis.
MGRS was diagnosed.
Bortezomib based treatment protocol was started.
Renal response was achieved: serum urea and creatinine ↓, proteinuria ↓



SCAN ME

Godanci Kelmendi Vjollca[1] Kovaci Anita[1], Cavolli Viola[2]
UCCK University Clinical Centre of Kosova Nephrology and Hematlogy departments

CONCLUSION

How these renal lesions found on biopsy relate to a specific hematological disorder is yet unknown, however we want to raise awareness in diagosing and treating MGRS in order to prevent the progression of kidney failure.

AL/AH AMYLOIDOSIS – A CASE REPORT

Osmani Besa[1], Kasumović Dino[1], Lucijanić Marko[2], Strizić Ana[1], Šenjug Petar[3,4], Galešić Krešimir[1], Galešić Ljubanović Danica[3,4]

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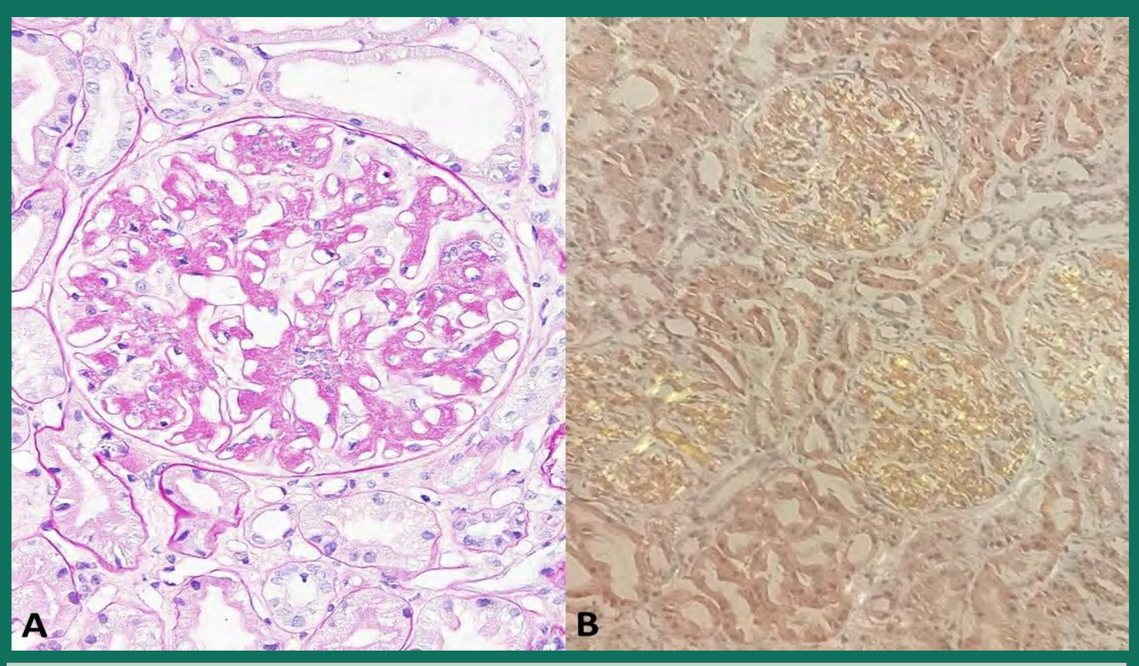
Introduction: *We present a case of a 74-year-old female patient, admitted to the hospital due to nephrotic syndrome with reduced renal function. She had a history of arterial hypertension with target organ damage (heart and kidney) and hyperlipidemia. A kidney biopsy was performed.*



On light microscopy the mesangial expansion (Figure 1A) with accumulation of the Congo Red positive material in glomeruli with birefringence under polarized light (Figure 1B) were found. Immunofluorescence was positive for IgG (3+), lambda chains (2+), C3 (1+) and weakly positive for IgM.

Figure 1. A) Mesangial expansion. PAS stain, original magnification x400. B) Birefringence under polarized light, Congo Red stain, x200.

Staining for the IgG subclasses showed only IgG2 positivity (Figure 2A). The electron microscopy showed deposition of fibrillar material (Figure 2B) with the average fibril's diameter of 8 nm. Pathology report concluded that according to available diagnostic methods this is a case of light and heavy chains renal amyloidosis caused by monoclonal IgG-lambda chains.

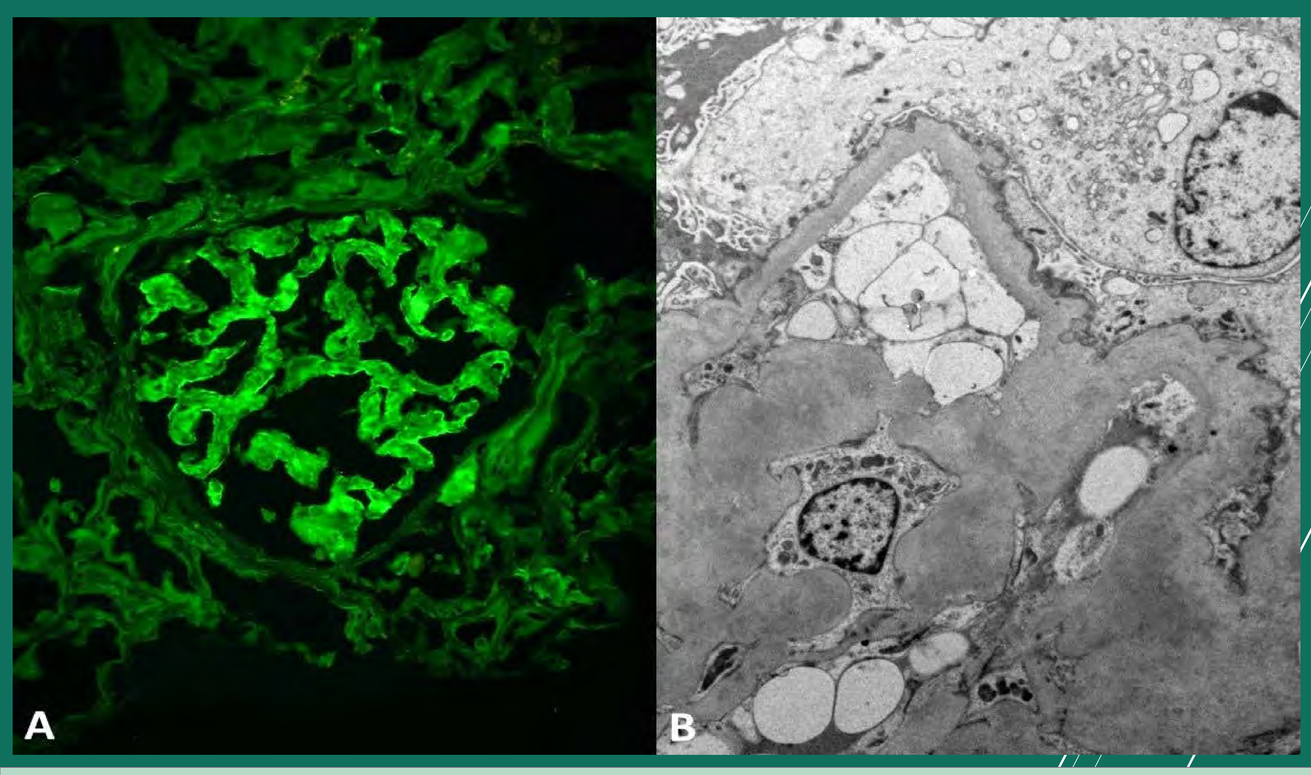


Figure 2. A) Positive IgG2, immunofluorescence on frozen section, original magnification x400. B) Fibrillar material in mesangium and basement membrane. TEM, original magnification x5000.

Additional tests were performed to find underlying hematologic disorder. Bone marrow fine needle aspiration showed more lush hematopoiesis without major morphological peculiarities. Immunofixation analysis showed IgG lambda with decreased kappa/lambda ratio. The patient was admitted to the Hematology department and started on VMP protocol and continued with her checkups via Hematology clinic where she received less than 4 cycles of chemotherapy. During that time a deterioration of her general condition was noted. Soon after, she died out of our institution, without a known cause of death.

The most common type of amyloidosis that affects the kidneys is AL amyloidosis. Here we presented a rare form of AL/AH amyloidosis with accumulation of both lambda light chains and IgG2 heavy chains.

DIAGNOSTIC CHALLENGE IN A YOUNG PATIENT WITH KIDNEY AMYLOIDOSIS

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Male patient at the age of *40 years*, was hospitalized due to nephrotic syndrome and anasarca. He has been *paraplegic* for the past 20 years.
Family history was negative for kidney diseases.
Initially he presented with deep vein thrombosis of the leg and *severe nephrotic syndrome* (proteinuria of 20 g/dU) was detected. A large *decubitus ulcer* of sacral region, which has been deteriorating for a past year, was also verified at that time.

Prior to kidney biopsy extensive diagnostic tests were performed, and *immunofixation* results showed positivity for *monoclonal IgG lambda*. Also, *7% of monoclonal plasma cells* in the bone marrow were found (expressing the CD38 + CD138 + CD19- s phenotype by monoclonal expression of lambda immunoglobulin light chains).

A **kidney biopsy** was performed and a diagnosis of *renal amyloidosis* was made (Figure 1).
Immunohistochemical positivity for AA was found, while staining for the kappa and lambda chains showed a polytypical reaction. Given the fact that laser microdissection and mass spectrometry is considered the most reliable method for amyloidosis typing, formalin paraffin embedded block with kidney specimen was sent to National Amyloidosis Centre in London for **mass spectrometry** to be performed. Amyloid deposits were present in glomeruli, tubules and vessels.
The proteomic analysis of the amyloid supported the result of the immunohistochemical staining, diagnosis of AA kidney amyloidosis remained.

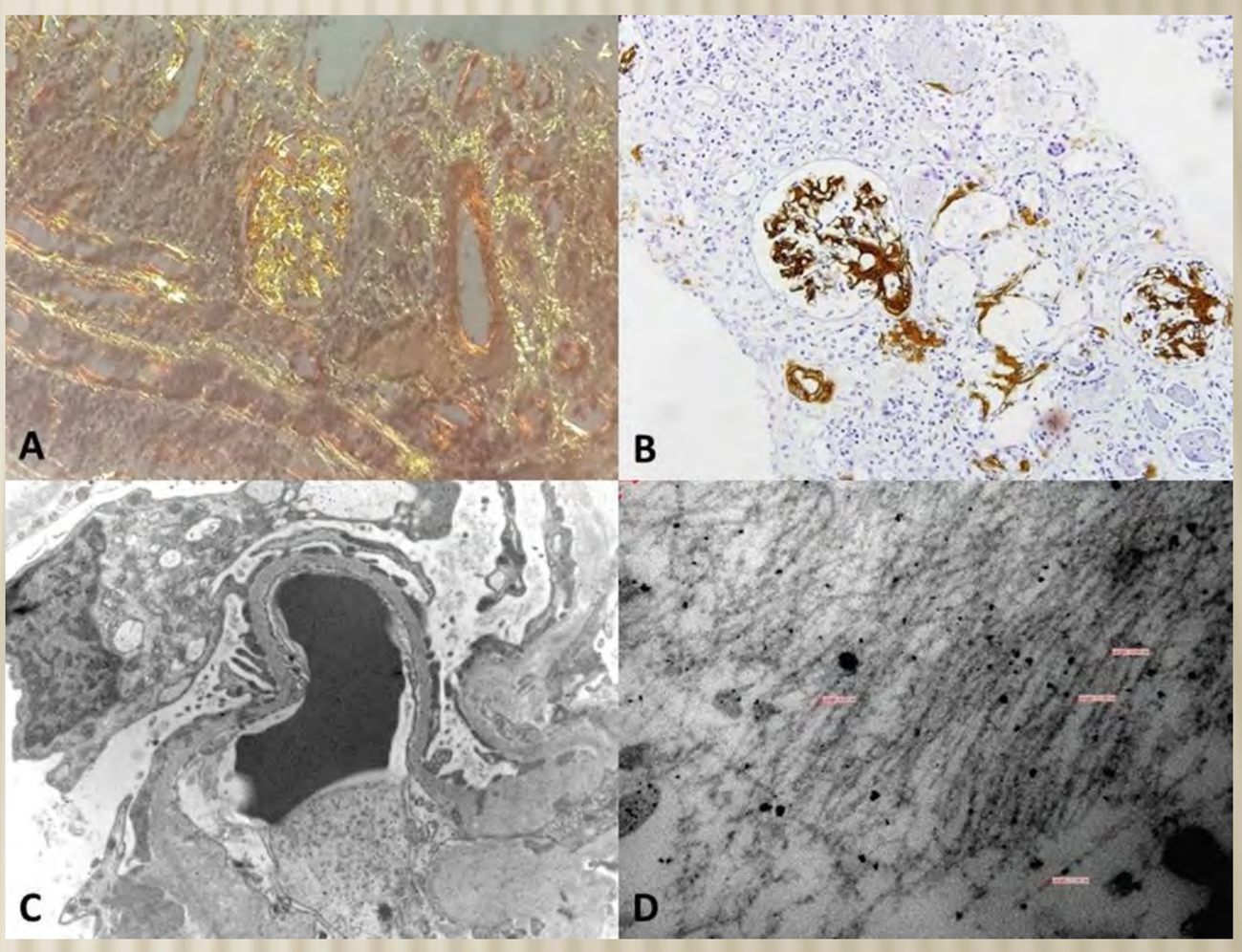

***Figure 1** A) Birefringence under polarized light, Congo Red stain, magnification x200. B) Positive AA, immunohistochemistry, magnification x 200. C) Amyloid in mesangium and basement membrane. TEM, original magnification x 10000. D) Amyloid fibrils measuring 10 nm in average. Fibrillar material in mesangium and basement membrane. TEM, original magnification x 100000*

Department of Nephropathology and Electron Microscopy, University Hospital Dubrava, Zagreb, Croatia

The concluding opinion was that there were no criteria for diagnosis of monoclonal gammopathy of renal significance. The main cause of *AA kidney amyloidosis* was probably a *chronic inflammation* derived from massive decubitus ulcer. Only option to prevent further kidney deterioration was to treat the underlying inflammatory disease.

New Frameshift Variant-associated Familial Hereditary Fibrinogen A-chainAmyloidosis.

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* These authors shared the second authorship of the work, and each made an equal contribution.

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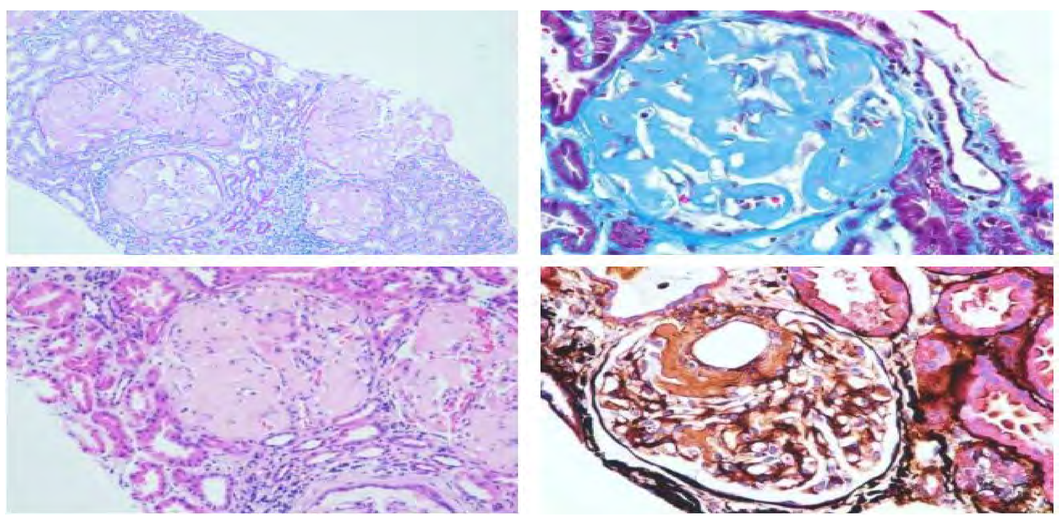
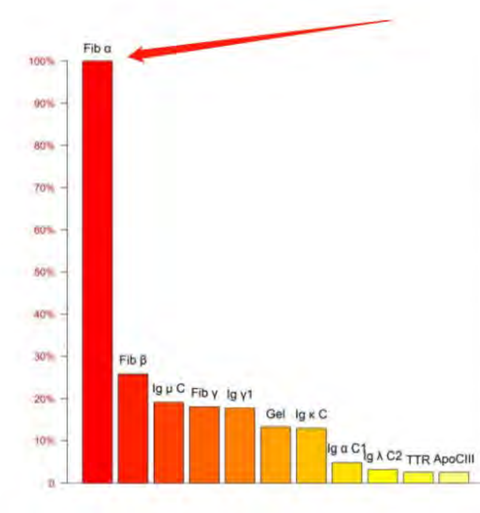
Abstract

Hereditary fibrinogen Aα-chain amyloidosis is a type of autosomal dominant systemic amyloidosis caused by mutations in the fibrinogen Aα-chain（AFib) gene and is typically accompanied by renal disease. Here, we describe a case of familial hereditary fibrinogen Aα-chain amyloidosis associated with a novel frameshift mutation in the fibrinogen Aa-chain gene. The patient is a 36-year-old Chinese woman presenting with proteinuria and edema in both lower limbs. Her edema of both lower limbs and foam urine persisted for 15 days, accompanied by a family history of early deaths from uremia in his uncle and second uncle. She was identified as having urinary protein cause (serum albumin, 31g/L, urinary protein 9.9g/day). Renal biopsy revealed that amyloid deposits in most of the glomeruli without interstitial or vascular involvement, and the amyloid depositions were selectively stained with fibrinogen-specific antibodies (Figure 1). As determined by mass spectrometry study, the relative abundance of Fibα is the highest among known typing proteins in renal tissue, raising the likelihood of hereditary fibrinogen amyloidosis (Figure 2). And in the FGA gene testing, this patient possessed a frameshift mutation (c.1673del) in FGA gene testing, which was a deletion-mutation to be reported in patients with Aα-chain amyloidosis (Figure 3). Therefore, to lessen urine protein leakage, we have mostly provided treatment including symptom assistance, dietary advice, and valsartan. With blood albumin levels of 36.5 g/L and urine protein intake of 6.01 g/day, the patient's symptoms considerably improved after six months of treatment. Up to now, this is the ninth case of "familial hereditary fibrinogen Aα-chain amyloidosis" reported in an Asian individual. The distinctive renal histology, mass spectrometry analysis and gene testing all provided significant clues to the diagnosis.



ure 1. Findings of renal biopsy in a Chinese Aα-chain amyloidosis patient at the age of 36 years. Renal biopsy shows: 1) Heavy amyloid deposits mainly in glomerulus; 2) The positive staining is observed in immunohistochemistry. 3) PASM-MASSON shows homogeneous amyloid deposits mainly in the mesangial area and capillary loops. 4) The results of immunofluorescence show that IgG (-), IgA (+/-), IgM (+), C1q (-), PLA2R (+), C3 (+), Alb (-), Fib (-). 5) The results of special staining: Congo red (+), Congo red oxide (+).



	Identified Proteins	#spectra
•	Apolipoprotein E	243
•	Apolipoprotein A-IV	121
•	Apolipoprotein A-I	39
•	Serum amyloid P-component	24
1	Fibrinogen alpha chain	287
2	Fibrinogen beta chain	74
3	Fibrinogen gamma chain	52
4	Immunoglobulin heavy constant mu	55
5	Immunoglobulin gamma-1 heavy chain	51
6	Gelsolin	38
7	Immunoglobulin kappa constant	37
8	Immunoglobulin heavy constant alpha 1	14
9	Immunoglobulin lambda constant 2	9
10	Apolipoprotein C-III	7
11	Transthyretin	7
12	Leukocyte cell-derived chemotaxin-2	0
13	Serum amyloid A-1 protein	0
14	Apolipoprotein A-II	0
15	Apolipoprotein C-II	0
16	Lysozyme C	0
17	Cystatin-C	0
18	Beta-2-microglobulin	0

Figure 2. Findings of mass spectrometric typing of renal amyloidosis in a Chinese Aα-chain amyloidosis patient at the age of 36 years. Among the currently known typing proteins, the relative abundance of Fibα is the highest, suggesting AFib type which is hereditary amyloidosis.

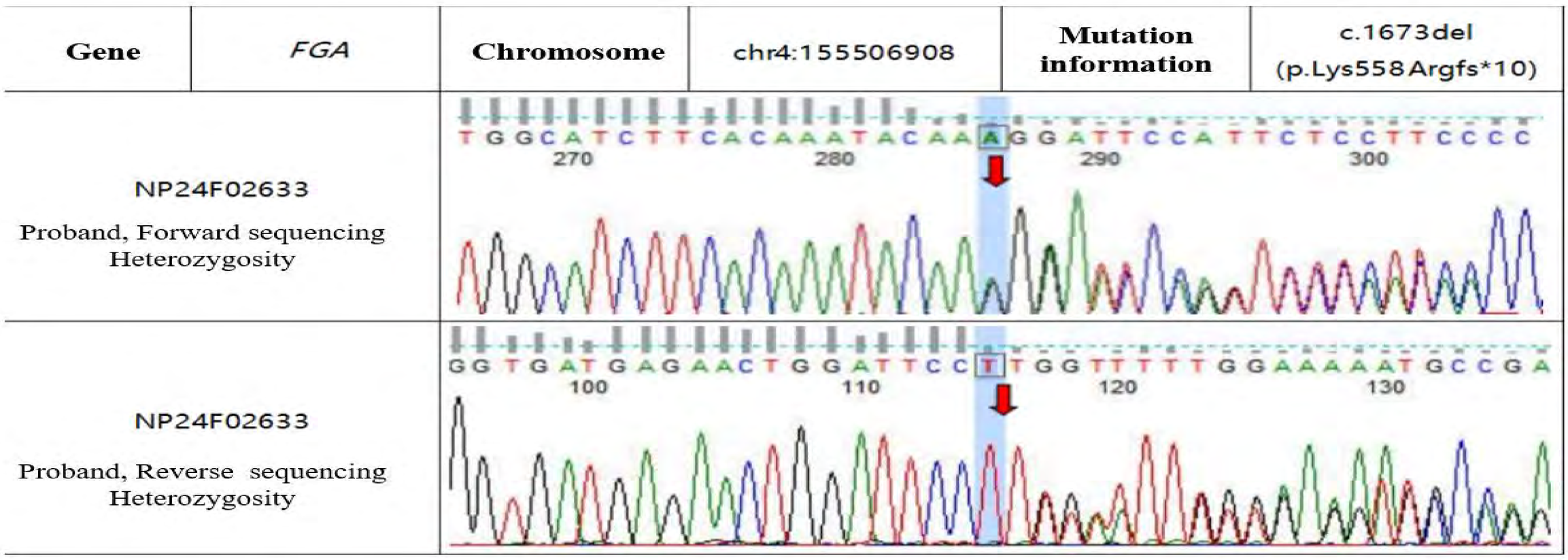


Figure 3. Findings of FGA gene sequence in a Chinese Aα-chain amyloidosis patient at the age of 36 years. In the coding region of FGA gene related to familial amyloidosis, c.1673del (p. Lys558Argfs × 10) carried by the patient was detected. This mutation is a frameshift mutation caused by base deletion in the coding region of FGA gene, which can lead to loss of protein function (through nonsense mediated mRNA degradation or early termination of coding amino acid sequence).

Profiling of membranous nephropathy based on immunohistochemical expression of antigens NELL-1, PlA2R, and THSD7A

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INTRODUCTION

- Membranous nephropathy (MN) is one of the commonest causes of nephrotic syndrome in adults
- It is an immune complex-mediated disease characterized by deposition of antigen-antibody immune complexes in subepithelium of glomerular basement membrane (GBM)
- MN has been characterized by deposition of M-type phospholipase A2 receptor (PLA2R) and Thrombospondin type - I Domain containing 7A (THSD7A)
- Recently, a novel antigen called Neural epidermal growth factor-like protein (NELL-1) was identified in PLA2R and THSD7A negative MN cases by mass spectrometry
- Data on PLA2R-related & THSD7A-related MN in Indian literature is limited
- Role of NELL-1 in MN has been described in few studies in world literature to date
- The present study aims to profile membranous nephropathy based on the expression of NELL-1, PLA2R & THSD7A immunohistochemically

MATERIALS & METHODS

- It was a prospective & retrospective observational study and included renal biopsies received in the Department of Pathology at AIIMS, Jodhpur over a period of 5 years
- Two cores were received for each case. One was fixed in formalin and processed for light microscopy evaluation & second core was received in normal saline and processed for immunofluorescence(IF) microscopy
- All the formalin-fixed paraffin-embedded tissue sections were stained with H&E, PAS, Trichrome, & Jones methenamine silver stains.
- IF was done against the antisera specific for IgG, IgA, IgM, Complements C3 & C1q, and Kappa and Lambda light chains
- Immunohistochemistry (IHC) for NELL-1, PLA2R, & THSD7A was put up on all cases diagnosed as MN on light and immunofluorescence
- Diffuse granular capillary wall staining of glomerular capillary walls was considered positive for all 3 stains

RESULTS

- Of the total 474 renal biopsies received, 50 cases were diagnosed as MN
- Of the 50 cases, 47 had adequate tissue for all 3 immunohistochemical stains and were included in the study
- Median age of patients with MN was 40years; Males=27, Females=20
- N=47, Primary MN=32 (68.08%), Secondary MN=15 (31.92%)
- One patient of primary MN exhibited triple antigen positivity, while there were no such cases in secondary MN
- 5 triple negative, 1 was primary and 4 were secondary MN
- 25/47 had only PLA2R positive, only NELL1 was positive in only 2 cases

Table 1: Co-morbidity status of patients with MN

Variables		n(%) [N=47]
Hypertension		12 (25.50)
Diabetes mellitus		3 (6.40)
Systemic Lupus Erythematosus		11 (23.40)
Hepatitis B infection		2 (4.30)
Malignancies		0 (0.0)
Dyslipidemia		33 (70.20)
Proteinuria	1^+	2 (4.30)
	2^+	10 (21.30)
	3^+	31 (66.0)
	4^+	4 (8.50)
Hematuria		12 (25.50)

Table 2: Light microscopy findings in primary vs. secondary MN

Variables	Primary MN [N=32] n (%)	Secondary MN [N=15] n (%)	χ^2 value	p value
Mesangial hypercellularity	4 (12.5)	10 (66.7)	14.3	<0.001
Endocapillary hypercellularity	0 (0.0)	7 (46.7)	-	-
Crescent formation	0 (0.0)	2 (13.3)	-	-
Focal segment glomerulosclerosis	17 (53.1)	7 (46.7)	0.75	0.686
Arteriosclerosis	18 (56.3)	8 (53.3)	0.03	0.851
Arteriolosclerosis	12 (37.5)	5 (33.3)	0.07	0.782

Empty boxes indicate that chi-square is not applicable

Table 3: Profiling of antigens in MN

	Primary MN [N=32] n (%)	Secondary MN [N=15] n (%)	Total [N=47] n (%)
Single antigen positives			
Only NELL 1	1 (3.12)	1 (6.67)	2 (4.24)
Only PLA2R	19 (59.38)	6 (40.0)	25 (53.19)
Only THSD7A	1 (3.12)	1 (6.67)	2 (4.24)
Double antigen positives			
NELL1 and PLA2R	7 (21.89)	2 (13.32)	9 (19.14)
NELL1 and THSD7A	0 (0.0)	1 (6.67)	1 (2.12)
PLA2R and THSD7A	2 (6.25)	0 (0.0)	2 (4.24)
Triple antigen positive	1 (3.12)	0 (0.0)	1 (2.12)
Triple antigen negative	1 (3.12)	4 (26.67)	5 (10.63)

Table 4: IHC expression of antigens in primary vs. secondary MN

Antigens	Primary MN [N=32] n (%)	Secondary MN [N=15] n (%)	Total [N=47] N (%)	χ^2 value	p value
NELL 1	9 (28.1)	4 (25.7)	13 (27.65)	0.011	1.000
PLA2R	29 (90.6)	8 (53.3)	37 (78.72)	8.48	0.007
THSD7A	4 (12.5)	2 (13.3)	6 (14.89)	0.006	1.000

Fischer's-Exact test

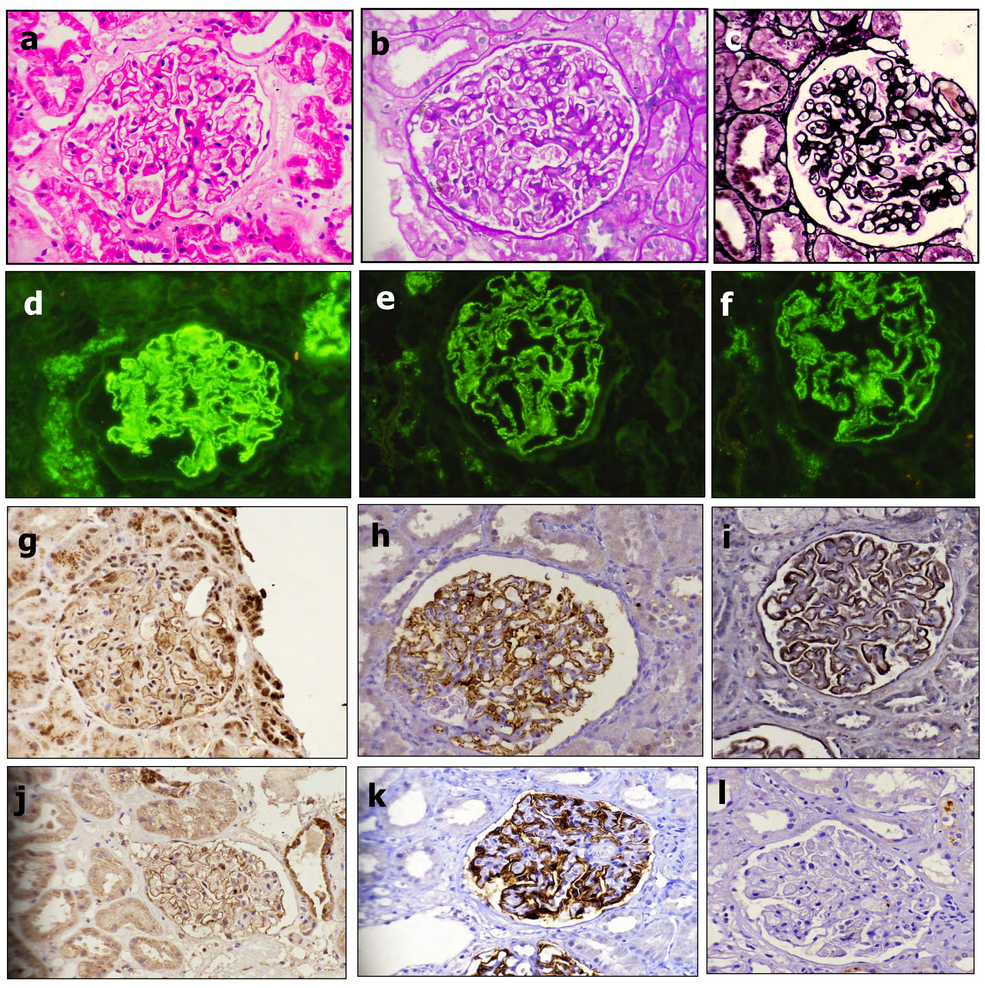

Figure1 a& b)uniform thickening of the glomerular capillary loop; H&E x400 & PAS x400 c) JMS showing craters & spikes x400, d-f) Intense capillary wall granular staining on IF with IgG, kappa, & lambda; x200 g-i) Bright capillary wall granular staining with IHC for NELL-1 (g, x400), PLA2R (h, x400), & THSd7a(I, x400)

DISCUSSION

- MN is broadly classified as primary and secondary depending on the cause
- Primary membranous nephropathy earlier considered to be idiopathic has now been characterized by deposition of PLA2R & THSD7A which accounts for approximately 75% and 5% of cases respectively
- NELL-1 is a gene that is strongly expressed in neural tissue encoding a protein with EGF- like repeats
- In kidney, tubules have highest expression of NELL-1
- Globally, many studies have shown that primary & secondary MN account for approximately 5-80% & 20-25% of cases respectively
- Many studies debate on strong association of malignancies with secondary MN; no malignancies were observed in the present study
- Caza et al have suggested NELL-1 as target antigen in malignancy-associated MN, No association with malignancy was seen in the NELL-1-positive MN cases in the present study
- 24-hour urinary protein levels were found to be significantly higher among patients with PLA2R positivity, similar result was seen by Subramanian P et al and Gudipati A et al
- PLA2R antigen was most commonly expressed in primary MN similar to studies by Beck et al
- Most studies suggest that NELL-1 is more commonly expressed among PLA2R-negative cases
- Wang G et al in 2021 showed t expression of NELL-1 with PLA2 in 1 case in their study
- Similarly present study showed NELL-1 & PLA2R positivity in 9 cases & NELL-1 &THSD7A positivity in 1 case
- It is believed that PLA2R & THSD7A are expressed together due to a common antigenic motif in N terminal region in these antigens that activates B cells to produce antibodies
- These antibodies may be directed against both antigens or any one of the two
- Recent studies have shown that these antigens are expressed even in secondary MN, which is also affirmed by the findings in the present study

CONCLUSION

- Among the cases, majority showed PLA2R positivity exclusively
- Expression of NELL-1 was more common than THSD7A in the study group
- Double antigen positive were found among a quarter of patients in the study
- No definite association of NELL-1 positivity and malignancy was seen in the study
- PLA2R although considered marker for primary MN was also seen in cases of secondary MN in the present study
- Lack of statistical associations in present study cannot be considered conclusive, given the smaller sample size

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PREVALENCE, CLINICAL FEATURES AND TARGET ANTIGENS ASSOCIATED WITH MEMBRANOUS NEPHROPATHY IN CHILDREN IN INDIA

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AIM

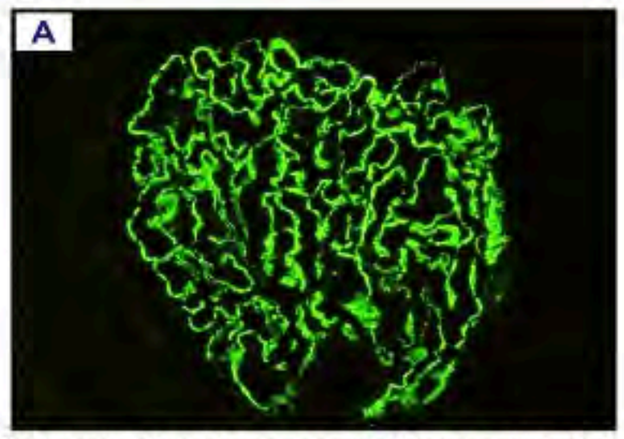
- Membranous nephropathy (MN) is rare in children
- While MN in adults is well characterised on the basis of the target antigens, the antigenic distribution of pediatric MN is largely unknown
- We report the prevalence, clinical features and target antigens associated with MN in children in our population

METHODS

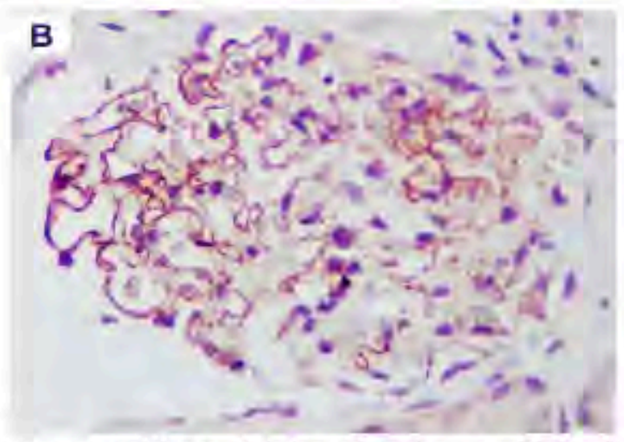
- Of the 17532 native kidney biopsies reported between January 2018 to December 2022, there were 2177 biopsies (12.42%) with the diagnosis of MN
- Eighty two (3.77%) of the MN biopsies were in children (<18 years)
- Tissue was available for immunostaining for PLA2R, NELL-1, Semaphorin-3B and Exostosin-1 in 70 biopsies

RESULTS

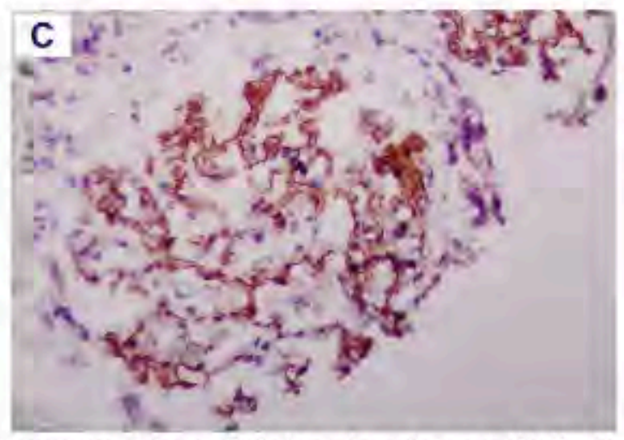
- The eighty two children included 29 boys and 53 girls
- Patients ranged from 2–18yrs of age
- Nephrotic-range proteinuria (uPCR >3.5) was found in 62%
- Mean creatinine was 0.76mg/dl
- Co-morbid conditions were uncommon, with one patient having diabetes mellitus and two with hypertension
- No patients had a known history of SLE or other autoimmune disease
- Hepatitis B infection which is considered as a cause for MN in children was not identified in our cohort
- Five of six patients with NELL1 positive MN had a history of traditional indigenous medicine use, which are known to contain mercury, and the remaining patient had a history of use of skin whitening cream contaminated with high levels of mercury



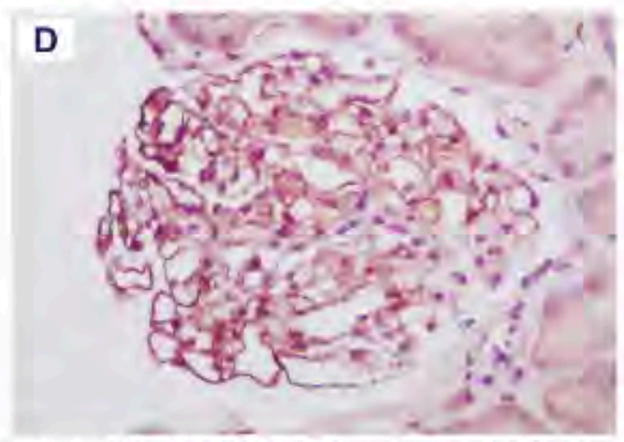
A) Paraffin immunofluorescence showing positivity for PLA2R



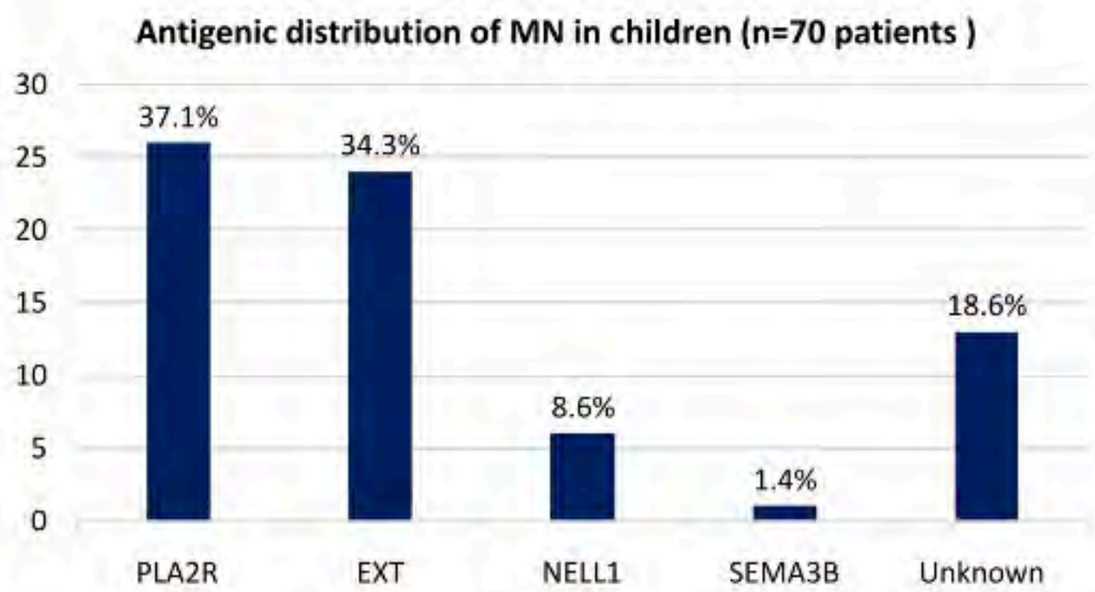
B) Immunoperoxidase staining showing positivity for EXT1



C) Immunoperoxidase staining showing positivity for NELL1



D) Immunoperoxidase staining showing positivity for SEMA3B



Characteristics of children with MN by antigen type (n=70 patients)

Antigen	Frequency N (%)	Mean Age (years)	Male	Female	Mean proteinuria (g)	Mean Creatinine (mg/dl)
PLA2R	26 (37.1)	15.8	12	14	7.774545	0.780476
EXT1	24 (34.3)	14.7	5	19	10.03462	0.796842
NELL1	6 (8.6)	15	0	6	3.34	0.68
SEMA 3B	1 (1.4)	6	1	0	8.8	0.4
Unknown	13 (18.6)	13.9	6	7	6.2	0.685

CONCLUSION

- Of the 2177 cases of MN reported during the study period, 82 (3.77%) were in children
- The majority of pediatric MN occurred during adolescence
- Renal function was preserved for patients of all antigen types
- The majority of patients presented with nephrotic range proteinuria
- There was significant female predominance in EXT1 MN and NELL1 MN
- NELL1 MN was associated with mercury toxicity
- Semaphorin-3B MN was rare in our pediatric population
- There was a lower frequency of PLA2R positivity, increased EXT1 positivity, and an increase in the proportion of cases in which the causative antigen remained unknown compared to that reported in adults
- Further work is needed to understand whether EXT1/2 positivity precedes later development of autoimmune disease in children

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Focal segmental glomerulosclerosis in adults related to low birth weight – case series

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Aim

Lower nephron endowment due to low-birth weight (LBW), prematurity (PM) or perinatal complications like sepsis or asphyxia is well-known risk factor for development of chronic kidney disease (CKD) and arterial hypertension (AH) in later life.[1] We aimed to investigate clinical and pathohistological characteristics of adult patients with focal segmental glomerulosclerosis (FSGS) presumed to be related to LBW or PM.

Methods

Patients with diagnosis consistent with secondary FSGS were assessed for underlying condition related to FSGS. Patients were recruited from Registry of kidney biopsies (Department of Nephrology and Dialysis, Dubrava University Hospital, Zagreb) in the period from 2010 to 2021. The birth weight ≤ 2500 and ≤ 1500 g was considered as LBW and very LBV, respectively. PM was defined as delivery before 37th gestational week. Clinical, laboratory and pathohistological characteristics of patients were analyzed and results are shown descriptively.

Results

Of 180 patients with histologic findings consistent with secondary FSGS, we identified 5 patients with LBW and PM, two patients with very LBW (P1 and P7) and one patient (P3) born in 32nd week of gestation (birth weight 2800 g). Table 1 summarizes relevant clinical, laboratory and histopathological data.

All analyzed patients presented with arterial hypertension, hyperlipidemia and isolated proteinuria (in absence of other elements of nephrotic syndrome) without erythrocyturia. Seven patients had perihilar variant of FSGS (Figure 1) and one (P1) had classic (NOS) variant. All patients had at least nodular arteriolar hyalinosis and three of them (P1, P6, P7) hyalinosis in full arteriolar circumference. Majority of patients were overweight or obese. There were no immune deposits on electron microscopy, glomerular basement membrane had normal thickness and podocyte foot processes were almost preserved.

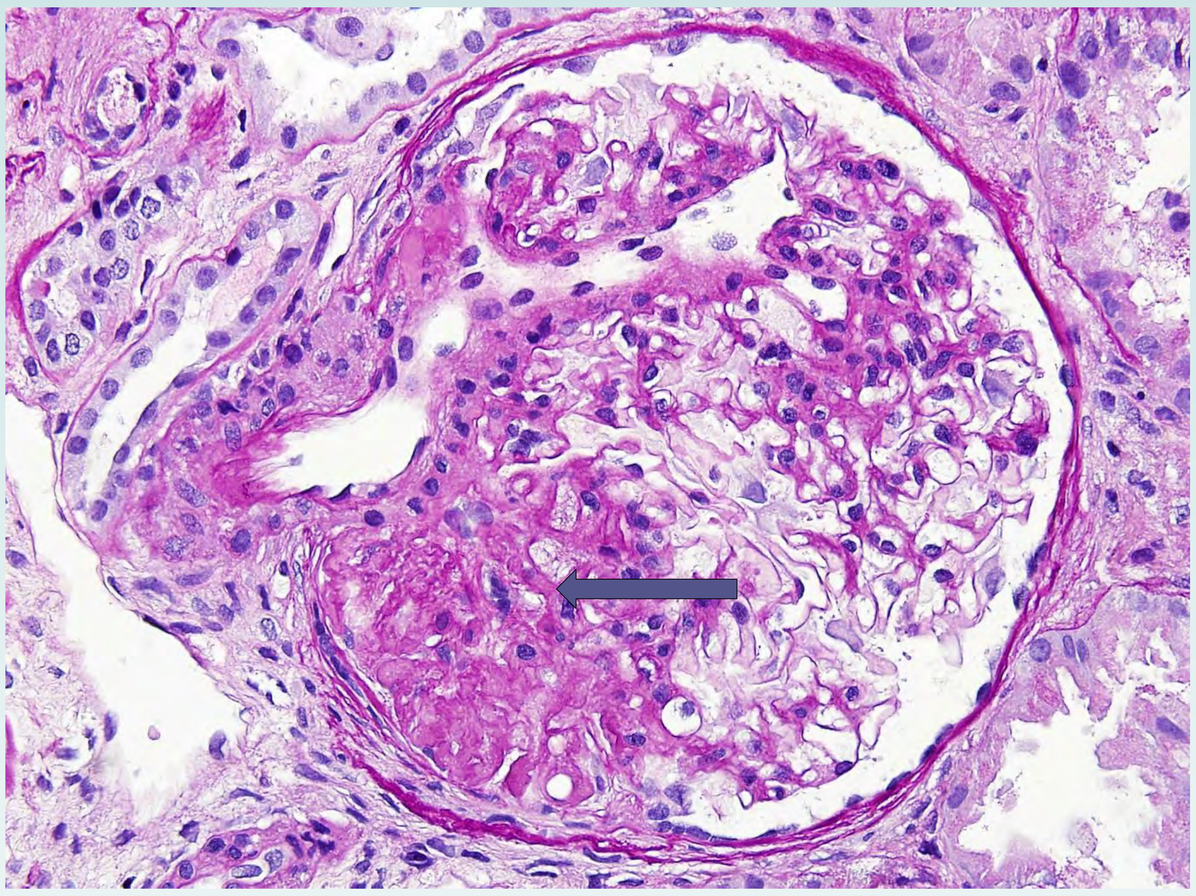

Figure 1 Kidney biopsy – light microscopy. Perihilar segmental sclerosis (arrow) in enlarged glomerulus (P2). PAS stain, original magnification x400.

Table 1 Relevant clinical and histological data of included patients.

Patient/sex	P1/M	P2/M	P3/F	P4/M	P5/M	P6/M	P7/F	P8/F
Age at Bx (years)	24.3	27.4	43.5	32.1	24.9	39.7	20.7	24
Age of first symptoms	18	26	40	32	21	39	16	17
BW (g)	1500	2500	2800	2200	2000	2000	900*	2400*
GW	n/a	n/a	32nd	n/a	36th	n/a	27th	n/a
BMI (kg/m^2)	31.8	28.7	32.3	27.5	32.7	26.5	22.0	26.4
24h proteinuria (g/day)	3.78	1.03	1.60	1.90	4.10	5.50	0.91	1.0
eGFR (ml/min/1.73m^2)	84	58	102	82	71	50	109	109
HU (yes/no)	no	yes	yes	yes	yes	yes	no	no
No of glomeruli	14	15	35	20	27	25	11	20
GSG (%)	7	8	6	0	30	80	0	20
SSG (%)	14	16	6	15	19	8	18	15
IF (glomeruli)	IgM1+	IgM1+	IgM+/-	neg	neg	IgM+/-	IgM1+	IgM+/-
IFTA (%)	15	5	3	5	15	35	0	15

** Additional perinatal asphyxia; M, male; F, female; Bx, kidney biopsy; BW, birth weight; GW, gestational weeks; BMI, body mass index; eGFR, estimated glomerular filtration rate; HU, hyperuricemia; GSG, globally sclerosed glomeruli; SSG, segmentally sclerosed glomeruli; IF, immunofluorescence; IFTA, interstitial fibrosis and tubular atrophy.*

Conclusion

FSGS related to LBW or PM usually presents with arterial hypertension and isolated proteinuria in absence of erythrocyturia. Perihilar variant of FSGS is the most common histological finding underlying kidney disease related to LBW or PM.

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KETOROLAC INDUCED MINIMAL CHANGE DISEASE

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Introduction: Commonly reported renal complications of non-steroidal anti-inflammatory drugs include acute renal failure and/or acute interstitial nephritis; in rare cases a nephrotic syndrome was also observed. Minimal change disease and acute tubular necrosis were also described in rare cases. We report patient with acute tubulointerstitial nephritis and minimal change disease caused by ketorolac.

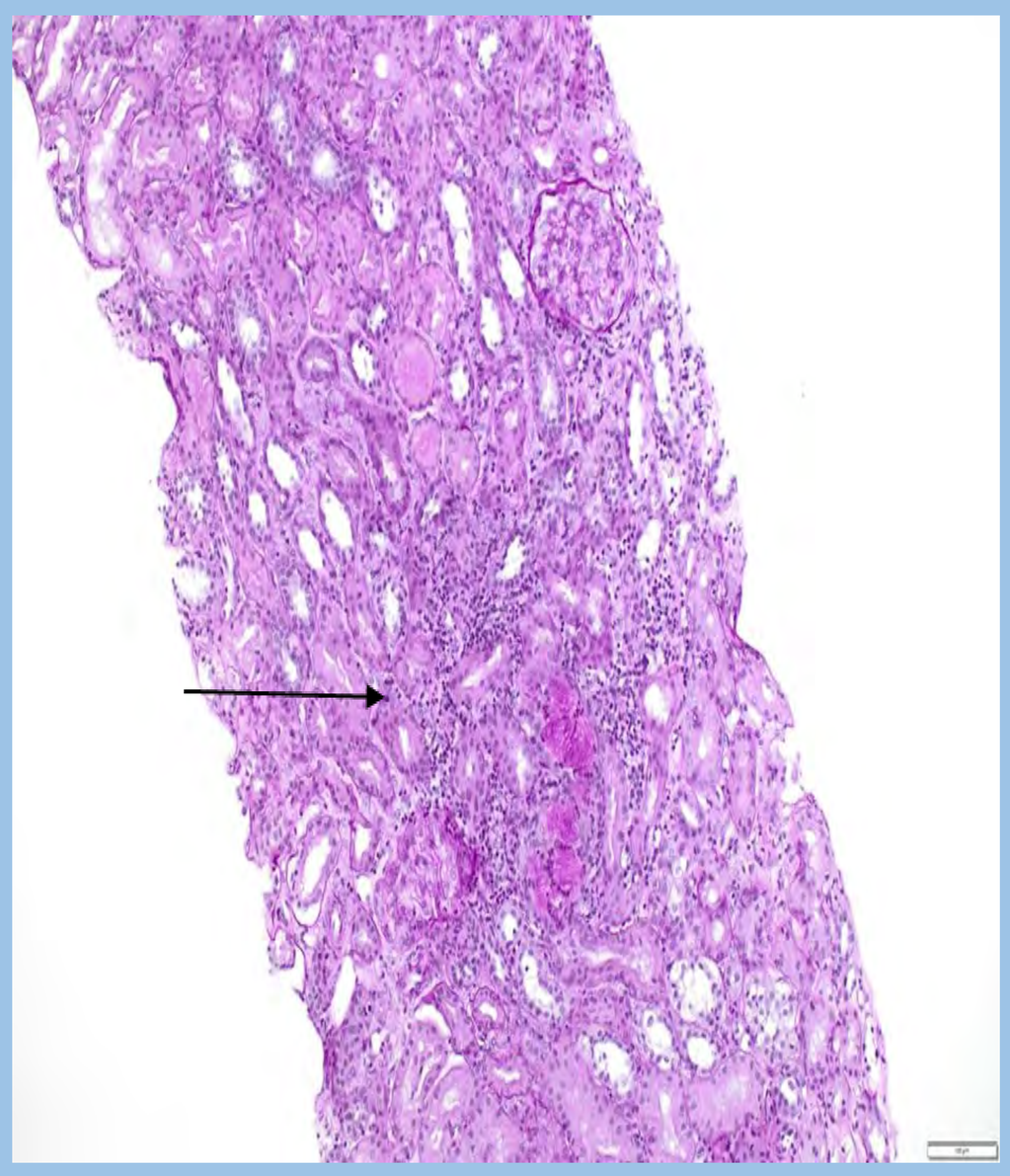

Figure 1. Focal interstitial infiltrate of mononuclear cells (arrow). The glomerulus is well preserved with no evidence of proliferation or inflammation. (PAS stain, original magnification × 100x).

Case report: Male patient, 24 years old, treated for 5 days with intravenous ketorolac in a standard dose, because of pain in the lumbosacral part of the spine, with confirmed discopathy, without other chronic or hereditary illnesses, developed hypertension and severe peripheral edema shortly after ketorolac administration. His serum creatinine was in referent rage, renal function was in hyper filtration and proteinuria was in nephrotic range, 23,25 gr/24h, followed by hypoproteinemia and hypoalbuminemia, with body fluid overload of 18 kg and conserved urine output with polyuria. We performed kidney biopsy. On light microscopy in the interstitium, focal clusters of mononuclear cells were observed, with a few neutrophils, along with the penetration of inflammatory cells into the canaliculi walls on few sites (figure 1.). Tamm-Horsaffal's protein was found in the lumen of the some tubules. The glomeruli were normal, with delicate basement membranes and no hyper cellularity. Moderately abundant deposits of IgM were found in the mesangium by immunofluorescence. Serum IgA, IgG, C1q, C3, fibrin, kappa and lambda chains were negative. In the findings of electron microscopy, podocytes showed a complete loss of foot processes and several mononuclear inflammatory cells in the interstitium.

Consclussion: histopathological finding is referable primarily to drug-induced minimal change disease. Patient was treated with symptomatic therapy with full clinical recovery in two weeks.

DIFFUSE PODOCYTOPATHY COMPATIBLE WITH COLLAPSING GLOMERULOPATHY POST COVID-19 VACCINE

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INTRODUCTION

- Collapsing glomerulopathy (CG) considered as an aggressive and distinct histological variant of focal segmental glomerulosclerosis (FSGS) has aroused great interest recently
- Involve rapid loss of renal function, high incidence of nephrotic syndrome, poor response to therapy and an early need for renal replacement therapy in about 50% of the cases
- Common risk factors: patients with Apolipoprotein L1 (APOL1) gene mutation, patients with HIV and SARS-CoV-2 infection.

CASE PRESENTATION

BACKGROUND

A 51 year old male previously healthy received his first Pfizer boost for COVID-19 on July 13th 2021. No adverse reactions were reported. 21 days later he received his second Pfizer boost.

- Few hours later after vaccination: headache, malaise, and shivering
- 2 weeks later: bipalpebral edema and edema in lower limbs were added
- At emergency room: BP 180/120 An ABPM was performed and hypertension of recent diagnosis was confirmed. Laboratory tests showed normal result. Patient started antihypertensive drugs.
- 3 months later the patient continued with edema and foamy urine was added. Significant proteinuria in 24 hours (386.7 mg/24h) was reported. The patient started Furosemide
- 2 renal ultrasounds scans were performed with no alterations reported
- Laboratory test showed an altered lipid profile, a slight increase in serum creatinine (from 0.89 to 1.04), proteinuria in 24 hours continued altered (1478.8 mg/24h) and subclinical hypothyroidism was diagnosed
- 7 months later a nephrotic proteinuria (12799.2 mg/24h) was reported for the first time
- 9 months later additional tests were requested (VDRL, HBsAg, Anti-HBs, ANA, pANCA, cANCA, Anti-PLA2R, HIV ELISA). All results were negative
- 1 year later: Renal biopsy was taken. Histopathological findings were compatible with Collapsing glomerulopathy (CG).
- During the entire period of illness, the patient denied having respiratory symptoms. 2 more serologic tests were requested, Anti-N-SARS-CoV-2 was found negative yet Anti-S-SARS-CoV-2 resulted positive
- The patient lost a total of 18 kg in 1 year.

DISCUSSION

- No specific etiology for CG has yet been established.
- APOL1 gene mutation, patients with HIV and SARS-CoV-2 infections are the closest relations we have to causality
- Glomerulopathies have been reported after receiving COVID-19 vaccines, however none of them has presented as a CG.
- The following case is the first reported with this histological pattern after receiving an RNA COVID-19 vaccine
- 2 serologic tests helped defined that the patient had not got infected by COVID-19 but he had developed antibodies because of vaccination.
- Anti-N-SARS-CoV-2 (-), Anti-S-SARS-CoV-2 (+)
- Pathogenesis of emerging cases of CG associated with COVID-19 infection have been established as multifactorial proposing on the one hand direct toxic viral effect on podocytes and on the other, injury in podocytes caused by virus induced cytokine release syndrome

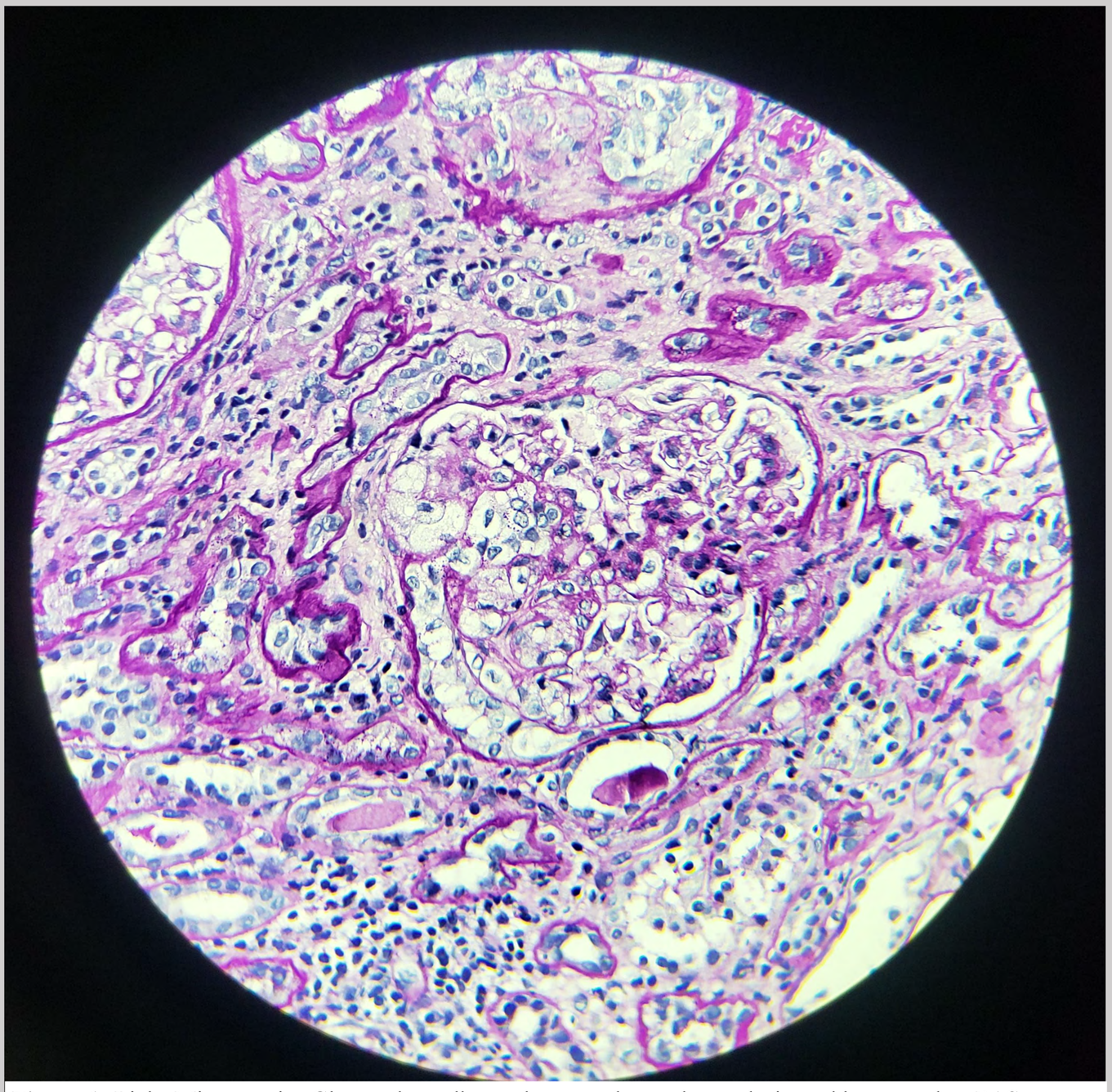

Figure 1: Light Microscopic: Glomerular collapse due to podocyte hyperplasia and hypertrophy. PAS staining 40X

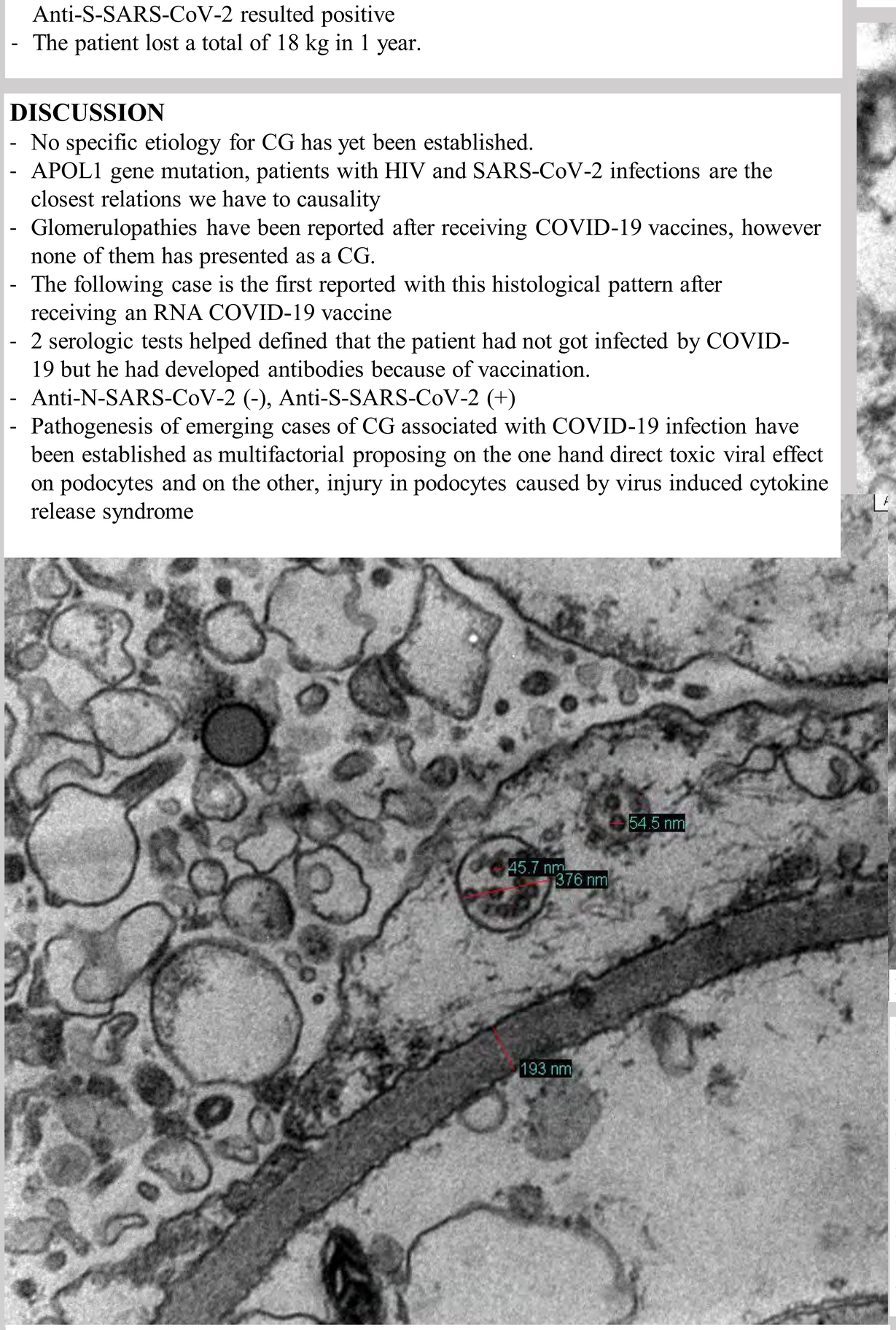

Figure 2: Microscopic electron: Particles inside podocytes which could be attributed to viral particles

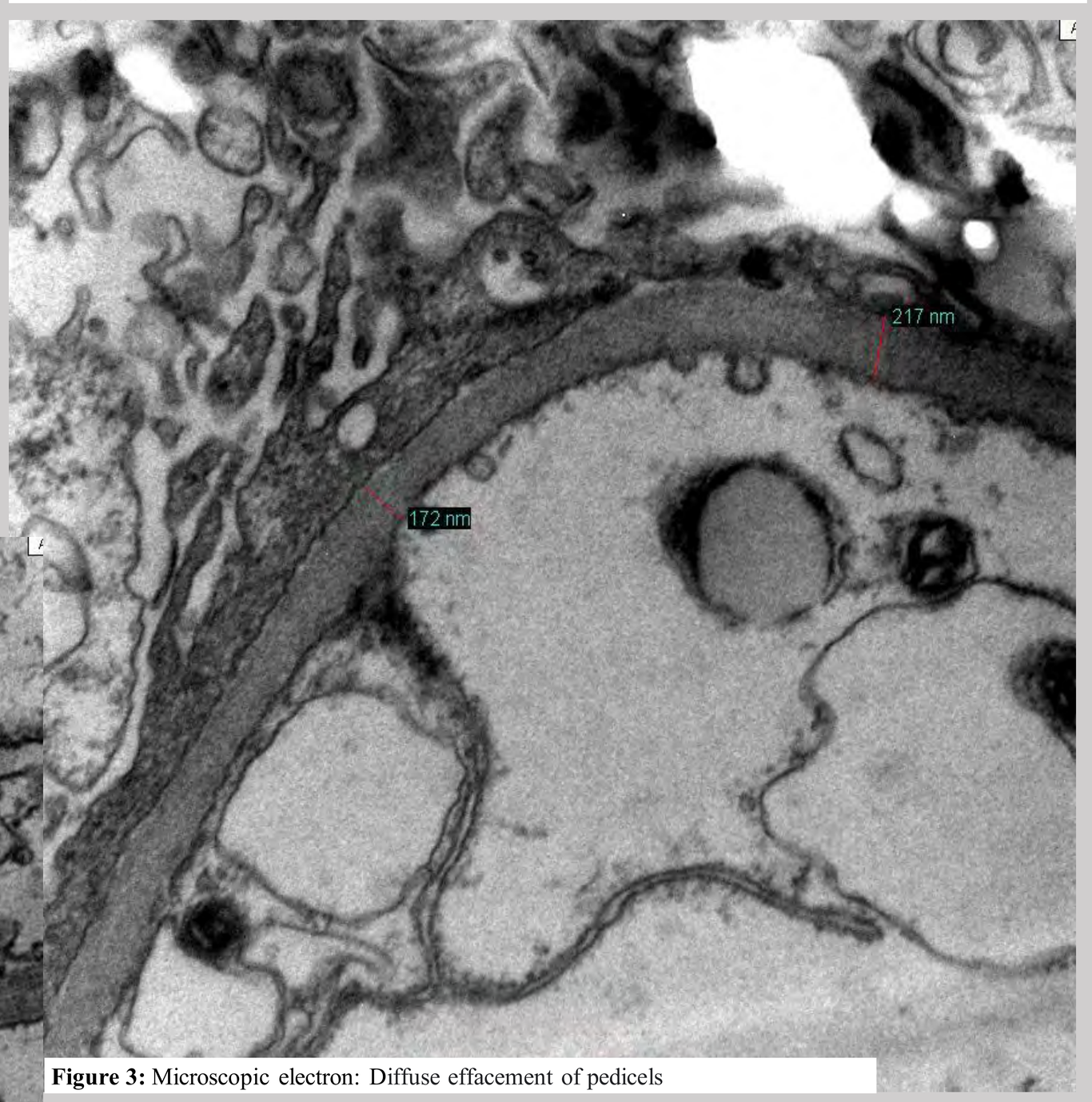

Figure 3: Microscopic electron: Diffuse effacement of pedicels

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SAFETY OF KIDNEY (INCLUDING PROTOCOL KIDNEY TRANSPLANT) BIOPSIES IN UNIVERSITY HOSPITAL MERKUR

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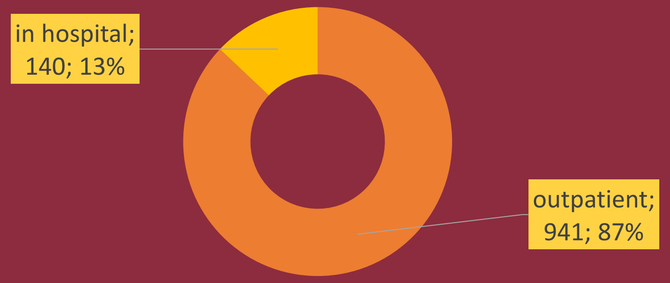
Background

Kidney biopsy (BX) is frequently performed in our center as an outpatient procedure.

Outpatient *vs* in hospital kidney biopsies
2018 - 2022
in University Hospital Center Merkur, Zagreb, Croatia
N = 1081



Aim

To evaluate the safety of biopsy in the outpatient setting.

Patients and methods

We analyzed kidney biopsies performed from 2018 to 2022 (5 years). 941 of 1081 biopsies (87 %) were performed in the outpatient setting. Other patients underwent BX during the ongoing hospitalization or were hospitalized for the BX, as assessed as at higher risk for complication by nephrologist. All were performed under ultrasound guidance using an 18G needle, with freehand technique. In all patients with estimated glomerular filtration rate < 30 mL/min/1.73m^2 body surface area desmopressin was administered. Patients were observed after the BX and complete blood count test and urinalysis were done after 4 h.
Data on complications of BX - drop in serum hemoglobin concentration, bleeding, hospitalization, transfusion, surgery or radiological intervention, death (the last three being the major) were analyzed.

Results

The presented results included outpatient BXs only. Each BX was performed by one of 8 nephrologists (the report authors). Protocol kidney transplant biopsies were done at 2, 6 or 12 months after transplantation.

Outpatient biopsies (n = 941), patients' characteristics and outcomes	
Patients age (years)	median 53 (min. 15 – max. 86)
Patients gender (male)	609 (64.7 %)
Transplant kidney	754 (80.1 %)
Received desmopressin for bleeding prevention	100 (10.6 %)
Protocol transplant kidney biopsy	518 (55.1 %)
Complications	19 (2 %)
Macrohematuria	16 (1.7 %)
Perirenal hematoma	2 (%)
Need for hospitalization	11 (1.2 %)
Drop in hemoglobin	2 (%)
Need for blood transfusion	0 (0 %)
Need for surgery	0 (0 %)
Need for radiologic intervention	0 (0 %)
Death	0 (0 %)

Conclusion

Kidney BX performed in outpatient setting in our center in selected patients was only rarely associated with complications, never a major one, and is generally a safe procedure.

SIGNIFICANCE OF LIPOCALIN-2 (NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN [NGAL]) DETECTION IN THE DIAGNOSIS OF KIDNEY TRANSPLANT REJECTION

Ljiljana Bogdanović[1], Sanja Simić-Ogrizović[2]

Introduction:

Many kidney diseases eventually lead to chronic renal deficiency. In these cases transplantation is the best method of treatment. However, in time renal transplant may lose its function too. As certain causes of dysfunction can lead to graft death in a very short time it is necessary to make a rapid and correct diagnosis. Today, one of the most significant early biomarkers is neutrophil inflammatory protein, lipocalin-2 (LCN2/NGAL). LCN2/NGAL is a protein which is normally secreted in very small amounts from various organs, but its expression increases in a number of pathological conditions including ischemic injury, urinary infections and immunological graft rejection.

Aim of the study: To examine the significance of immunohistochemically expression of the neutrophil inflammatory protein, lipocalin-2 (LCN2/NGAL) in renal transplant as well as its correlation with histopathological changes.

Matherials and methods:

Patients and Tissue Samples:

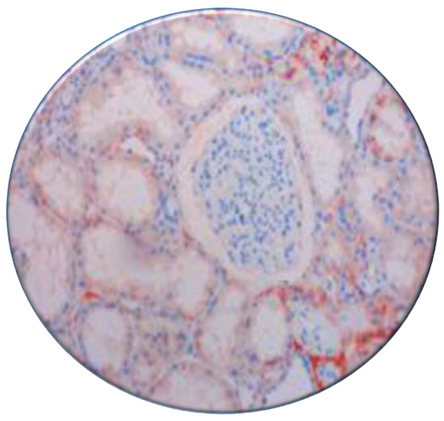
Archival tissue specimens of kidney obtained from 29 patients treated by kidney transplantation were retrospectively studied. In 17 cases there was an acute rejection, of which all were positive to LCN2/NGAL staining.

Immunohistochemistry with Neutrophil inflammatory protein, lipocalin-2 (LCN2/NGAL) Antibody:

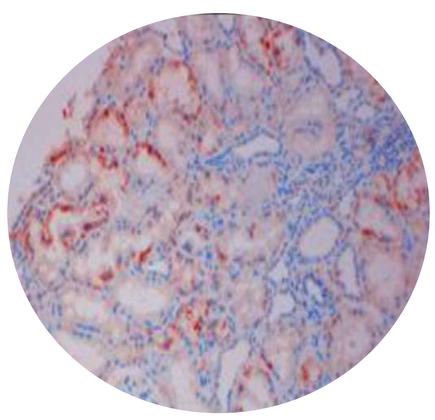
Immunohistochemical staining of archival tissue specimens was performed by the labeled streptavidin–biotin–peroxidase method.

LCN2/NGAL immunoexpression was evaluated as the percentage of cells with positive cytoplasmic staining. Accumulation of LCN2/NGAL was examined in correlation with 4 morphological changes that, according to the BANFF classification, indicate acute rejection: 1. transplant glomerulitis, 2. tubular expression of MHC class II antigen, 3. transplant endarteritis, 4. interstitial cellular rejection.

Semiquantitative scoring was performed:



+ **FOCAL** if less than 50% LCN2/NGAL positive cells (X200)



++ **DIFFUSE** if more than 50% LCN2/NGAL positive cells (X400)

Results:

Patient characteristic	Number of patients n (%)
Gender	
Male	22 (75.9%)
Female	7 (24.1%)
Transplantation	
Cadaveric	18 (60.07%)
Living donor	11 (37.93%)

	Score n (%)	
	++	+
LCN2/NGAL expression in kidney transplant biopsy with acute rejection	**9/17 (52.9%)**	**8/17 (47.1%)**
Transplant glomerulitis	5/17 (29.4%)	2/17 (11.8%)
Tubular expression of MHC class II antigen	2/17 (11.8%)	5/17 (29.4%)
Interstitial cellular rejection	0 (0%)	1/17 (5.8%)
Transplant endarteritis	0 (0%)	2/17 (11.8%)

Expression of LCN2/NGAL is in acute rejection samples in corelation to tubular expression of MHC class II antigen and transplant glomerulitis

p<0.01

Conclusion:

In this study we have confirmed that LCN2/NGAL is strongly associated with acute rejection and other forms of acute injury. Tubular MHC class II expression was correlated with LCN2/NGAL accumulation and was accompanied by the presence of transplant glomerulitis. All of this contributes to the idea of its further use in clinical practice.

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Frequency of Glomerular Diseases Post Renal Transplantation in Oman- a single center experience

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Aim and Specific Objectives

Overall aim: This study aims to determine the frequency of recurrent and de novo glomerular diseases post-renal transplantation in Oman.

Specific objectives:

To determine the frequency of glomerular disease occurrence after renal transplant.

To determine whether the glomerular diseases are recurrent or de novo.

To identify glomerular causes of end-stage renal disease (ESRD) in these patients.

To determine the time from transplantation to identification of the glomerular disease.

Background

Although kidney transplantation offers the best outcome for patients with end stage renal disease, one of the factors associated with graft failure is recurrence of a previous glomerulonephritis or development of a new kidney disease, known as 'de novo'.

In a study conducted in Pakistan, out of 163 live-related transplant kidney biopsies, 27% cases showed recurrence of the original disease, whereas the prevalence of de novo glomerulonephritis was 1.9%.

Data from the Dialysis and Transplant (ANZDATA) Registry accumulated over 30 years showed that recurrence was reported in 479 of 4637 patients, and of these, 212 lost their allograft due to recurrence. Transplant recipients with recurrent disease were twice as likely to lose their allografts compared to those without recurrence (adjusted hazard ratio 2.04 [1.81-2.31])

Materials and Methods

A retrospective study that included renal allograft biopsy samples received in the department of Pathology between biopsies between 1st January 2010 and 1st January 2021. Biopsies with clear evidence of GN based on light microscopy and immunofluorescence and/or electron microscopy were included. Patients' age, sex, date of transplant cause of ESRD (if available) were recorded. The time from transplantation to diagnosis was also recorded.

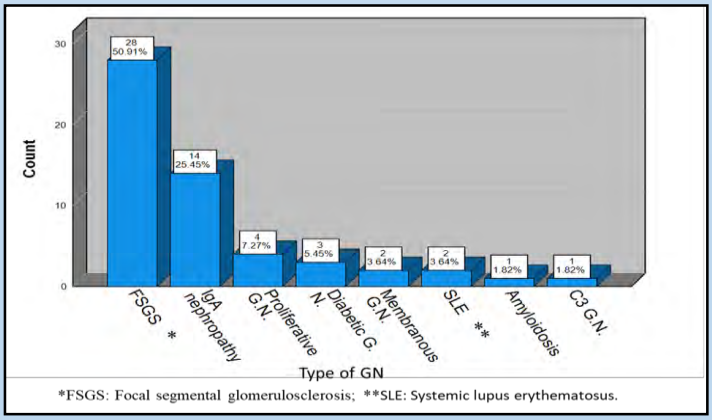
Results

There were a total of 523 allograft biopsies during the study period out of which 55 (10.5%) had a biopsy-proven report of glomerular disease.

17 were females (30.1%) biopsies and 38 (69.9%) males to with an average age of 41.1 ±14 years. The most frequent GN was FSGS in 28 cases (50.9%), followed by IgA nephropathy in 14 cases (25.5%). Recurrence of GN was seen in 25 (45.5%), including 12 cases of FSGS, 9 IgA nephropathy, 1 case of C3 GN, 1 proliferative GN, and 1 of amyloidosis.

Conclusion

The frequency of glomerular disease post-transplantation was 10.5%. The most common glomerulonephritis and recurrent GN was FSGS (50.91%), followed by IgA nephropathy (25.45%). 4 de novo cases, including FSGS and IgA Diabetic GN, and Membranous GN, 47.3% of cases was unknown whether de novo or recurrent. Time to occurrence post-transplantation ranged from 0 – 19.6 years with a mean of 5.3 years.



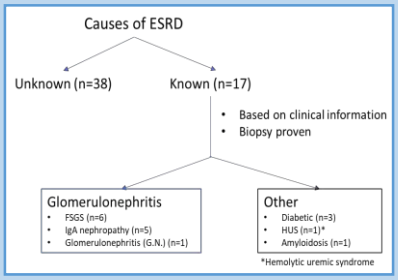
*FSGS: Focal segmental glomerulosclerosis; **SLE: Systemic lupus erythematosus.

Prevalence of recurrence and de novo disease

Variable	Frequency	Percent
Recurrence	25	45.5%
De novo	4	7.3%
Unknown	26	47.3%
Total	55	100.0%

the time from transplantation till the appearance of the disease (n=55)

Variable	Range (yrs)	Mean (yrs)	Median (yrs)	SD
FSGS (n=28)	0 – 19.6	3.9	2.8	3.8
IgA nephropathy (n=14)	0 – 16.6	6.4	6.7	0.6
Others (n=13)	1 – 13.4	7.2	6.5	3.9



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Opportunistic infection of renal allograft in renal transplant recipients

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Introduction:

Potent immunosuppressive regimen have reduced risk of graft loss due to acute rejection but have increased risk of infectious complications. Infections are now considered to be the second leading cause of death in renal allograft recipients after cardiovascular complications. Urinary tract infection (UTI) is considered to be the most common infection following renal transplantation. BK polyoma virus nephropathy is considered most important viral infection causing allograft failure. There is paucity of literature regarding spectrum of opportunistic infection affecting the renal allograft itself.

Materials and Methods:

Retrospective study from December 2011 to December 2021. Renal graft biopsies and graft nephrectomy performed during this period were retrospectively analyzed for presence of bacterial, viral and fungal opportunistic infections. Epidemiological, clinical details and laboratory workup including microbiological investigations for these patients were retrieved from electronic records.

Results:

A total of 2019 renal transplants were performed during study period. 47 episodes of renal opportunistic infections were diagnosed in allograft biopsies or graft nephrectomy specimen of 47 renal allograft recipients. Table 1 shows details of these infections. BK virus nephropathy was the most common infection followed by graft pyelonephritis. Mucor was most common fungal infection. On follow up, 14 patients died, 17 became dialysis dependent and only 13 had stable graft function.

	Total cases of Renal opportunistic infections N= 47
Age	44.59±13.72 years
Gender	39 males 8 females
Serum Ceatinine at biopsy	1.85±0.30 mg/dl
Mean eGFR at biopsy	47.06±10.48 ml/min/1.73 m^2
BK virus	22
CMV	2
Bacterial graft pyelonephritis	12
Granulomatous interstitial nephritis	2 (tubercular) 1 (E.coli)
Mucor	3
Aspergillus	2
Candida	2
Cryptococcus	1

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AMYLOIDOSIS AND KIDNEY TRANSPLANTATION – OUR EXPERIENCE

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INTRODUCTION: Amyloidosis is a group of diseases characterized by the extracellular deposition of insoluble fibrils composed of misfolded aggregated proteins. Localized or systemic disease may occur de novo or secondary to infectious, inflammatory, or malignant conditions. Organ failure occurs due to the local impact on the tissue structure, but also because of cytotoxic effects of amyloid. Kidney involvement in ~1% can lead to end-stage renal disease (ESRD) and organ transplantation as replacement therapy. The aim of this presentation was to share our experiences with transplanted patients with amyloidosis as an underlying disease .

PATIENTS: In Clinical Hospital Center Rijeka from 1st January 2003 until 28th February 2023. 449 kidney transplantations (KT) were performed. Amyloidosis as the main cause of renal failure was diagnosed in 3 (0.7%) patients. Their clinical and biopsy data as well as KT outcome were retrospectively analysed.

RESULTS: During the observed period, two male and one female patient with the amyloidosis diagnosed by biopsy of the native kidney, were transplanted. Femal patient had AL, one male had transtiretin type of amyloidosis and in one patient we could not establish the origin of fibrills. At the time of the transplantation, patients were of 66, 68 and 80 of age and received deceased kidney transplant. In one patient dual KT was performed with delayed and never fully recovered graft function. This patient died shortly after KT and autopsy showed amyloid deposition in the arteries of the transplanted kidneys. The oldest patient died two years after successful KT because of urosepsis. The female patient is alive, eight years after KT, with good function of the graft.

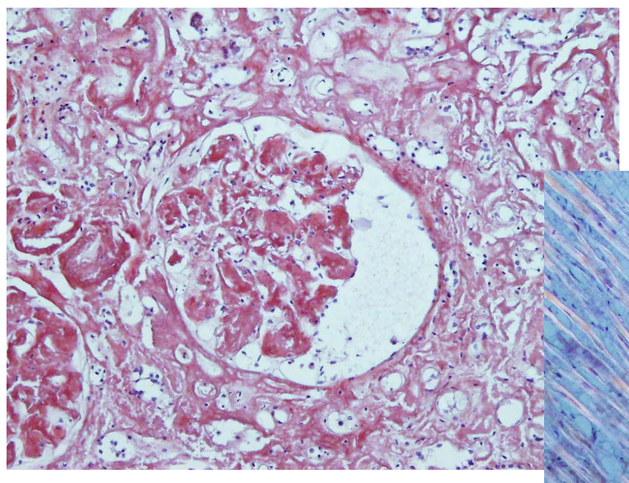

Fig.1A Microphotograp of amyloid deposits in glomeruli and tubulo-interstitium of the native kidney (Congo red , 100x)

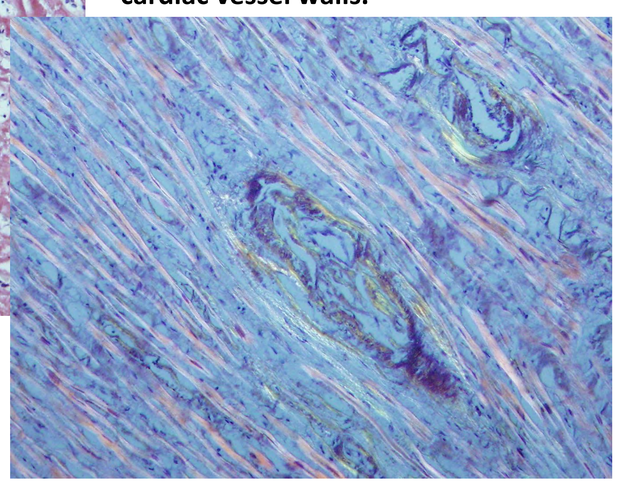

Fig 1BCongo red stain under polarized light (apple-green birefringence) in cardiac vessel walls.

MICROPHOTOGRAPHS show autopsy findings in our patient with dual KT with abundant amyloid deposits in native kidney(1A) and heart blood vessels whith green birefringence under the polarized light (1B). In patient acute Tcell mediated Banf IIA rejection was found in the previous graft biopsy, and active chronic cellular type rejection at autopsy 4 months later (2A;B). Intimal arteritis, hypertensive changes can be seen in the blood vessel wall (2C) where Congo staining also revealed discrete amyloid deposition (2D).

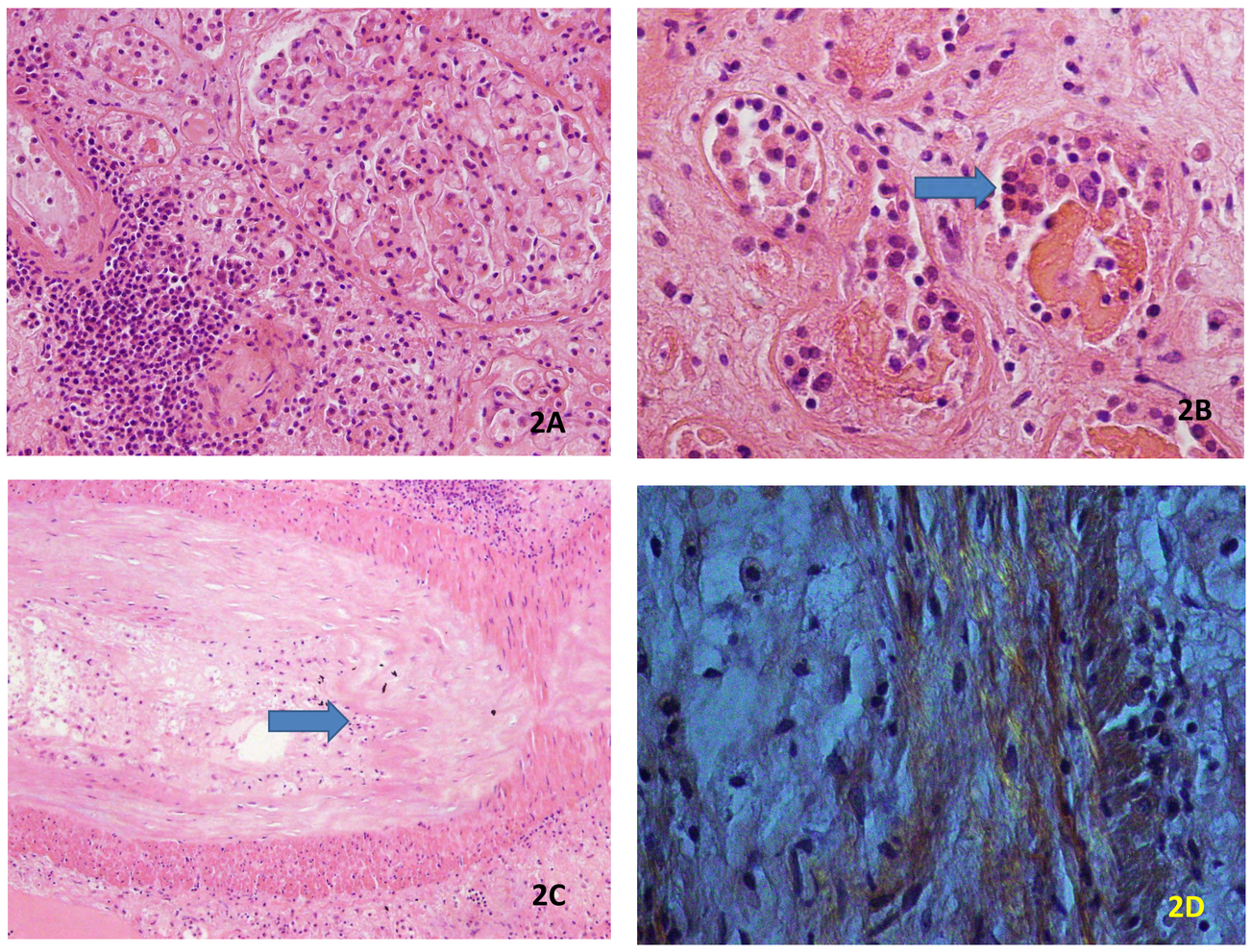

Fig 2. (A;B)Microphotography of active chronic cellular rejection of kidney transplant, arrow points to tubulitis (HE, 200x) (C)Disruption of the internal elastic lamina (arrowhead) is detected in transplant arteriopathy (he staining, 100x). Congo red stain under polarized light (apple-green birefringence 400x)

CONCLUSION: In our population amyloidosis is a rare cause of ESRD. KT is a possible and effective treatment. Transplanted patients must be closely followed because of the possibility of the amyloid deposition in graft soon after the transplantation. Larger studies indicate a shorter survival of patients on renal replacement therapy with a median survival of 2.1 years on dialysis and after kidney transplantation but are comparable to other high-risk subgroups. Recent studies show that transplant outcomes improve, especially in the group with AL amyloidosis due to better control of the underlying disease, which is also the case with our patient with a preserved allograft.

Steven Law, Oliver Cohen, Helen J Lachmann, Tamer Rezk, Janet A Gilbertson, Dorota Rowczenio, Ashutosh D Wechalekar, Philip N Hawkins, Reza Motallebzadeh, Julian D Gillmore, Renal transplant outcomes in amyloidosis, Nephrology Dialysis Transplantation, Volume 36, Issue 2, February 2021, Pages 355–365

TRANSMISSION OF PANCREATIC ADENOCARCINOMA BY A SINGLE DONOR TO TWO KIDNEY TRANSPLANT RECIPIENTS

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INTRODUCTION

Despite careful donor selection, cancer transmission remains a rare but serious, life-threatening complication of renal transplantation, with an estimated incidence of 0.01% to 0.05%. Here, we report a single center's experience with the transmission of adenocarcinoma of the pancreas from a deceased multiorgan donor to two kidney transplant recipients.

CASE

Autopsy of the donor revealed adenocarcinoma of the pancreas that had already metastasized locally to the regional lymph nodes and had not been detected at the time of organ retrieval. The donor was a 46-year-old female who died due to spontaneous intracerebral hemorrhage without a known history of malignancy. Because the patient's history of diabetes, concurrent kidney-pancreas transplantation was not considered.

CASE

Both kidney transplant recipients were male and were carefully monitored, as neither consented to nephrectomy of the graft. In one patient, the tumor was discovered incidentally during a surveillance biopsy of the graft approximately 14 months after transplantation. The biopsy showed no evidence of rejection, but two of four tissue samples showed moderately to poorly differentiated adenocarcinoma. According to the immunohistochemical findings (positive immunohistochemical staining for cytokeratin 19 and 7 and mucin 1), the tumor was compatible with metastasis from the pancreaticobiliary tract (Figure 1).

A sample of tumor tissue from the transplanted kidney was analyzed based on the detection of selected genetic markers on the X and Y-chromosomes using the quantitative fluorescence polymerase chain reaction (QF-PCR) method. In the second patient, ultrasound-guided aspiration needle biopsy of a growing cystic formation in the lower pole of the graft revealed a poorly differentiated metastatic adenocarcinoma. Both patients were successfully treated with graft nephrectomy and complete discontinuation of immunosuppression, and follow-up revealed no persistent or recurrent disease (Figure 2,3).

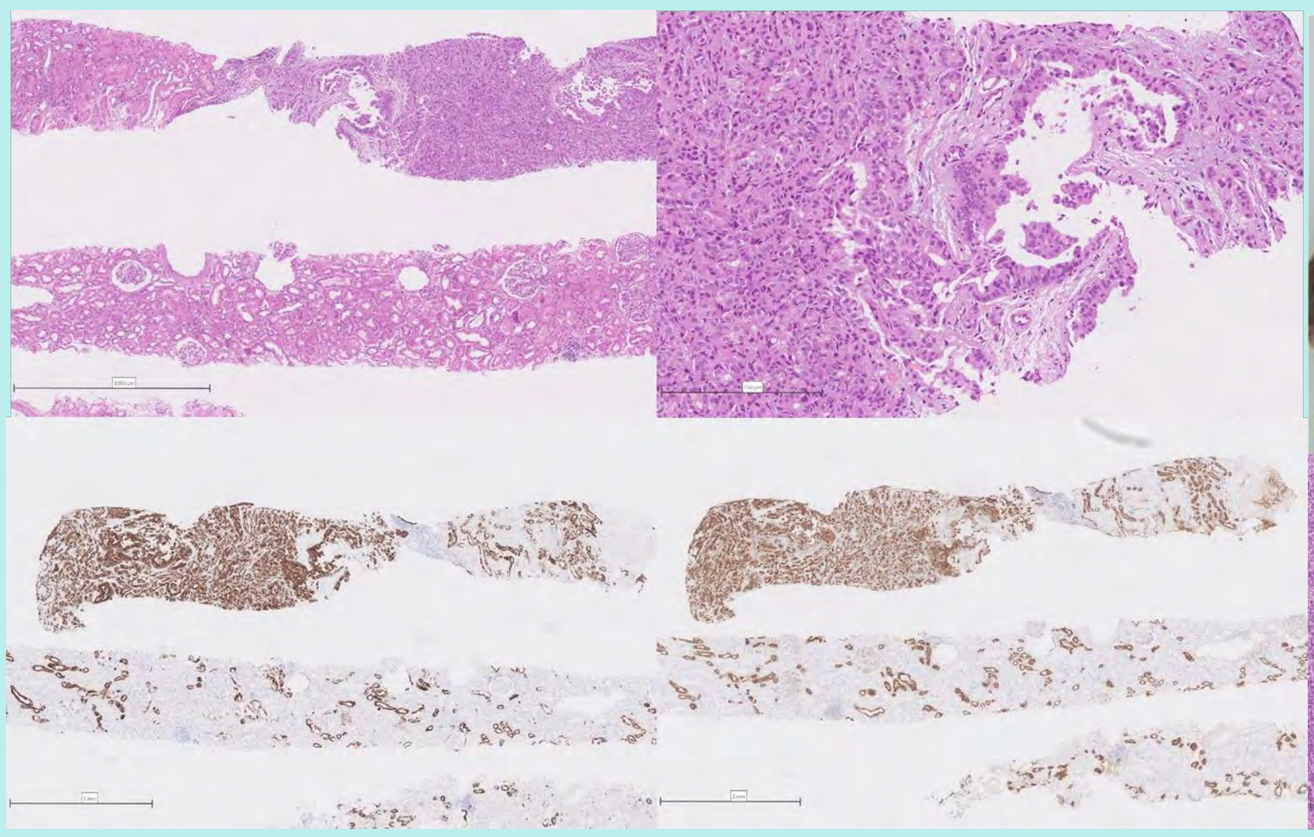

Figure 1. A. Adenocarcinoma in surveillance transplant kidney biopsy 1-year after transplantation; B. closer view; Adenocarcinoma expressed C. CK7 and D. MUC1 indicating origo from pancreas.

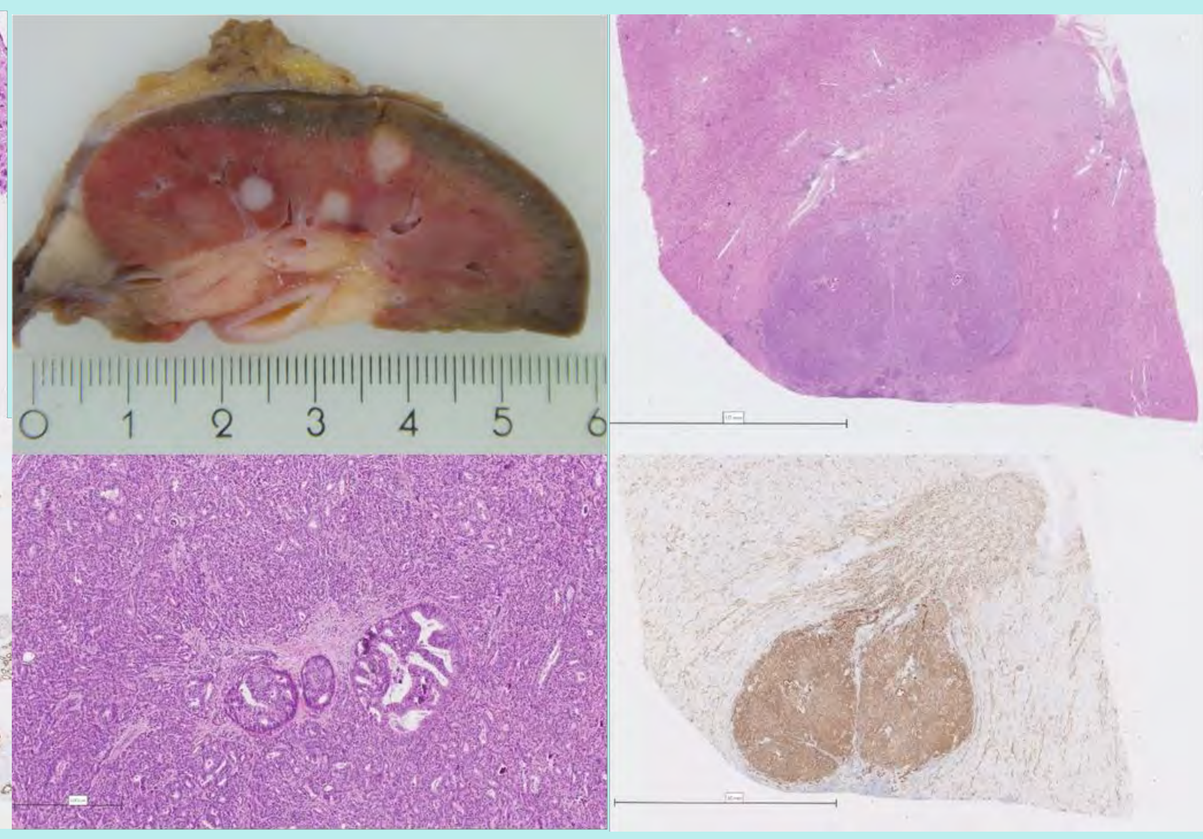

Figure 3. Explanted kidney with foci of adenocarcinoma. Adenocarcinoma expressed CK19.

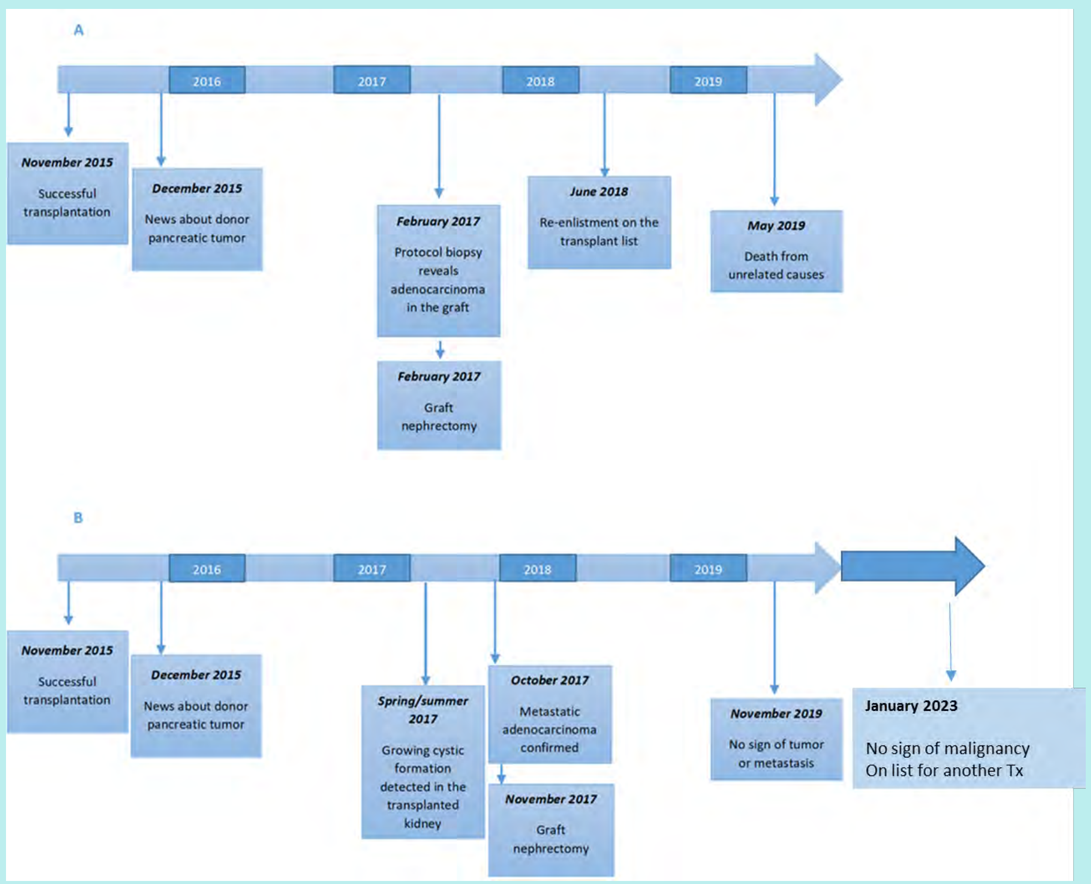

Figure 3. Timelines of major events in both kidney transplant recipients. A. left-kidney recipient. B. right-kidney recipient.

CONCLUSION

Autopsy of the donor is mandatory.
Microscopic tumor transmission can be detected on surveillance/indication kidney graft biopsy.

Early intervention upon discovery of tumor cells is mandatory and can prevent a poor outcome. In case of kidney graft lesion suspected for tumor transmission, ultrasound guided biopsy is recommended for patohistological evaluation. The best treatment options are immunosuppression withdrawal and graft nephrectomy offering potential full recovery even in suspicious metastatic disease.

INSTITUTE OF PATHOLOGY
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RIFAMPICIN AS THE CAUSE FOR HEMOGLOBIN CAST NEPHROPATHY: A SERIES OF 28 CASES

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INTRODUCTION

- Rifampicin is an important component in the multidrug treatment regimen for tuberculosis, nontuberculous mycobacterial infections and leprosy
- We describe a case series of rifampicin induced acute tubular injury with hemoglobin cast nephropathy (HCN)

METHODS

- A total of 83 cases of HCN were reported at our institution between March 2015 and February 2023
- Among them, 28 patients (33.7%) had rifampicin induced intra vascular hemolysis and haemoglobin casts
- The clinical and histopathological findings and clinical outcome of these cases were evaluated

RESULTS

- The mean age was 47.9 years (range 21 -72year), with 16 male and 12 female patients
- 26 patients had pulmonary tuberculosis, one had tuberculous lymphadenitis and one patient had tuberculosis of spine
- Eight patients were treated for relapse of tuberculosis and 4 patients were on irregular treatment
- Six patients presented with rapidly progressive renal failure, 21 patients presented with acute kidney injury and one patient presented with acute on chronic kidney injury
- The mean s. creatinine was 9.1 mg/dl (range: 2.9 to 20 mg/dl)
- Histological features of acute tubular injury with pigment cast formation were observed in all the biopsies
- The casts did not show light chain restriction on immunofluorescence study
- In all the cases, the pigment casts were intensely positive for hemoglobin and negative for myoglobin immunostaining

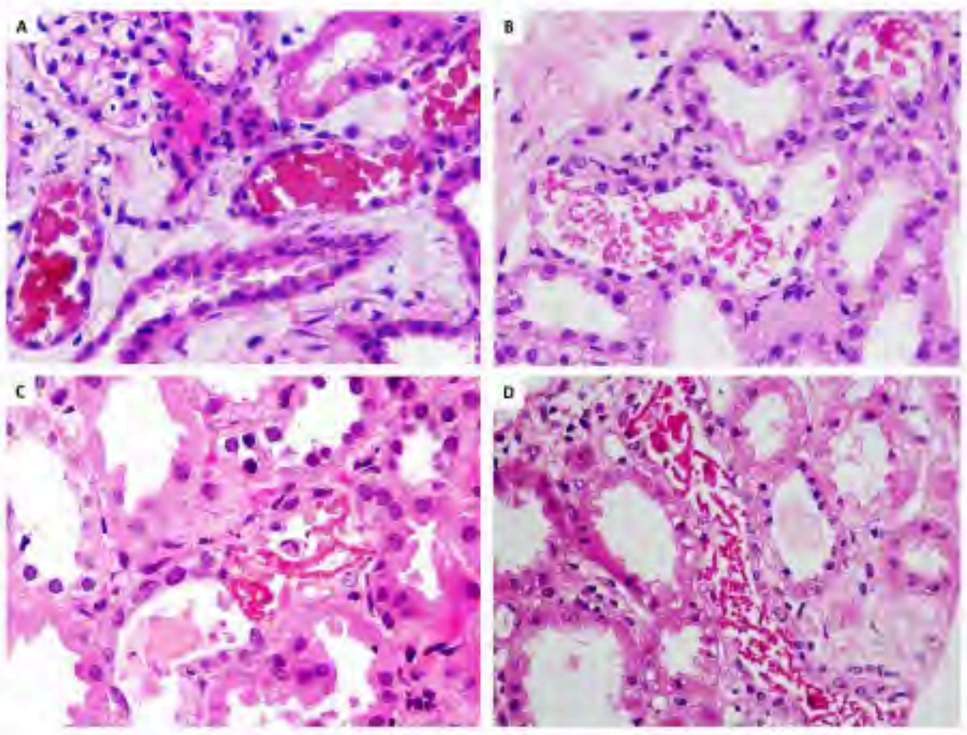
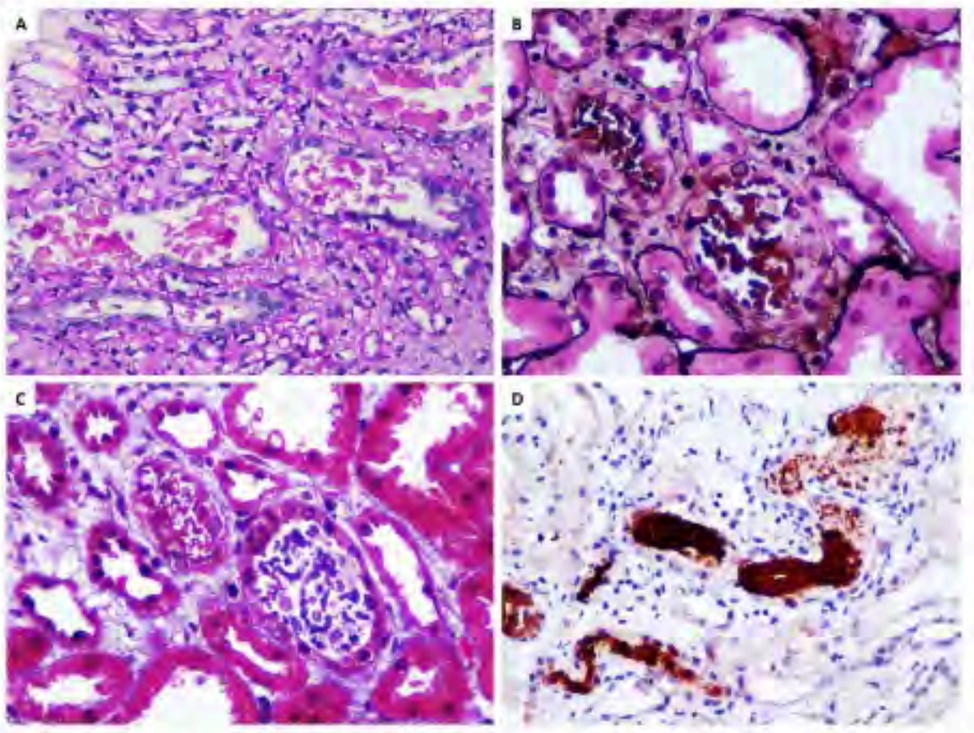


Image 1. The haemoglobin casts were globular (A) or a granular (B) appearance. Some had a ropy (C) or beaded (D) pattern. (Hematoxylin and eosin)



The casts were weakly positive on periodic acid–Schiff stain (A), they were brown to black on Jones methenamine silver stain (B). The colour of the casts ranged from blue to red on Masson trichrome stain (C). Immunohistochemistry for hemoglobin showed intense positive staining on the casts (D).

- At the time of biopsy, 24 patients were dialysis dependent
- Follow-up details were available in 21 out of 28 patients
- Kidney function improved with prompt drug withdrawal and supportive care in 17 patients
- Four patients continue to be dialysis dependent

DISCUSSION

- Hepatotoxicity is a well-known side effect of rifampicin and liver function is routinely monitored during treatment. Acute kidney injury (AKI) is a rare complication
- The biopsy findings in rifampicin associated AKI are acute interstitial nephritis and acute tubular injury with hemoglobin casts
- Rifampicin is known to cause immunologically mediated hemolytic anaemia. Intravascular hemolysis occurs due to the formation of anti-rifampicin antibodies (IgG and IgM) which cross react with blood group I-antigen present on the surface of red blood cells resulting in complement mediated hemolysis
- Heme pigment is toxic to the tubular epithelial cells. It can precipitate with Tamm Horsfall protein to form pigmented tubular casts
- The I-antigen is also expressed in renal tubular cells. Anti-rifampicin antibodies can target I-antigen on renal tubular cells and directly cause their destruction

CONCLUSION

- As tuberculosis is re-emerging as one of the most prevalent infectious diseases worldwide, clinicians should be aware of this severe complication
- HCN should be suspected if a patient on rifampicin therapy develops acute kidney injury
- The accurate diagnosis requires a kidney biopsy with immunostaining for hemoglobin
- To the best of our knowledge this is the largest series of rifampicin induced HCN

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Harsha Graphics, Porur, Ch-116, 9444 044 112

Shrinking lung syndrome and tubulointerstitial nephritis – Sjögren's syndrome or systemic lupus erythematosus manifestation?

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Introduction

- Shrinking lung syndrome (SLS) is a rare complication of several autoimmune diseases with unclear pathogenesis[1].
- SLS manifests with progressive dyspnea, pleuritic chest pain, diaphragmatic elevation, dry cough, restrictive pattern in respiratory function tests and lack of objective parenchymal abnormalities[1].
- Due to a small number of patients with this syndrome, there are no available guidelines, so the treatment is based on the physician's judgment[2-4].
- So far there has not been described an association between SLS and renal damage due to tubulointerstital nephritis (TIN).

Case report

- A 33-year-old woman
- Presented with arthralgia, fever, xerophthalmia, dyspnea and right thoracic wall pain with elevation of the right hemidiaphragm
- Laboratory findings showed anemia, lymphopenia, elevated erythrocyte sedimentation rate, positive anti-nuclear, anti-Sm, anti-dsDNA, Anti-Ro/SSA and anti-La/SSB antibodies, low C3 complement level, polyclonal hypergammaglobulinemia, proteinuria (up to 0.95 gr/24 hour)
- Acid-base status revealed the presence of moderate metabolic acidosis(HCO_3^-=17mmol/l), with hypokalemia (K^+ down to 2.9 mmol/L) and alkaline urine (pH=8).
- Restrictive pattern on pulmonary function testing and few atelectatic segments without signs of pulmonary embolism or interstitial lung disease on high resolution CT scan were found
- Due to clinical symptoms and laboratory findings, primary Sjögren syndrome (SS), systemic lupus erythematosus (SLE) or overlap syndrome were suspected.
- Kidney biopsy revealed TIN with moderate activity and chronicity, primarily as SS manifestation. Interestingly, the biopsy didn't show any typical signs of lupus nephritis (Figure 1).
- Changes in skin biopsy associated with the clinical picture probably corresponded to SLE (Figure 2).
- The patient was treated with high dose glucocorticoids, azathioprine and theophylline
- Application of the therapy resulted in regression of clinical symptoms, improvement of laboratory findings, including acid-base status and proteinuria normalisation.

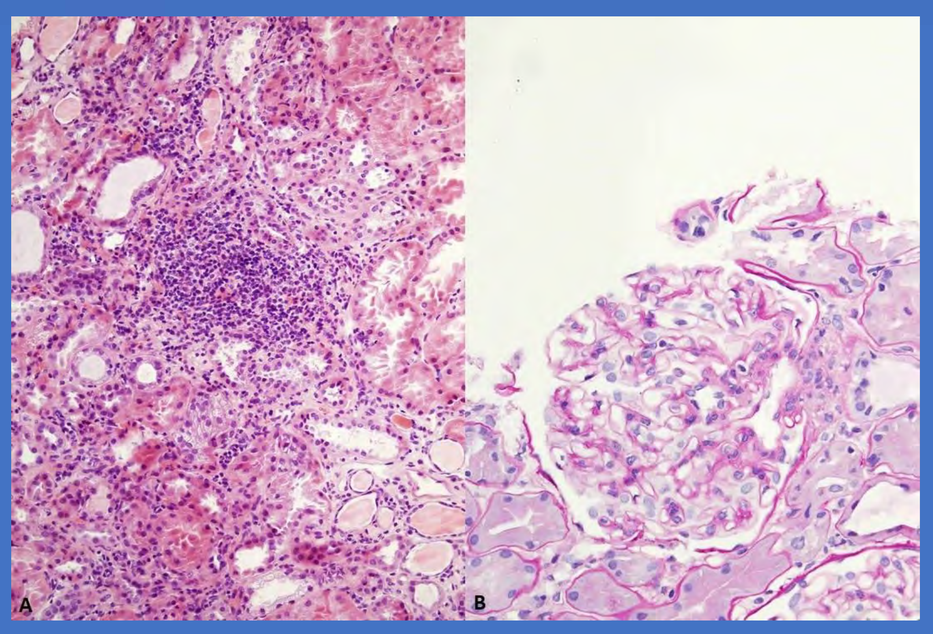

Figure 1. Kidney biopsy specimen. A) Dense mononuclear interstitial inflammation. HE stain, original magnification x200. B) Glomerulus with normal morphology. PAS stain, original magnification x400

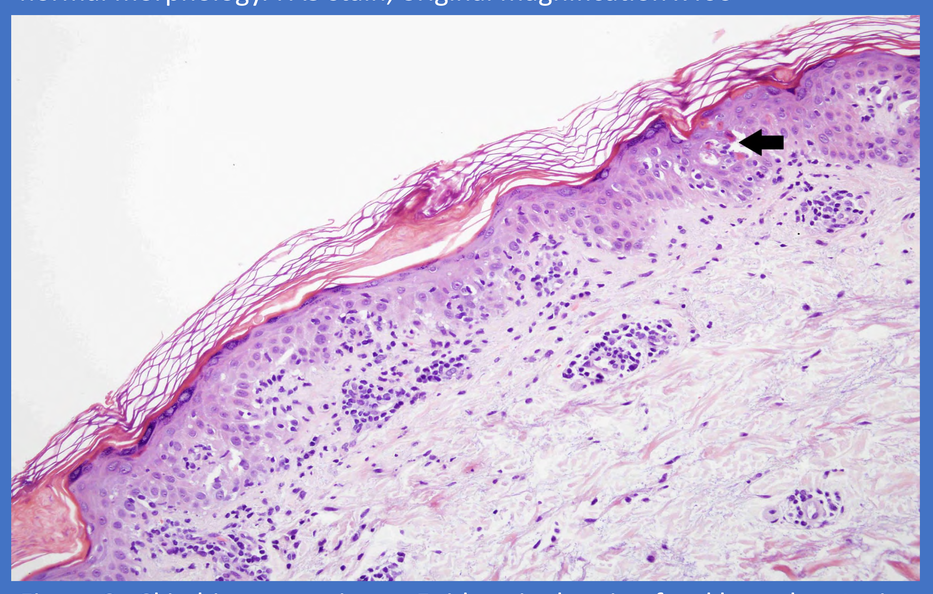

Figure 2. Skin biopsy specimen. Epidermis showing focal hyperkeratosis. There is interface change with basal vacuolisation and focal keratinocyte apoptosis (arrow). In the dermis there is perivascular mononuclear inflammation. HE stain, original magnification x200.

Conclusion

- This is to our knowledge the first case report of patient with SLS and TIN, nephrocalcinosis and renal tubular acidosis, probably related to SS.
- Concomitant presence of SLE might have influence on pathophysiological mechanisms in development of SLS.
- Simultaneous renal and pulmonary affection should encourage additional researches in pathophysiological mechanisms of these two rare disorders.

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TUBULOINTERSTITIAL NEPHRITIS AND ACUTE KIDNEY INJURY DUE TO SIMULTANEOUS HANTAAN AND PARVO B19 VIRUS INFECTION

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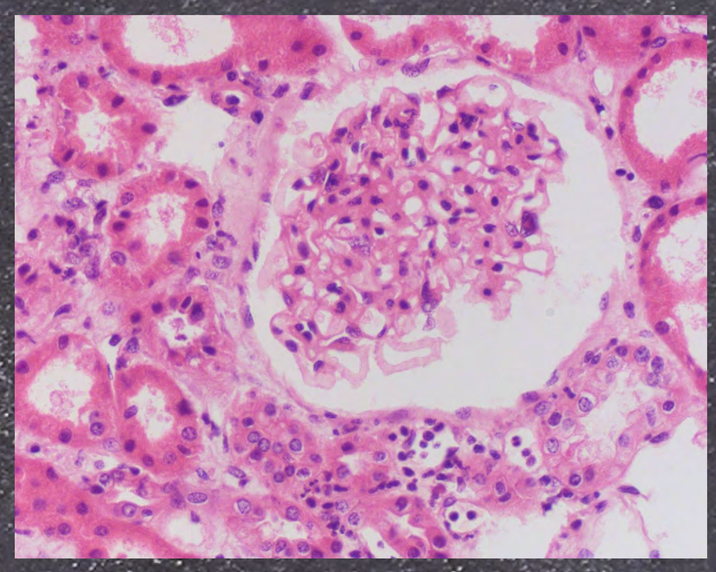
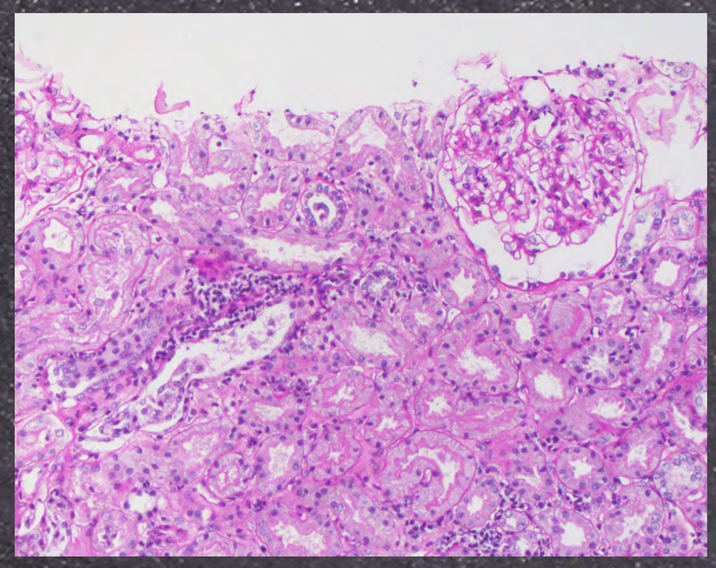
This is a case of a 41-year-old male previously healthy patient who developed AKI due to a dual viral infection. The present illness started as a fever of 38 ◦ C, weakness, muscle pain, inappetence, oliguria, and hypertension. He was a Bosnian on temporal work in Croatia with the hobby of hunting in Bosnian woods.

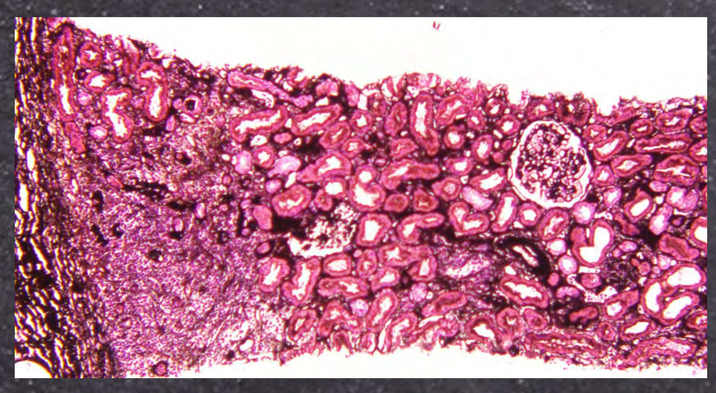
Laboratory findings revealed slightly elevated leukocytes 10.1x109/L with 5% of atypical lymphocytes and no other abnormality of a peripheral blood smear, mild normocytic anemia, and regular thrombocytes. Creatinine was 1032 µmol/L without electrolyte disturbances. Hepatic markers were regular, with a mild elevation of LDH (270 U/L), sedimentation (72 mm/3.6 ks), and CRP 28.8 mg/L. Urine analysis revealed proteinuria 2+, erythrocyturia 1+, leukocyturia, and granular casts. The immunological panel was negative. In daily urine, there were 221 mg of proteins and normal β2M and α1M. Ultrasound presented swollen kidneys and splenomegaly.

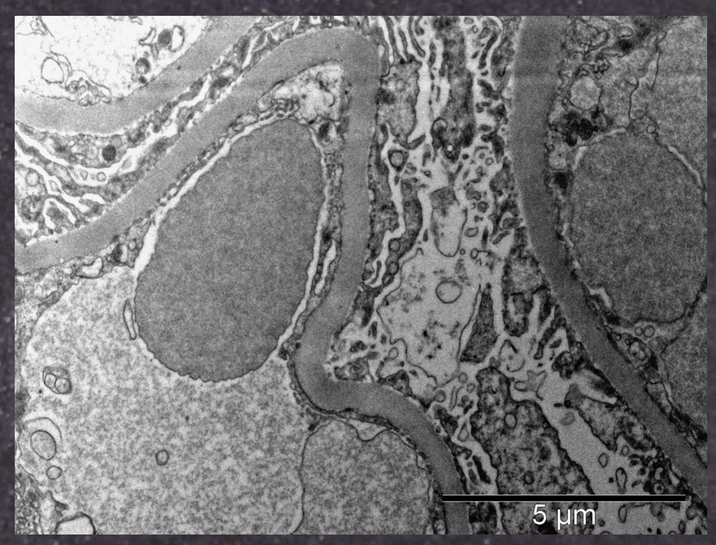
A kidney biopsy set the diagnosis of acute interstitial nephritis. The light microscopy showed edematous interstitium with lymphocyte and plasma cell infiltration. There were no IgA, IgM, IgG, Cq1, C3, C4, kappa, and lambda light chain deposits. Electron microscopy revealed an open lumen of capillaries, lined with neat endothelial cells. Only in a few lumens, there was an initial collapse with the folding of the glomerular basement membrane. GBM on average measured 362 nm, 226 - 531 nm, SD 84 nm. Podocytes had a regular ultrastructure and preserved legs. The mesangial areas were wider due to the increased amount of mesangial matrix. Immune deposits were not found. Tubules were regular or atrophic.

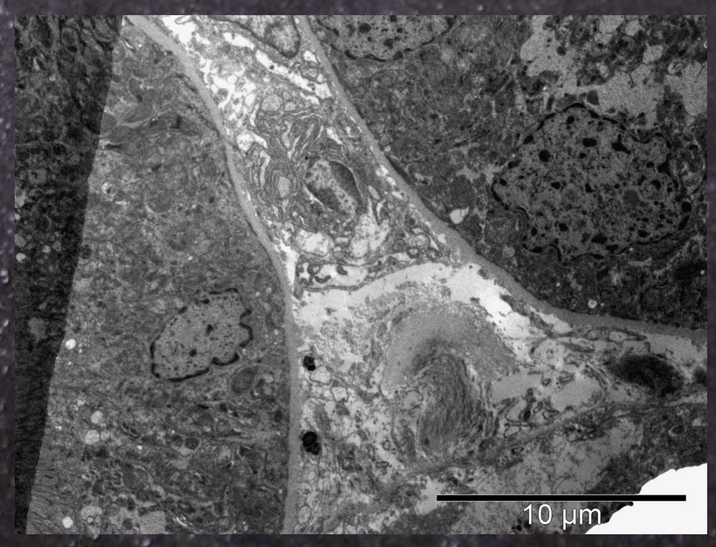
He was treated with methylprednisolone of 80 mg/day tapering through two months. After three days kidney function recovered with diuresis of 5500 ml/day. Finally, microbiological tests have shown simultaneous acute Parvo B19 and Hantaan virus infection, but with good therapeutical success.











AUTOSOMAL DOMINANT TUBULOINTERSTITIAL KIDNEY DISEASE. CHALLENGE IN DIAGNOSING WITHOUT ACCESS TO GENETIC TESTING: A CASE REPORT

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Abstract

In nephrological practice we usually face to glomerular diseases that have well-recognized symptoms. Therefore, when we see a patient with an impaired kidney function but without any sings of glomerular diseases it is always more challengeable to discover the reason of it. The present case illustrates a tubulointerstitial lesions due to possible genetic reason. A 38-year-old non-hypertensive female presented with impaired renal function (urea 10,04 mmol/L; serum creatinine 143,1 mcmol/L; GFR-Epi 40 ml/min), family history of CKD (her brother had impaired renal function and her father died in the age of 48 due to CKD), proteinuria 0,5 g/day, urinary sediment unremarkable. As on admission an elevation of serum creatinine was discovered without severe daily proteinuria and the family background showed a progressive trend to the end stage kidney disease the kidney biopsy was considered mandatory even despite the fact that at advanced stages of CKD benefits from kidney biopsy with diagnostic purposes appear to be lower. In our opinion, a primary glomerulopathy should not be the reason of impaired kidney function in this case due to the absence of a long-term, severe proteinuria and hypertension. The pathology report proved our thoughts reveling interstitial kidney disease without any other signs **(Figure)**. This fact has led us to the thought of some genetic disorders. Relying on her family history, the middle age of an onset and the progression to end stage kidney disease an autosomal dominant tubulointerstitial kidney disease was suspected. In conclusion, initially diagnosed tubulointerstitial kidney disease is likely to be secondary to mutation in genes encoding mucin-1 **(Table)**. Pathology findings in this case played the pivotal role in establishing diagnosis. However, it still needs to be proved by genetic tests.

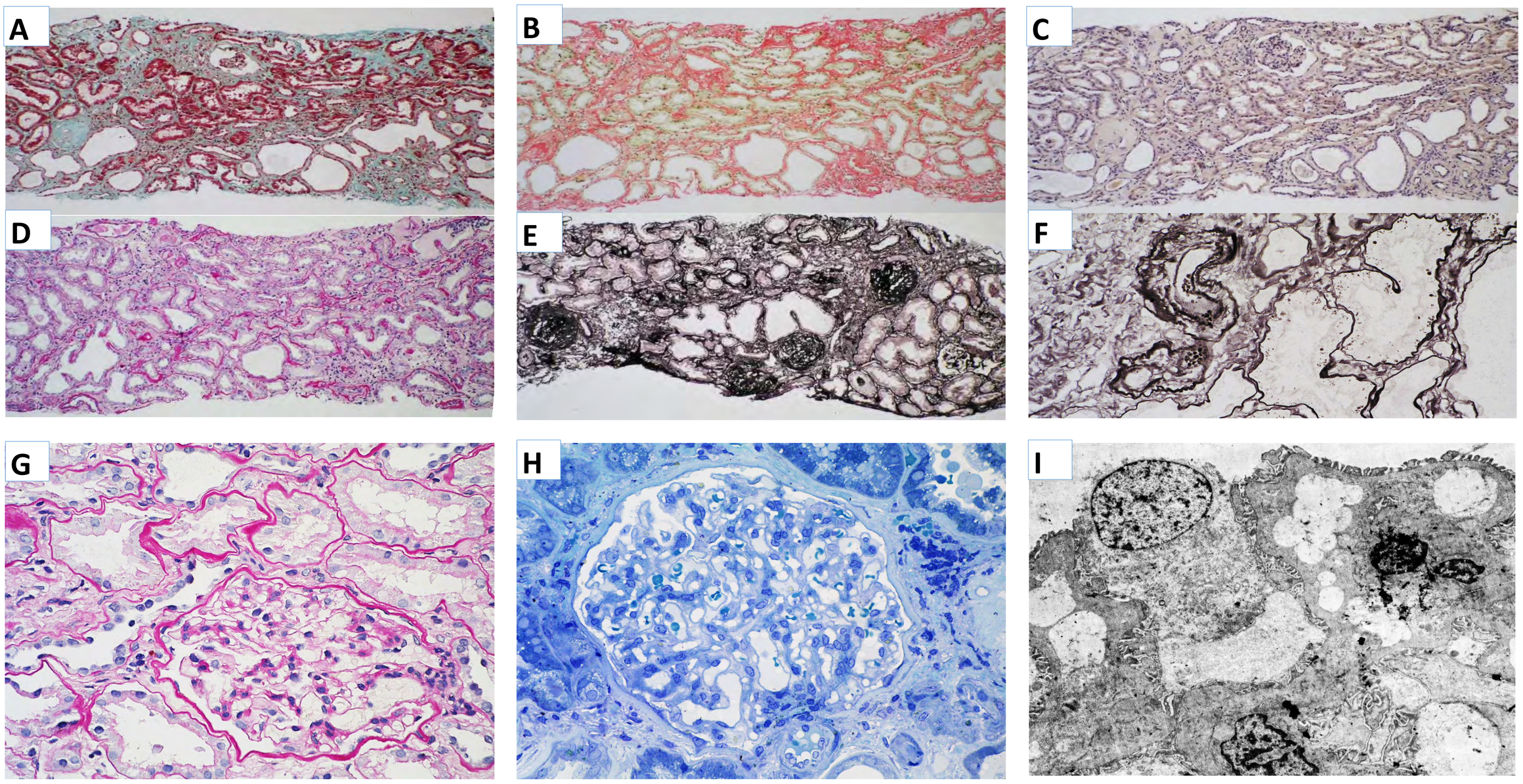

Figure. The chronic tubulointerstitial kidney disease with 58% (14/24) glomerulosclerosis and unremarkable other glomeruli.
A – Masson-Goldner stain, x100. B – Picro-Sirius, x100. C – Congo Red, x100. D – PAS, x100. E – PAMS, x100. F – PAMS, x400 G – PAS, x400, H – Semithin section, methylene blue, x400. I - Transmission electron micrograph of the relatively normal glomerulus, original magnification x2400

Table. Types of autosomal dominant tubulointerstitial kidney disease (medullary cystic kidney disease)

Characteristic	ADTKD due to UMOD mutations (ADTKD-UMOD)	ADTKD due to REN mutations (ADTKD-MOD)	ADTKD due to MUC1 mutations (ADTKD-MUC1)
Inheritance	Autosomal dominant	Autosomal dominant	Autosomal dominant
Urinalysis results	Bland without protein	Bland without protein	Bland without protein
Renal ultrasound	Normal or small kidneys, occasional cysts	Normal or small kidneys, occasional cysts	Normal or small kidneys, occasional cysts
Age of ESRD (years)	20 to 70	40 to 80	20 to 80
Kidney biopsy	Interstitial fibrosis, nondiagnostic	Interstitial fibrosis, nondiagnostic	Interstitial fibrosis, nondiagnostic
Definitive diagnosis	Genetic analysis	Genetic analysis	Genetic analysis
Associated findings	Many family members with gout, some in the teenage years	Low or low-normal blood pressure, hyperuricemia, anemia in childhood, mild hyperkalemia	No associated findings
Treatment	No specific treatment; allopurinol for gout	High-sodium diet or fludrocortisone	No specific treatment
Frequency	Rare	Very rare	Rare
Other names	Uromodulin kidney disease (UKD), medullary cystic kidney disease type 2 (MCKD2), familial juvenile hyperuricemic nephropathy (FJHN)	Familial juvenile hyperuricemic nephropathy type 2 (FJHN2)	Mucin-1 kidney disease (MKD), medullary cystic kidney disease type 1 (MCKD1)

RHABDOMYOLYSIS AND ACUTE KIDNEY INJURY: A REPORT OF 2 CASES

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Introduction:

Rhabdomyolysis is a clinical syndrome characterized by elevated serum creatine kinase (CK) and myoglobin levels due to muscle injury and the release of intracellular components into the plasma. Most commonly, it can occur due to trauma, drug abuse, or the side effects of drugs and infections.

We report two cases of trauma-related rhabdomyolysis complicated by myoglobinuric acute kidney injury (AKI). Two male patients, aged 66 and 60, presented to the emergency department with a history of general weakness, myalgia, hematuria, and decreased urine output. Both cases had traumatic injuries, and the first patient also had physical exertion a few days before admission to the hospital. Laboratory findings showed elevated serum CK (21583 U/L and 41660 U/L), creatinine levels (929 umol/L and 1409 umol/L), and CRP (58,3 and 72,2). Medical treatment included fluid resuscitation, antibiotics, and hemodialysis. Two days after the admission, both patients underwent the kidney biopsy due to worsening laboratory findings and had similar findings. Light microscopy demonstrated preserved kidney architecture. The tubules showed acute tubular injury with several intratubular pigmented casts (fig. 1). Electron microscopy demonstrated glomerular corrugation of basement membranes without any deposits. An abundant accumulation of electron dense material was noticed in the lumens of extended tubules that morphologically correspond to myoglobin casts. Immunofluorescence testing was nonspecific. Both patients showed improvement of clinical symptoms and were discharged after 2 weeks of care.

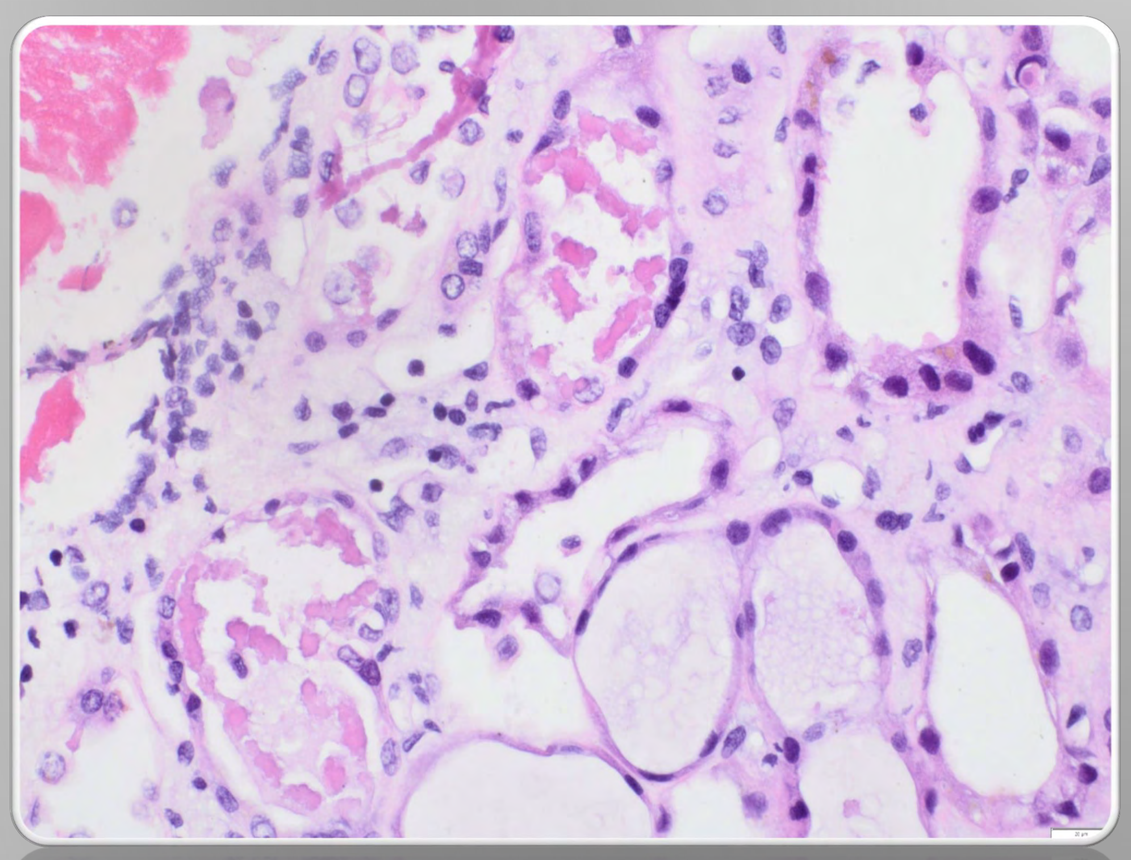

Figure 1. Intratubular pigmented casts, H&E 40x.

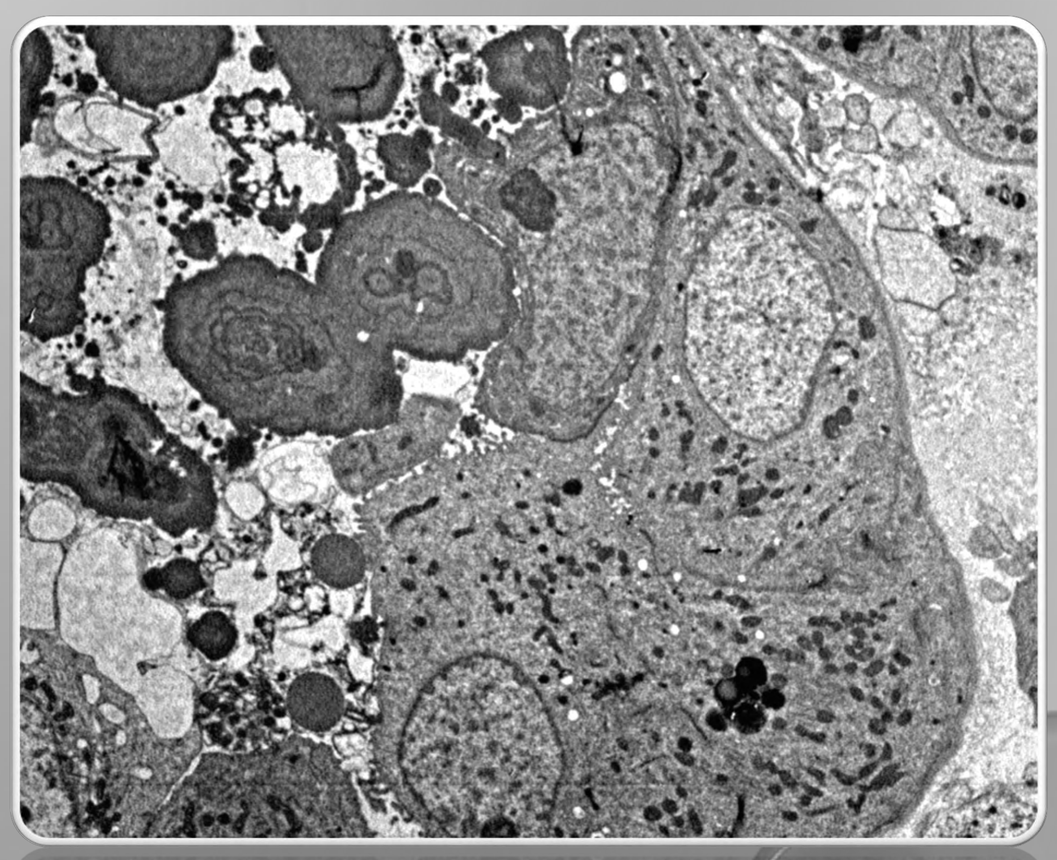

Figure 2. Electron dense material in the lumens of tubules, EM.

Conclusion:

These cases emphasize the importance of bearing in mind the possibility of rhabdomyolysis in a patient with acute kidney injury following a trauma. Rhabdomyolysis can lead to life-threatening multiorgan failure, so it is very important to timely recognize and treat such conditions.

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5th IRPC

5th International Renal Pathology Conference

18th – 20th May 2023
Zagreb, Croatia

Underlying Glomerulopathies in a Nationwide Colombian Pediatric Series with Atypical Hemolytic Uremic Syndrome

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Objective

To delineate clinical presentation, histopathological features and outcomes of Colombian pediatric patients with Atypical Hemolytic Uremic Syndrome (aHUS).

Methods

This multicenter cohort enrolled 27 Colombian children with aHUS (2010-2019). Patients grouped by age at onset. Clinical features compared using ANOVA/Fisher exact tests. Renal biopsy performed on six patients initially suspected of other renal diseases.

Results

Mostly male patients (70%) had aHUS onset before age 4 (60%), triggered mainly by gastroenteritis (52%). Pulmonary involvement (67%) was more frequent in the 1-7 age group (p=0.01) (Figure 2) Biopsies showed 3 MPGN type I, 1 MPGN type III, 1 C3GN, and 1 RPGN (Table 1) (Figure 1). Genetic screening in 5 patients identified 2xCFHR5, 2xMCP, and 1xADAMTS-13/THBD mutations. 15 relapses occurred, with 8 (72%) in 1-7 age group. Renal outcomes were similar across age groups.

Conclusion

Extrarenal involvement was frequent at presentation, particularly with pulmonary manifestations. The histopathological features support the alternative pathway hyperactivation mechanism in MPGN, C3GN and aHUS

Table 1. Clinical data of six pediatric aHUS patients with biopsy-proven glomerulopathy

ID	Age (years)	Sex	Glomerulopathy	IF (C3)	IF (IgG)	24-h PU (g)	Serum C3 (>80 mg/dl)	Serum C4 (>15 mg/dl)	Focused Therapy	Outcome
2	15	Male	MPGN I	+++	+	0,4	37	17	PE, steroids	ESKD
3	12	Male	MPGN I	+++	+	0.8	45	32	PE, steroids	ESKD
7	0,3	Male	MPGN I	+++	+	2.2	46	16	Eculizumab	Slight PU
11	3	Male	MPGN III	++	-	3.4	56	19	Eculizumab	ESKD, Died
19	0,2	Female	RPGN	-	-	0.4	132	30	PE, steroids	ESKD, Died
20	1	Male	C3GN	+++	-	0.9	28	24	Eculizumab	Slight PU

CKD, chronic kidney disease; ESKD, end-stage kidney disease; FSGS, focal and segmental glomerulosclerosis; C3GN, C3 glomerulonephritis; IF, immunofluorescence staining; MPGN, membranoproliferative glomerulonephritis; RPGN: Rapidly progressive glomerulonephritis; PE, plasma exchange; PU, proteinuria.

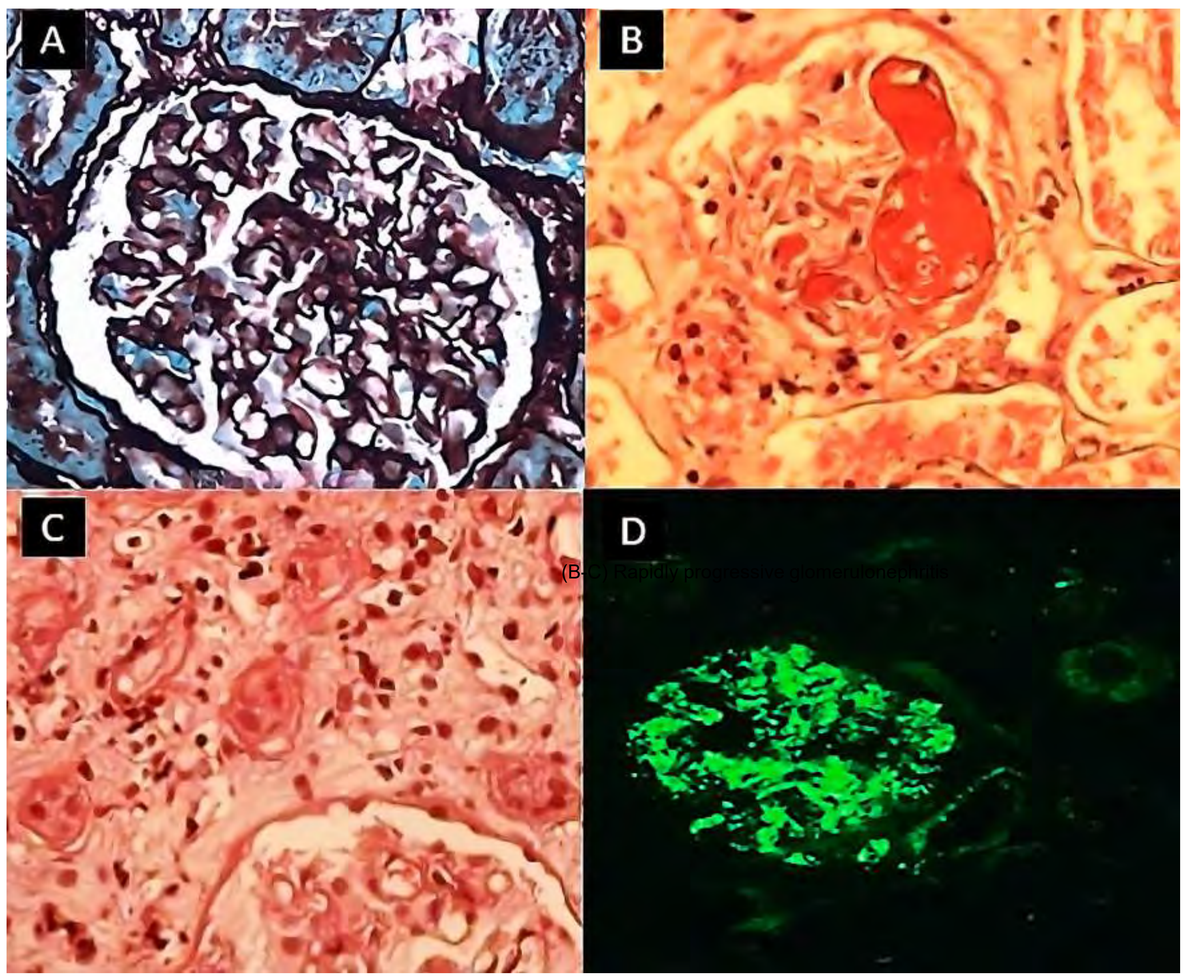

Figure 1: Light microscopy findings in children who developed glomerulopathy associated with aHUS

(A) Membranoproliferative glomerulonephritis

(B-C) Rapidly Progressive glomerulonephritis

(B) Thrombotic Microangiopathy
(C) Endothelial Proliferation and Fibrinoid Microthrombi

(D) C3 Glomerulopathy

(Anti-C3c antibody × 400)
Diffuse granular deposition

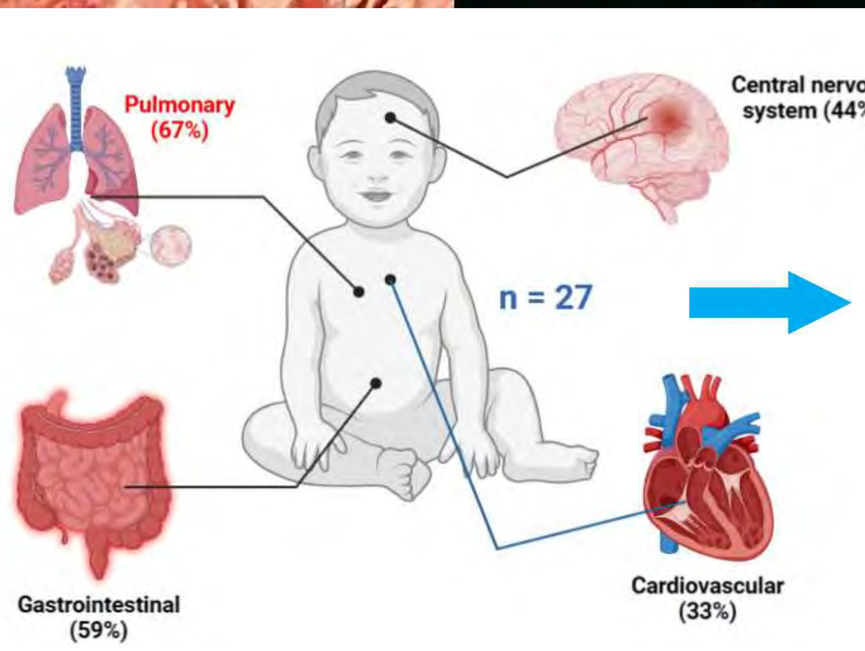

Figure 2: Extrarenal involment in Colombian Pediatric patients with Atypical Hemolytic Uremic Syndrome

Pulmonary manifestations were the most frequent extrarenal involment

SCAN ME

5th International Renal Pathology Conference
Zagreb, Croatia May 18 - May 20, 2023

MICROANGIOPATHIES IN RENAL PERCUTAENOUS BIOPSIES AT THE UNIVERSITY HOSPITAL OF SPLIT

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AIM: to present 29 years of experience in nephropathological diagnostics in University Hospital of Split, with special emphasis on microangiopathies.

METHODS: we collected clinical and pathological data of all renal biopsied patients since the first renal biopsy performed in December 1994. The most common were glomerular diseases, but here we point microangiopathies, which are relatively rare but need to be mention because sometimes dramatic clinical presentation and subtle histological features are crucial for diagnosis.

RESULTS: From 1994 until April 2023, almost 600 biopsies were performed. In the last few years, the number of biopsies has increased from 12 to 64 per year, confronting us with a daily nephrological routine and high clinical expectations (Figure 1). Since 2004, the pre-analytical process has been carried out in our laboratory, and the electron microscopic analysis at the Faculty of Medicine of the University of Zagreb. The most common diagnosis in our cochort was IgA nephropathy in men and focal segmental glomerulosclerosis in women. In the last two years, microangiopathies were diagnosed in five cases (Table 1). Characteristic findings were hyperplastic vessel wall, subendothelial edema and fibrin thrombi in vessels and glomeruli (Figure 2). In four cases the correct diagnosis was made by light microscopy, but the last one had to be corrected after expert supervision.
An autistic 17-year old patient with a history of epilepsy, was presented with hypertensive encephalopathy and retinopathy caused by renal insufficiency. Antistreptolisin titer was high. During that hospitalisation, periapical granulomas were surgicaly removed and renal biopsy was performed. Mild endocapilary proliferation, acute tubular injury, arterial thrombosis and focal fibrinoid necrosis of arterial wall, surrounded by granulomatous inflammation was found (Figure 3) and the diagnosis of postinfective glomerulonephritis with granulomatous intestitial nephritis was made. A consultation led to final diagnosis - necrotising vasculitis, polyarteritis nodosa should be excluded.

CONCLUSION: With more biopsies comes experience. In challenging nephropathological diagnostics, the help of a supervisor is sometimes required for a correct diagnosis. With the now available transmission electron microscope at the University of Split, we have the opportunity to extend our knowledge and hope to establish complete and high-quality nephropathological diagnostics.

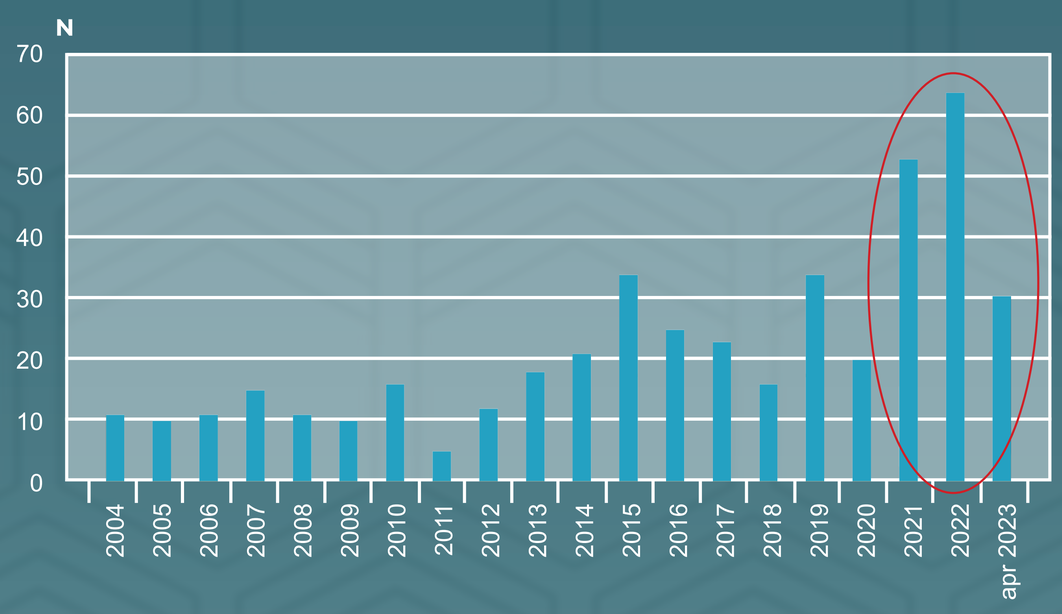

FIGURE 1. Number of percutaneous renal biopsies per year from 2004 to the present in the University Hospital of Split, Croatia

TABLE 1. Patients with microangiopathies in last few years (2020-2022)

Patient	Gender	Age	Disease
KA	m	24	Thrombotic microangiopathy
MA	f	50	Microscopic angitis
BA	m	37	Thrombotic microangiopathy
ČB	f	43	Thrombotic microangiopathy
BE	m	18	Polyarteritis nodosa

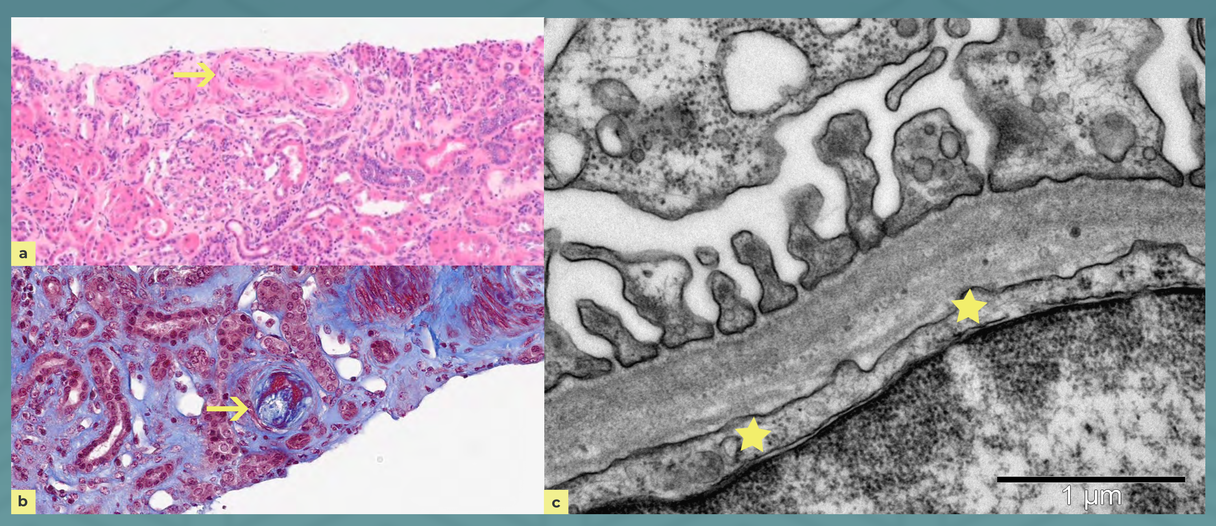

FIGURE 2. Thrombotic microangiopathy (Patient No. 3) - mucoid edema (a), luminal thrombi and fibrinoid necrosis in the wall of small blood vessels (arrows), as well as mucoid substance in the subendothelial space (asterisk) (HE and Masson trichrome 400x; EM 30.000x).

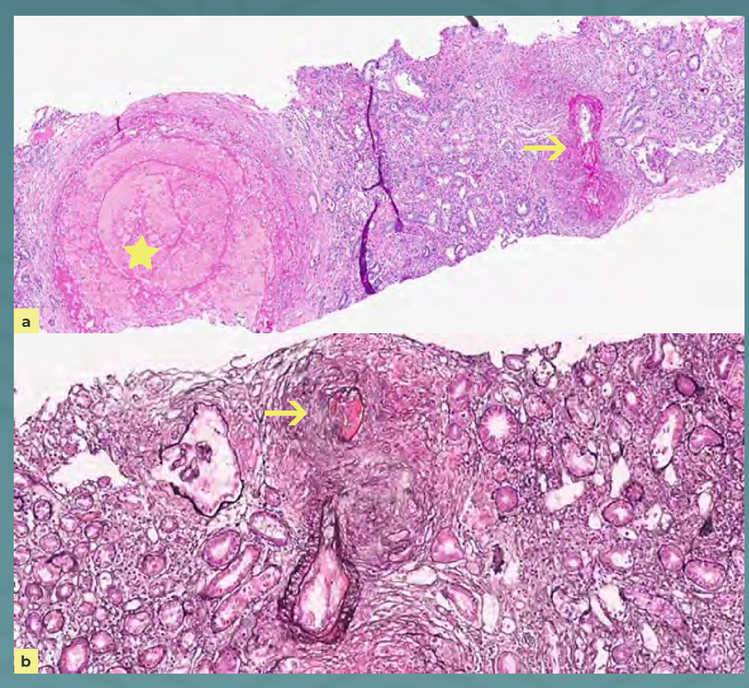

FIGURE 3. Polyarteritis nodosa (Patient No. 5) – acute necrosis and complete thrombosis of a muscular artery (a, asterisk), and fibrinoid necrosis of the wall of a small artery with granulomatous inflammation (a and b, arrow) HE and Jones 400x)

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Renal cortical necrosis and thrombotic microangiopathy caused by Plasmodium vivax- a case series

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INTRODUCTION

- Malaria is one of the world's major infectious diseases, especially in endemic developing countries
- Estimated 241 million malaria cases and 627, 000 malaria deaths were reported worldwide in 2020
- In last two decades, there have been changing trends of plasmodium vivax from benign to severe life-threatening complications
    - multiorgan dysfunction,
    - thrombocytopenia,
    - haemolytic anaemia, and
    - renal impairment
- Herein, we present three cases of renal cortical necrosis and thrombotic microangiopathy (TMA) in young females having Plasmodium vivax (P. vivax) infection

CASE REPORT

Case 1:

- 25/F with fever for 10 days and anuric for 3 days. Tested positive for Vivax on malarial card test
- Clinical investigations showed anaemia, thrombocytopenia, haemolytic anaemia & renal impairment with Sr Cr 5.7mg%

Case 2:

- 35/F diagnosed with P.vivax malaria presented with fever, headache, abdominal pain , & high coloured urine, followed by anuria. She had thrombocytopenia and hemolysis and underwent six sessions of haemodialysis but Sr Cr remained elevated (6.1mg%)

Case 3:

- 22/F diagnosed with P. vivax malaria presented with anuria for 4 days. On Lab investigation LDH was 3200IU/L and Sr Cr of 5.8mg%. She had thrombocytopenia and was transfused packed red blood cells & platelets with initiation of haemodialysis

-All the 3 patients underwent renal biopsy in view of clinical suspicion of thrombotic microangiopathy

Light Microscopy

Case 1:

- Renal cortical necrosis with infarcted glomeruli & tubules
- Entrapped malarial schizonts with peripheral haemozoin pigment in the infarcted glomeruli
- Myoglobin casts

Case 2:

- 20/23 glomeruli showed mesangiolysis
- Presence of entrapped malarial schizonts in thrombosed glomerular capillary loops
- Multiple infarcted tubules & microvascular thrombi in arterioles

Case 3:

- Diffuse cortical necrosis with malarial schizonts and haemozoin pigment in thrombosed glomeruli.
- Fibrinoid necrosis, microvascular thrombi & entrapped schizont forms in artery

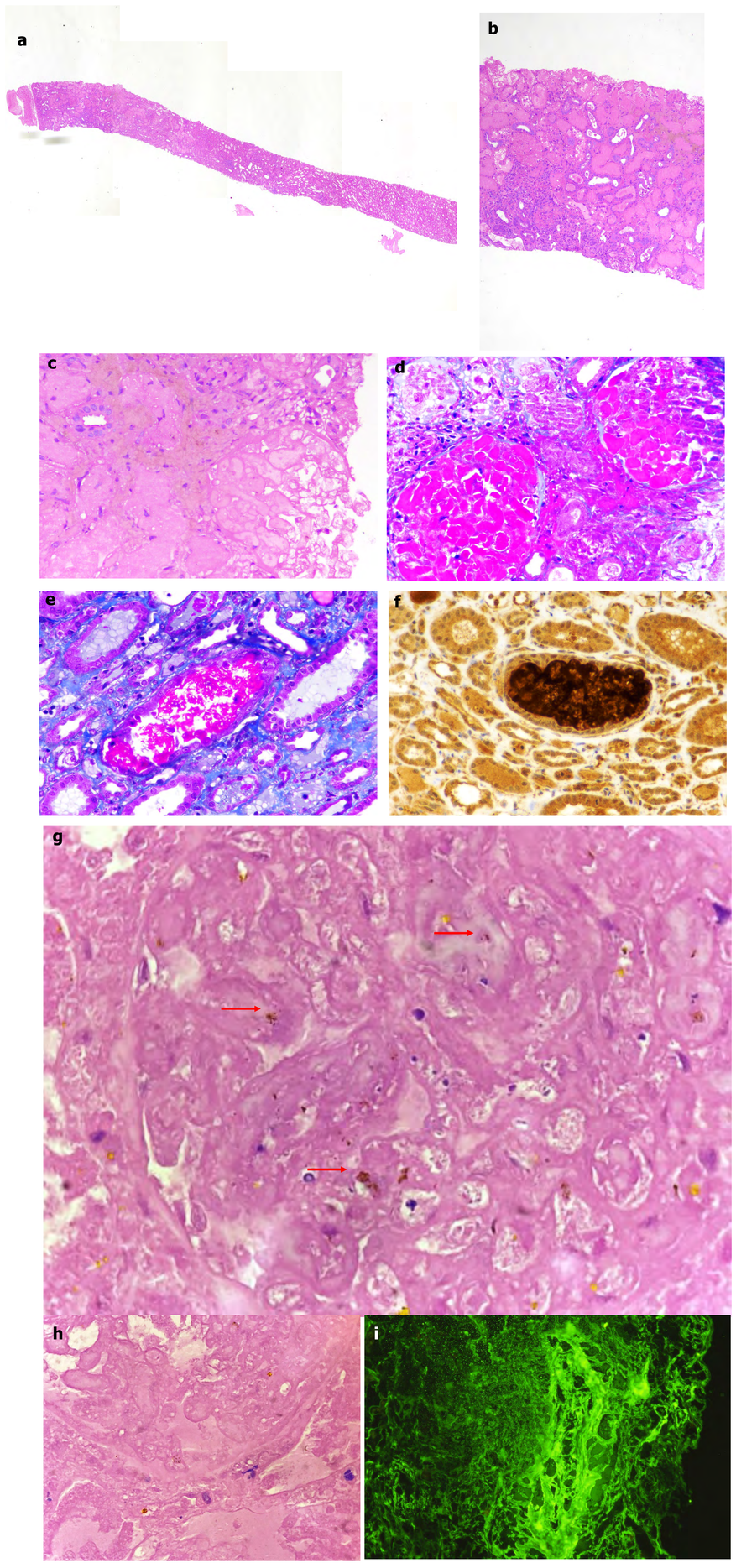

Figure a) shows patchy cortical necrosis of the biopsied core (H&E x40) b)Coagulative necrosis of tubules (H&E x100),c) The tubular & glomerular basement membranes are visible without any viable nuclei (H&E x400), d) The glomerulus shows fibrin thrombi (PAS x400),e) Occasional tubules show fragmented casts, brick-red in colour on masson's trichrome x400, f) Immunohistochemistry for myoglobin highlights the casts x400, g) & h) Many ring forms of malarial schizonts (red arrows) are seen in the infarcted glomerular capillary loops (H&E, oil immersion), i) Fibrinogen highlighted fibrinoid necrosis of the vessel on immunofluorescence microscopy x200

DIAGNOSIS

- Acute Cortical Necrosis
- Thrombotic microangiopathy
- Schizonts of P. vivax in thrombosed glomeruli
- Myoglobin casts

DISCUSSION

- P. vivax malaria is usually uncomplicated
    - Rarely fatal
- P. vivax is capable of inducing fever at levels of parasitemia lower than those causing fever in P. falciparum infection
- Host inflammatory response is activated to a greater extent
- Plasma levels of fever-inducing cytokines such as TNF-α is higher in vivax malaria compared to P. falciparum with similar parasitemia
- TNF-α is a myotoxin
- Red-cell sequestration in skeletal muscle, toxins derived from parasite, and lactic acidosis may cause myositis, myonecrosis, and rhabdomyolysis
- All three in our series developed severe complications
- They showed a spectrum of patchy to diffuse cortical necrosis with TMA along with entrapped malarial parasite schizonts within thrombosed and necrosed glomeruli & blood vessels
- Cases have been reported connecting TMA caused by p.vivax as part of an atypical hemolytic uremic syndrome(a HUS)
- The previous case reports mentioned in recent literature are majorly young females similar to our series

Article	Case- age/gender	Renal biopsy findings
V.B Kute et al. 2012	29/F	7/10 glomeruli necrosed, ACN
R kumar et al, 2014	17/f	9/15 glomeruli coagulative necrosis.
M.P Patel et al, 2015	1.20/f, 2. 24/f	PCN, organizing thrombi PCN, subintimal fibrin thrombi, endothelial swelling
R.K. Nair et al, 2019	24/f	PCN, TMA
Kaur et al, 2020	1.23 2.20 3. 22 4.30 5.50	Patchy ACN ACN Patchy ACN Multifocal cortical necrosis, scarring, chronic TMA Acute cortical necrosis, TMA
Our cases	1. 25/F 2.35/F 3.22/F	Focal ACN, TMA, myoglobin cast nephropathy Patchy ACN, TMA, ATI Diffuse cortical necrosis

CONCLUSION

To the best of our knowledge, this is the first case series displaying the presence of P. vivax schizonts in the thrombosed glomeruli & capillary loops, confirming their role in the development of TMA and related complications in infected individuals

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REVERSIBLE GLOMERULAR DAMAGE IN DISSEMINATED INTRAVASCULAR COAGULATION

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Brain death secondary to traumatic brain injury is one of the main sources of organs for transplantation. However, brain death can be associated with disseminated intravascular coagulation (DIC) in 30-50% of cases, which has been considered a relative contraindication for kidney donation.
Herein, we describe two successful transplantations in pediatric recipients who were transplanted with kidneys that had been harvested from the same donor with DIC.

The donor was a 17-year-old male who died after a head trauma. Twenty-four hours after the injury, the donor hemoglobin and platelets dropped to 8.3 g/dl and 32.000/mm^3, respectively. Serum creatinine reached 2.0 mg/dl, and urinalysis showed proteinuria (300 mg/dl).
The pre-implant biopsy (Figure 1) showed massive occlusion of glomerular capillaries by fibrin thrombi containing fragmented red blood cells and inflammatory cells, in addition to tubular damage. The glomerular capillary wall showed no damage. The arterioles and small arteries were spared, without features of thrombosis. These aspects suggested a diagnosis of DIC, allowing to rule out a thrombotic microangiopathy (TMA) (table 1).

The kidneys were transplanted in a 16-year-old girl and in a 13-year-old boy. Slow recovery of graft function was observed in both recipients. On post-operative day 3, platelets dropped to a minimum value of 66,000 and 86,000/mm^3, respectively (Table 2). None of the patients developed oliguria. On day 4, platelets started to rise in both patients.
Six months after transplantation both recipients had normal renal function. A protocol biopsy was performed in both patients showing unremarkable focal tubular atrophy, without capillaries microthrombi or other features of DIC (Figure 2)

Conclusions. Limited data are available in literature. The histology of graft DIC before transplantation may look very worrisome. However, most reports, including the present cases, indicate that this condition should not be considered a contraindication to transplantation.

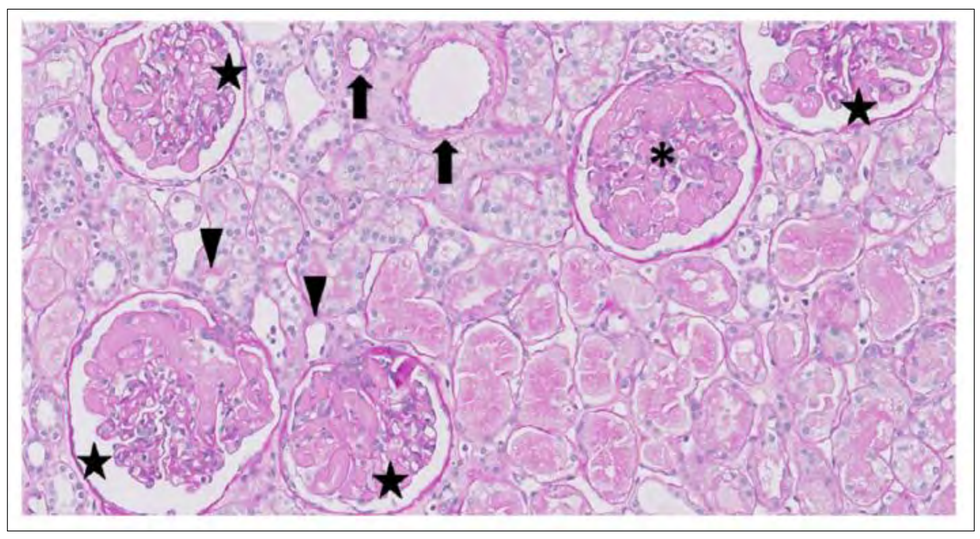

FIGURE 1 ***Massive intravascular coagulation involving all glomeruli, but segmentally (star), and globally (asterisk). Arterioles (arrowhead) and small/medium arteries (arrow) are normal***

	DIC	TMA
Localization	capillary	Small arteries, arterioles, capillaries
Vessel wall damage	minimal	severe
Thrombi formation	massive	multifocal
Fibrin stain	strong	weak
Factor VIII stain	weak	strong

TABLE 1. Differential diagnosis between DIC and TMA

	Recipient 1	Recipient 2
PT(sec)	13.9	16.9
PTT(sec)	27	30.6
Fibrinogen (mg/dl)	416	254
D-Dimer (μg/ml; vn<0.5)	3.69	0.6
Platelets (/μl)	66,000	86,000

TABLE 2. ***Hematological values of patients on POD*** **3**

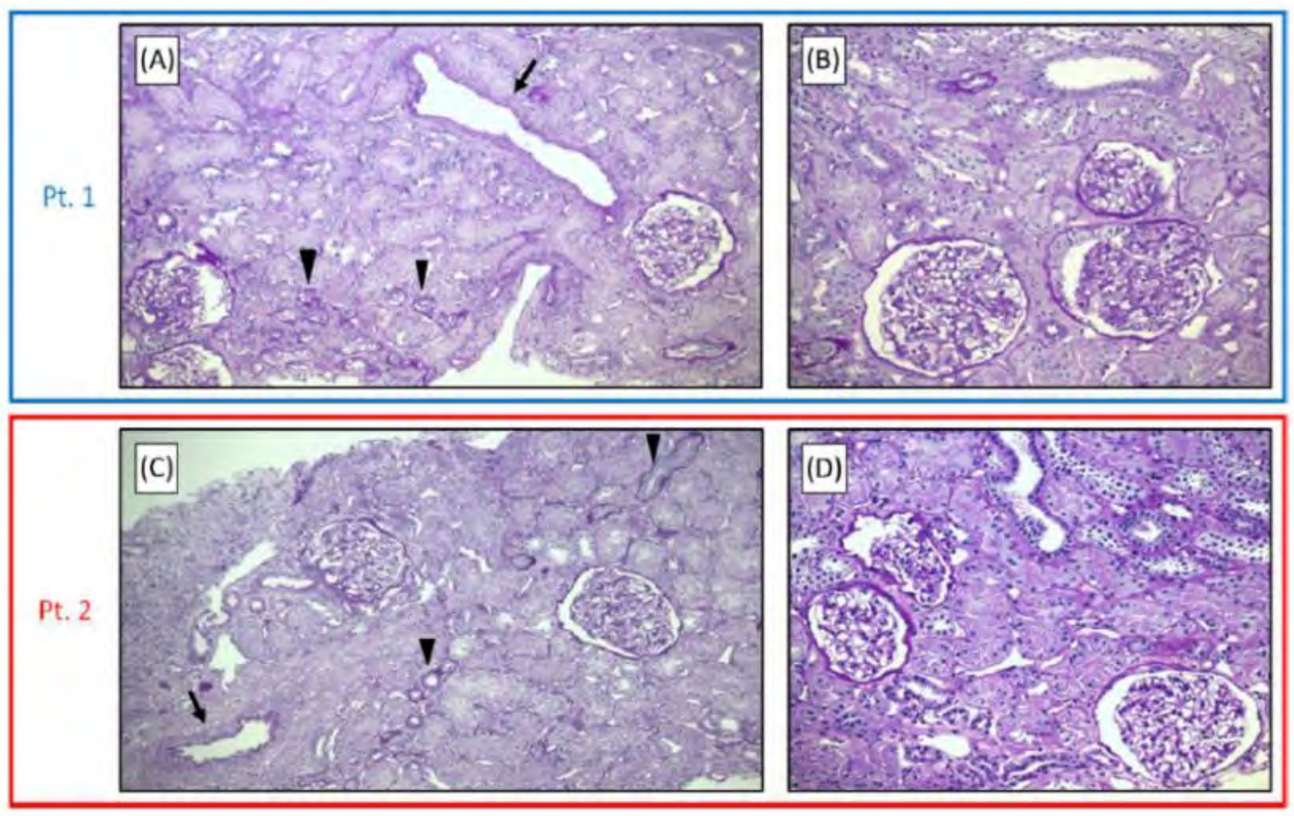

FIGURE 2 **Control biopsies at 6 months showed normal glomeruli and vessels (arrows) in both patients (patient 1: A and B; patient 2: C and D); unremarkable focal tubular atrophy was observed in both cases (arrowheads) but capillary thrombosis was not more evident in the whole tissue sample in either of them. (PAS stain; A, C: ×10; B, D: ×20).**

THROMBOTIC MICROANGIOPATHY AND CHRONIC KIDNEY DISEASE DUE TO EXCESSIVE COVID-19 VACCINATION - A CASE REPORT

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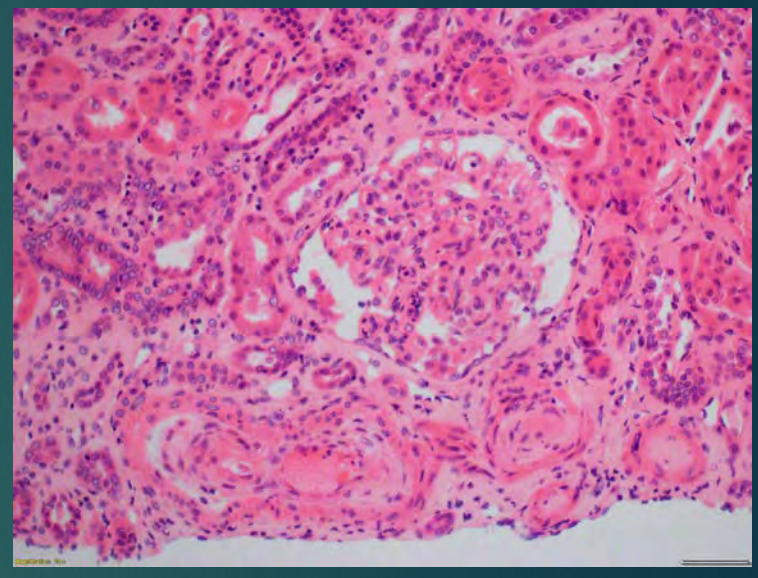
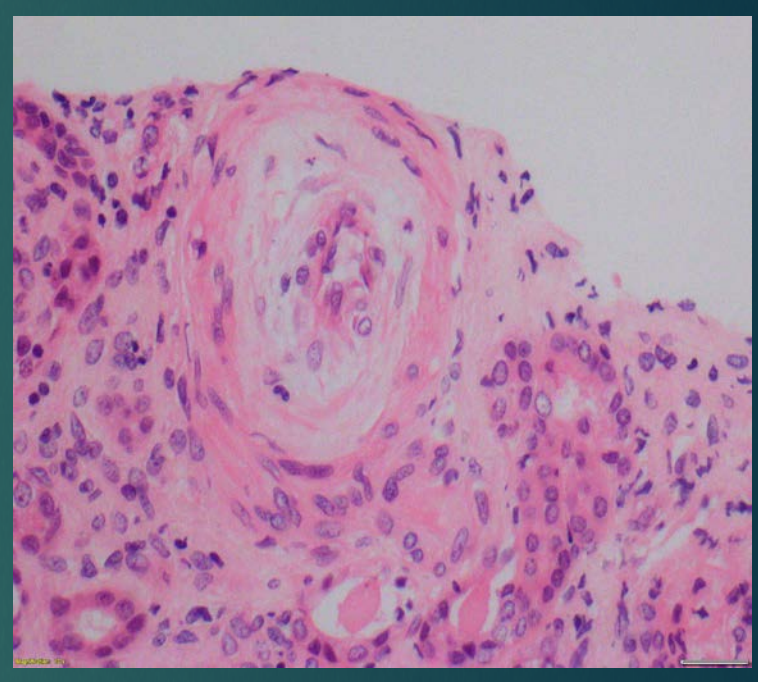
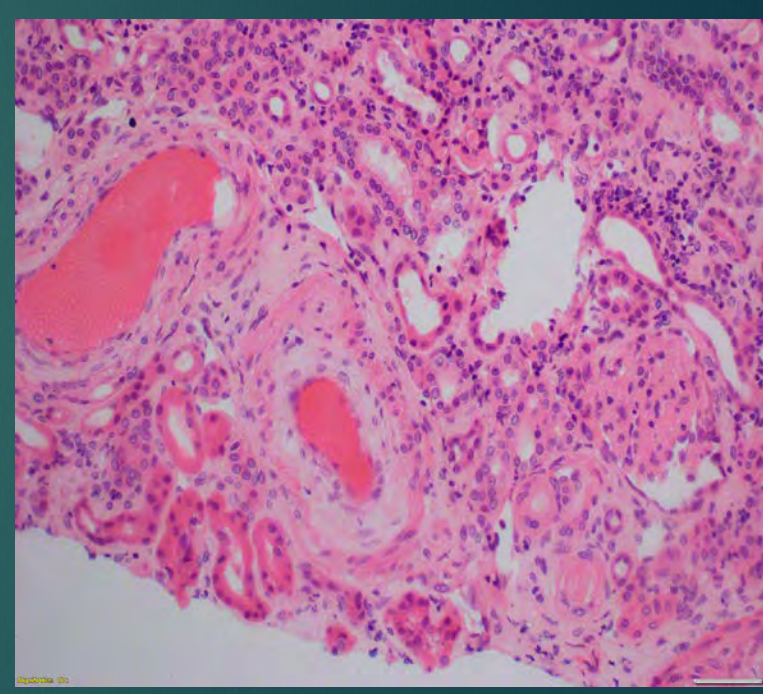
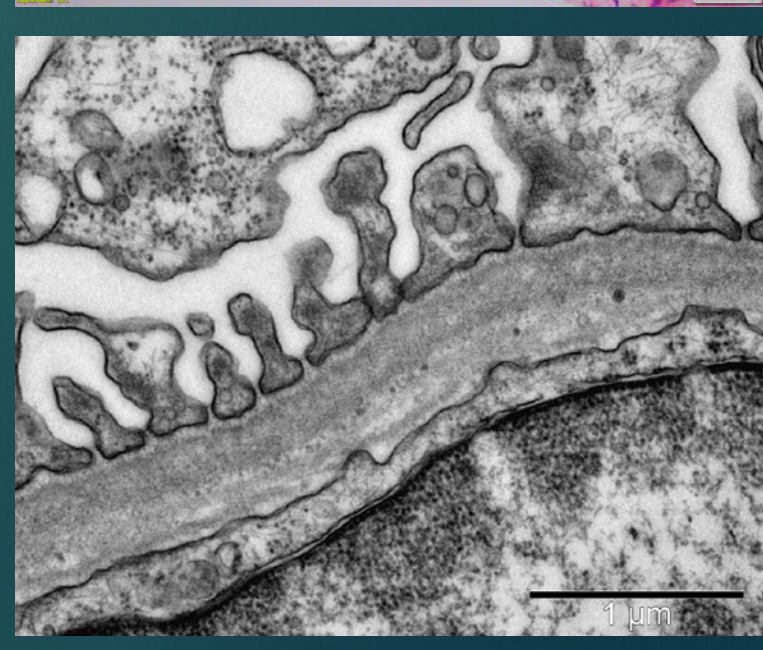
- This is a case of 36-year old male patient with thrombotic microangiopathy due to excessive COVID-19 vaccination (> 50 times) with the aim of making money. He suffered from hypertension but he did not take medications regularly and was prone to taking drugs and risk behavior.
- The present illness started as epigastralgia and inapetente. Laboratory findings revealed leukocytes 10.6x109/L, severe normocytic anemia (Hgb 79 g/L) and mild thrombocytopenia 116 x10^9/L. Creatinine was 1276 µmol/L without electrolyte disturbances. LDH was 732 U/L, sedimentation rate (128 mm/3.6 ks) and CRP 165.2 mg/L. Bilirubins were normal and haptoglobin low <0.1 g/L. Urine analysis revealed proteinuria 2+ and erythrocyturia 2+. Imunological panel was negative. In daily urine there were 295 mg of proteins and 1780 mg of B2M. Ultrasound showed normal sized kidneys with hyperehogenic parenhima. Kidney biopsy was finally performed.
- LM presented 15/48 completely connectively altered glomeruli, 3 with segmental sclerosis, 14 with collapsed capillary lumen and 4 with endothelial edema. Other glomeruli were regular. IFTA affected 46% of the cortex. Moderate tubular damage was present. The lumen of arteries was extremely narrowed due to concentric thickening of the muscle wall, somewhere with fibrinoid wall necrosis. There were no IgA, IgG, IgM and C4 deposits, but C3 and C1q deposits. In the analyzed glomerulus for EM, open capillary lumens were shown with neat endothelium. GBM had a regular ultrastructure and measured average 320 nm, 214 nm-607 nm, SD 80 nm. Podocytes had a regular ultrastructure and preserved legs. Immune deposits were not found. Tubules, interstitium and peritubular capillaries had a regular ultrastructure.
- **The diagnosis of thrombotic microangiopathy of open etiology, with moderate activity and moderate chronicity (aHUS suspected) was set.**
- However, he is still addicted to chronic hemodialysis.









IDIOPATHIC CRYOGLOBULINEMIC VASCULITIS AS A CAUSE OF THROMBOTIC MICROANGIOPATHY

Milanović Matea[1], Kasumović Dino[1], Šenjug Petar[2,3], Torić Luka[1], Zagorec Nikola[1], Galešić Krešimir[1], Horvatić Ivica[1,4], Galešić Ljubanović Danica[2,3]

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An 83-year-old male patient presented with **rash, edema and hemoptysis** in another hospital center. Laboratory signs showed **acute kidney injury, microhematuria, proteinuria and pancytopenia**. In the patient's history there were no significant chronic diseases. Kidney biopsy was performed and revealed **thrombotic microangiopathy**. Immunological findings (ANA, anti-dsDNA, ENA 6, antiphospholipid antibodies, p/c-ANCA, anti-GBM) were negative, as well as serology of HIV, hepatitis B and C. Stool samples were negative for Shigella and Salmonella and ADAMTS13 enzyme activity was within normal range. Lung biopsy indicated thickening of septa without hemorrhage. Renopulmonary syndrome was suspected, and the patient was treated with corticosteroids and cyclophosphamide. Since the patient's condition was not improving, he was transmitted to our tertiary center.

Because of the patient's state and incoherent results of the first biopsy analysis, kidney biopsy was repeated and revealed **membranoproliferative glomerulonephritis with extracapillary proliferation combined with active thrombotic microangiopathy** (Figure 1). Cytological analysis of bone marrow sample was normal and peripheral blood smears discovered few schistocytes. Levels of **cryoglobulins, rheumatoid factor and free IgM kappa chains** were high, levels of **C3** were slightly lower, whereas **C4** was extremely low. **Cryoglobulinemia type II** was found and primary TMA was excluded considering further analysis of complement parameters.

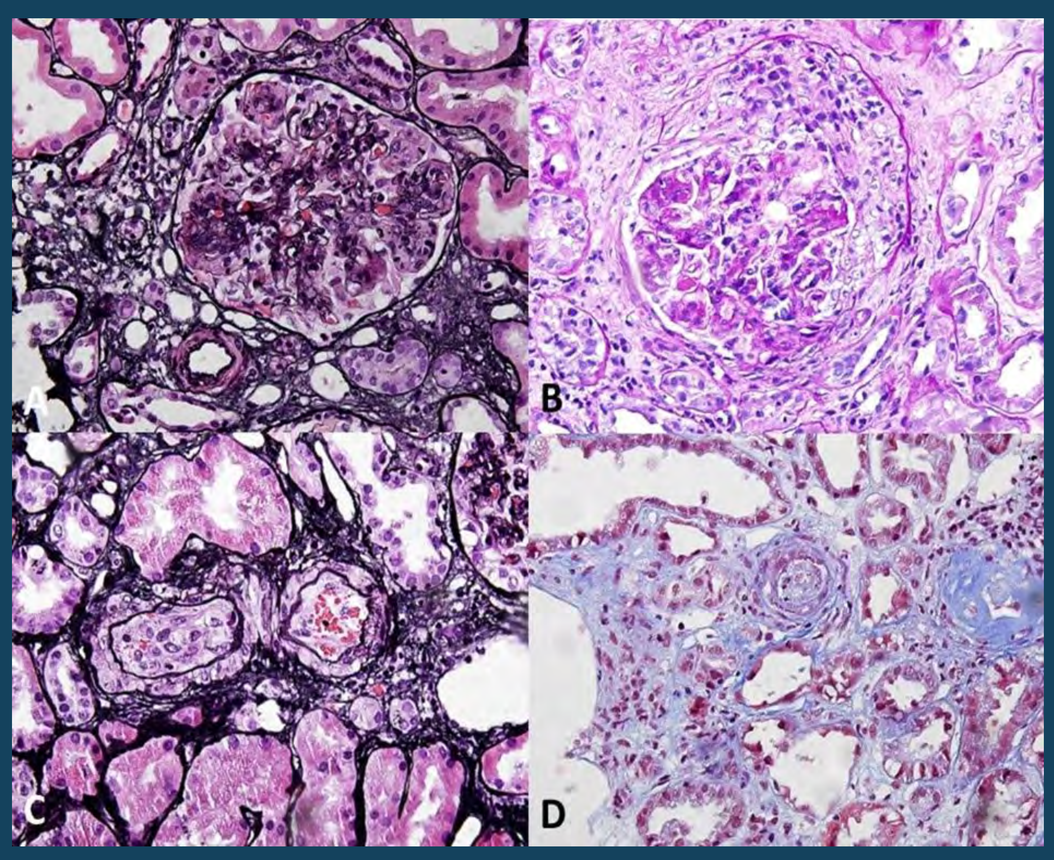

Figure 1. A) Glomerulus with membranoproliferative pattern. Jones methenamine silver, original magnification x400 B) Glomerulus with cellular crescent and hyaline thrombus. PAS stain, original magnification x400 C) Thrombus in arteriole, Jones methenamine silver, original magnification x400 D) Organized thrombus in arteriole, Masson trichrome staining, original magnification x400.

Considering **skin and lung involvement**, diagnosis of **idiopathic cryoglobulinemic vasculitis** was established and thereafter the patient was treated with one **plasmapheresis** and then 2 doses of **rituximab**. In the follow-up period renal function moderately improved, edema and rash diminished and quality of patient's life was better. In conclusion, cryoglobulinemic vasculitis, although rare, may cause thrombotic microangiopathy and only **detailed workup** can lead to proper diagnosis and treatment.

RENAL CORTICAL NECROSIS CAUSED BY TMA ASSOCIATED WITH VENOM-INDUCED CONSUMPTIVE COAGULOPATHY: REPORT OF TWO CASES.

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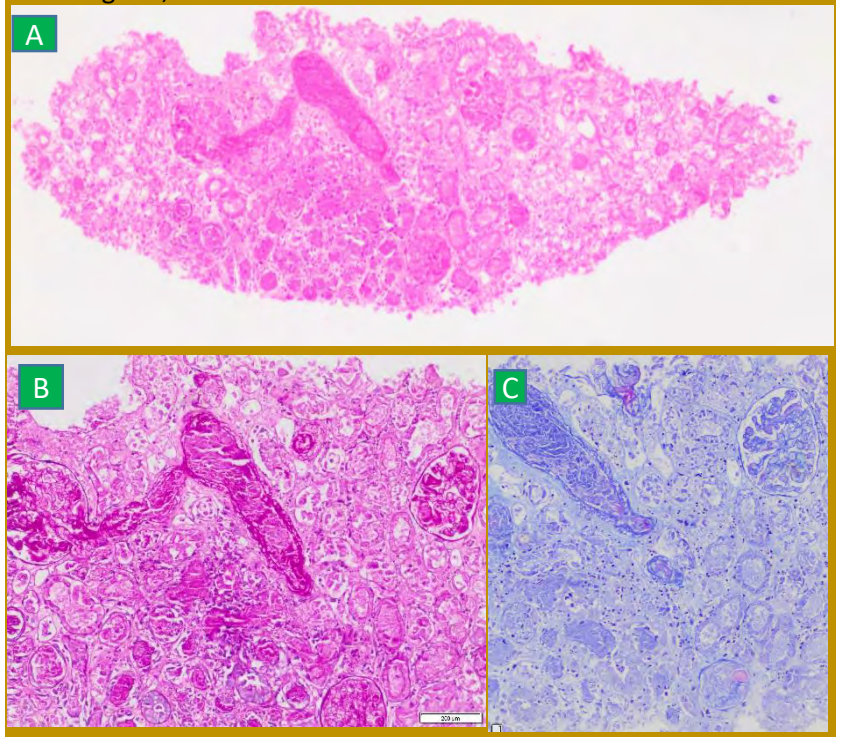
CASE 1

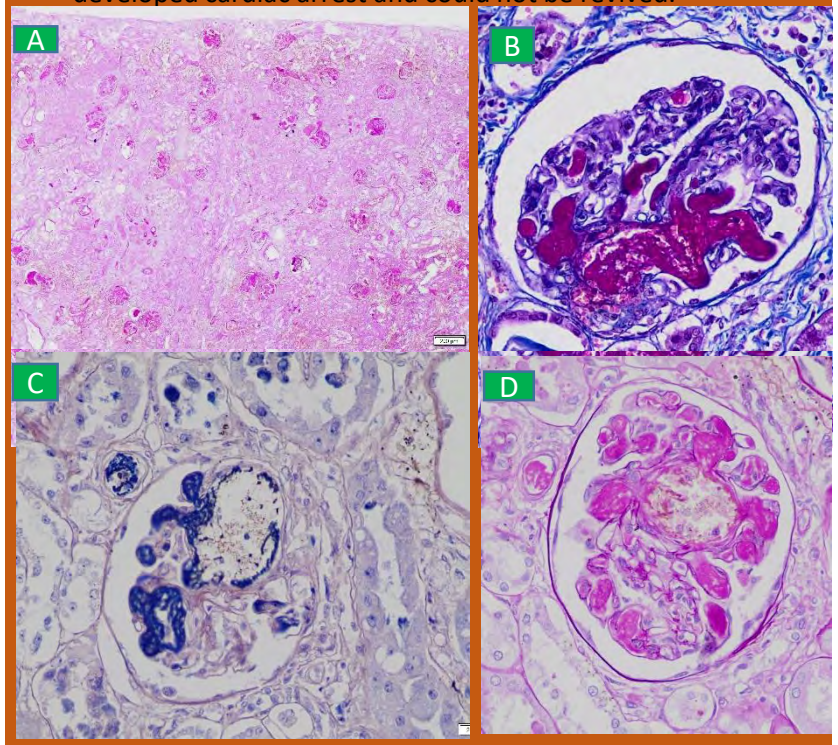
- A 10-year child had a snake bite following which 15 vials of anti-venom were given.
- Coagulation parameters revealed low fibrinogen, increased D-dimer, prolonged prothrombin time, and aPTT. Whole blood clotting time (WBCT) was >20 mins.
- During the course of admission, the child developed pallor, oliguria, and cola-colored urine.



Case 1: a) Renal cortical necrosis. b&C) fibrin thrombi in vessels and glomeruli(a & b- PAS, c-MT; a-40X, b&C- 100X)

CASE 2

- A 45-year-old female, who sustained a snake bite followed by hematuria. She received a total of 25 vials of anti-venom.
- On evaluation, WBCT was >20 mins. She had deranged INR, aPTT, low fibrinogen, raised D-dimer, and low platelet count.
- During the course of her illness, she developed oliguria and respiratory distress due to pulmonary edema. However, she developed cardiac arrest and could not be revived.



Case 2: a) Renal cortical necrosis. b&C) fibrin thrombi in glomerulus (a & d- PAS, c-PTAH, d-MT; a-20X, b-d- 400X)

Renal Pathology

Case 1: Biopsy revealed cortical necrosis involving nearly 60-70% of the sampled cortex. Fresh fibrin thrombi are noted in glomerular capillary loops and vessels.

Case 2: Renal tissue examined revealed extensive bilateral renal cortical necrosis. Thrombi are noted in the glomeruli and arterioles.

In both cases, the biopsy and clinical features were suggestive of TMA associated with venom-induced consumptive coagulopathy leading to renal cortical necrosis.

Immunofluorescence

All the immunoglobulins and complements were negative on direct immunofluorescence (DIF).

Follow-up

❖ Both cases succumbed to this snake bite.`

DISCUSSION & CONCLUSIONS

❖Snake venom-induced acute kidney injury (AKI) is multifactorial, which includes direct toxicity of venom, coagulopathy, disseminated intravascular coagulation, thrombotic microangiopathy (TMA), rhabdomyolysis, and secondary sepsis

❖Renal cortical necrosis induced by TMA is a rare and devastating complication following envenomation with hemotoxic snakes.

❖One should be watchful for the development of TMA in these cases.

RENAL INVOLVEMENT IN SYSTEMIC SCLEROSIS: SCLERODERMA RENAL CRISIS – A CASE REPORT

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- OBJECTIVE: SCLERODERMA RENAL CRISIS (SRC) IS A RARE COMPLICATION OF SYSTEMIC SCLEROSIS (SS), OCCURRING IN 5% OF PATIENTS. IT IS CHARACTERIZED BY MALIGNANT ARTERIAL HYPERTENSION (AH), ACUTE RENAL FAILURE (ARF) AND LEFT VENTRICULAR INSUFFICIENCY (LVI). SC IS MORE COMMON IN PATIENTS RECEIVING CORTICOSTEROIDS. THROMBOTIC MICROANGIOPATHY (TMA) IS DETECTED IN 40% OF SC. RENAL BIOPSY IS NOT NECESSARY IF CLASSICAL CLINICAL FEATURES ARE PRESENT, BUT HELPS TO DETERMINE THE DIAGNOSIS IN ATYPICAL FORMS.
- AIM: WE PRESENT A CASE REPORT OF A PATIENT PRESENTING WITH SC.
- CASE REPORT: A 71-YEAR-OLD FEMALE PATIENT WAS ADMITTED DUE TO DYSPNEA, CHEST PAIN, ARF, AND UNREGULATED AH. FOUR MONTHS EARLIER SHE WAS DIAGNOSED WITH DIFFUSE CUTANEOUS SS WITH THE AFFECTION PREDOMINANTLY OF THE SKIN, BUT ALSO PERIPHERAL CIRCULATION AND ESOPHAGUS. LOW DOSE CORTICOSTEROID THERAPY WAS STARTED. AT ADMISSION, HER BLOOD PRESSURE WAS UP TO 180/100 MMHG, SHE WAS DYSPNEIC WITH AUDIBLE CREPITATIONS IN THE LOWER THIRDS OF HER LUNGS. SHE HAD MICROSTOMIA AND TIGHT SKIN ON THE FINGERS AND HANDS WITH ULCERATION ON THE FINGERS. WORKUP SHOWED ARF REQUIRING DIALYSIS, AND A MYOCARDIAL AFFECTION WITH LVI. A KIDNEY BIOPSY WAS PERFORMED, FINDINGS CORRESPONDED TO TMA WITH INVOLVEMENT OF EXTRAGLOMERULAR BLOOD VESSELS AND MOSTLY SECONDARY GLOMERULAR CHANGES (MESANGIOLYSIS, ARTERIOLES WITH SWOLLEN ENDOTHELIUM IN THE VASCULAR POLES). FIBRINOID NECROSIS OF ARTERIOLES WAS FOUND (FIGURES 1,2, AND 3). ANGIOTENSIN-CONVERTING ENZYME (ACE) INHIBITOR WAS STARTED, BUT DUE TO AN UNFAVORABLE CLINICAL COURSE, PLASMA EXCHANGE, AND VASODILATORS WERE ALSO ADDED. THE PATIENT'S CONDITION DETERIORATED WITH A FATAL OUTCOME.

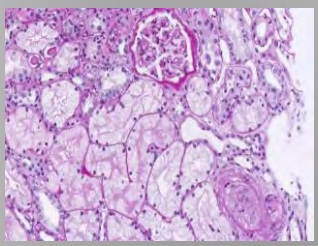

FIGURE 1. MUCOID PROLIFERATION WITH OBLITERATION OF ARTERY, ACUTE TUBULAR INJURY WITH ISOMETRIC VACUOLISATION AND BLOODLESS GLOMERULUS (PAS, 200X)

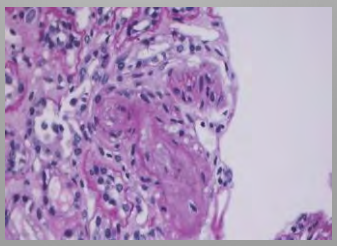

FIGURE 2. FIBRINOID NECROSIS OF ARTERIOLE (PAS, 400X),

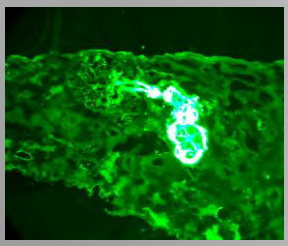

FIGURE 3. STRONG FIBRINOGEN POSITIVITY IN EXTRAGLOMERULAR ARTERIOLE AND VASCULAR POLE OF THE GLOMERULUS

- DISCUSSION: IN OUR PATIENT, THE DIAGNOSIS WAS PROMPT. HOWEVER, HER CONDITION DETERIORATED IN TERMS OF CARDIAC AND RENAL FAILURE.
- CONCLUSION: SRC IS A RARE BUT SEVERE COMPLICATION OF SS. IT IS CRUCIAL TO PROMPTLY INTRODUCE AND CONTINUE THE THERAPY WITH ACE INHIBITORS DESPITE THE END STAGE RENAL FAILURE BECAUSE IT GREATLY IMPROVES PROGNOSIS. HOWEVER, THE 5-YEAR SURVIVAL IS LOW (65%). ADDITIONAL THERAPIES ARE NEEDED TO IMPROVE THE PROGNOSIS.

MORPHOLOGY AND CLINICAL PRESENTATION OF PAUCI-IMMUNE SMALL VESSELS VASCULITIDES IN KINDEY

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Aim: Pauci-immune small vessels vasculitides in kidney, could be divided into ANCA associated vasculitides (AAV) and non-ANCA associated vasculitides (NAAV). Our aim was to examine and compare clinical and pathohistological characteristics of small vessel vasculitides in kidneys of patients with AAV and NAAV.

Methods: In this study we performed a retrospective analysis of pathohistological kidney biopsies, diagnosed between 2000-2020, at Institute for Pathology "Dr Đorđe Joannović" Faculty of Medicine University of Belgrade. Overall, 157 kidney biopsies with pauci-immune small vessels vasulitides were examined (79 patients with AAV and 78 patients with NAAV). Clinical data collected from available medical records, and pathohistological parameters were examined and compared between the AAV and NAAV patients.

Results: Table 1 summarized all investigated clinical and pathohistological parameters. ANCA positive patients were significantly older (55.5±17.6 years) than ANCA negative (45±18.1 years) patients, and they also had significantly higher frequency of glomeruli involved by crescent formations (≈40%) compared to patients without ANCA (≈30%), as illustrated in *Table 1*. Moreover, ANCA positive patients had slightly higher frequency of chronic lesions such as interstitial fibrosis, tubular atrophy and glomerular sclerosis. Laboratory parameters were high in both groups, without significant difference. However, mean CRP values were higher in ANCA positive patients. Clinically assessed BVAS score was similar in both investigated groups. The morphology of heterogenious presentation is illustrated on Figures 1 and 2.

Conclusion: Patients with ANCA positive renal vasculitis are significantly older and have higher frequency of chronic glomerular and tubulointerstitial lesion, while laboratory and clinical parameters are not significantly different between ANCA positive and ANCA negative patients.

Table 1. Clinical and pathohistological parameteres in patients with pauci-immune small vessels vasculitides in kidney.

		ANCA -	ANCA +	p
Gender n (%)	male	33 (48,5%)	35 (51,5%)	0,695
	female	46 (51,7%)	43 (48,3%)	
Age (years)		45±18,1	55,5±17,6	**<0,001**
Total crescents (%)		29,39	39,59	**0,013**
Cellular crescents (%)		7,19	9,05	0,331
Fibrocellular crescents (%)		21,9	28,06	0,136
Fibrous crescents (%)		1,11	2,49	0,159
Fibrinoid GBM necrosis (%)		30,08	33,85	0,357
Segmental glomerulosclerosis (%)		5,92	9,35	0,080
Global glomerulosclerosis (%)		7,57	7,42	0,948
Tubular atrophy (%)	+	74 (94,9)	74 (96,1)	0,712
	-	4 (5,1)	3 (3,9)	
Interstitial inflammation (%)	+	71 (49,3%)	73 (50,7%)	0,360
	-	7 (63,6%)	4 (36,4%)	
Interstitial fibrosis n(%)	+	72 (49%)	75 (51%)	0,099
	-	5 (83,5%)	1 (16,7%)	
CRP (mg/l)		29±41,96	77±65,96	0,124
Creatinine (µmol/l)		499,8±421,5	492,9±330,2	0,917
BVAS score		11,78±4,67	12,60±4,21	0,292

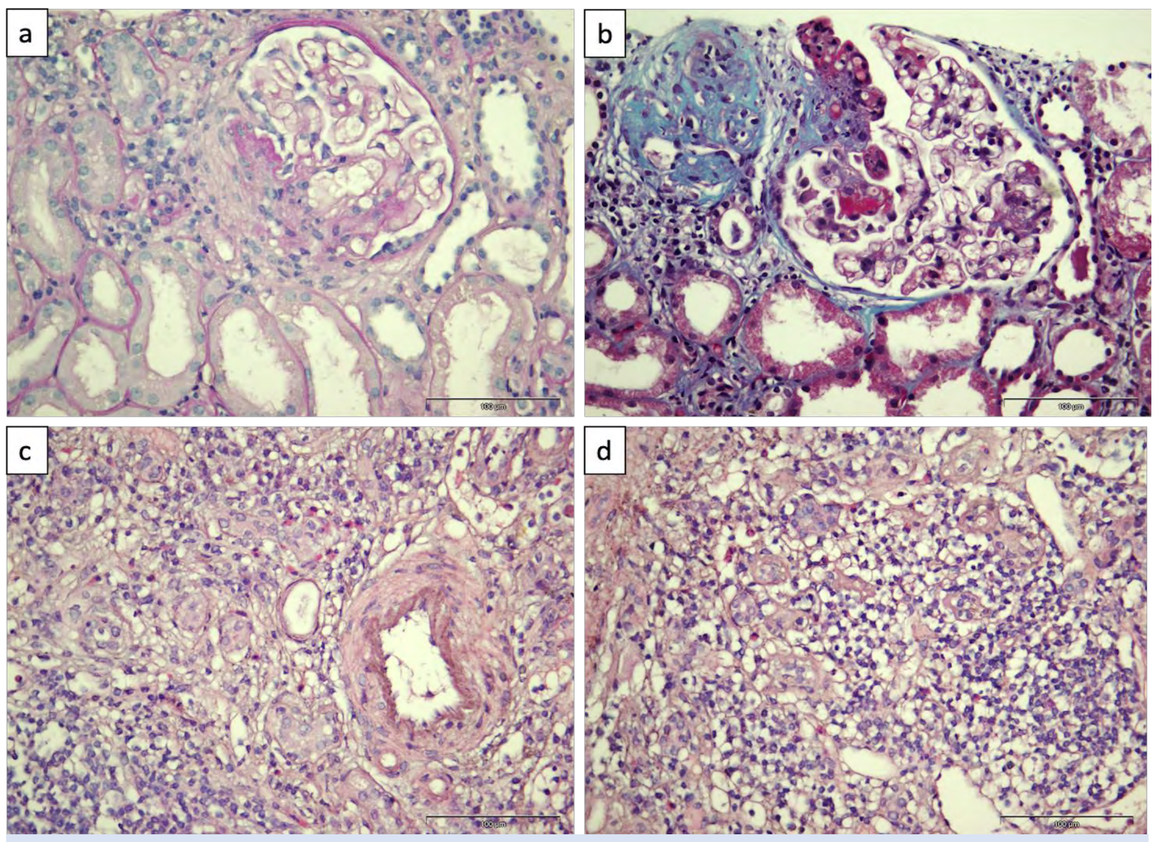

Figure 1. Interesting case of EGPA (Churg-Strauss syndrome). A) segmental fibrinoid necrosis of glomerular basement membrane (GBM) clearly visible on HE and B) MTS stained slides; C) tubulointerstitial compartment was extensively affected with diffuse infiltration of mononuclear inflammatory cells with focally prominent eosinophil-rich accumulation; eosinophils were found in a form of granulomatous eosinophil-rich inflammatory process surrounding blood vessels and D) occupying interstitial space between tubuli.

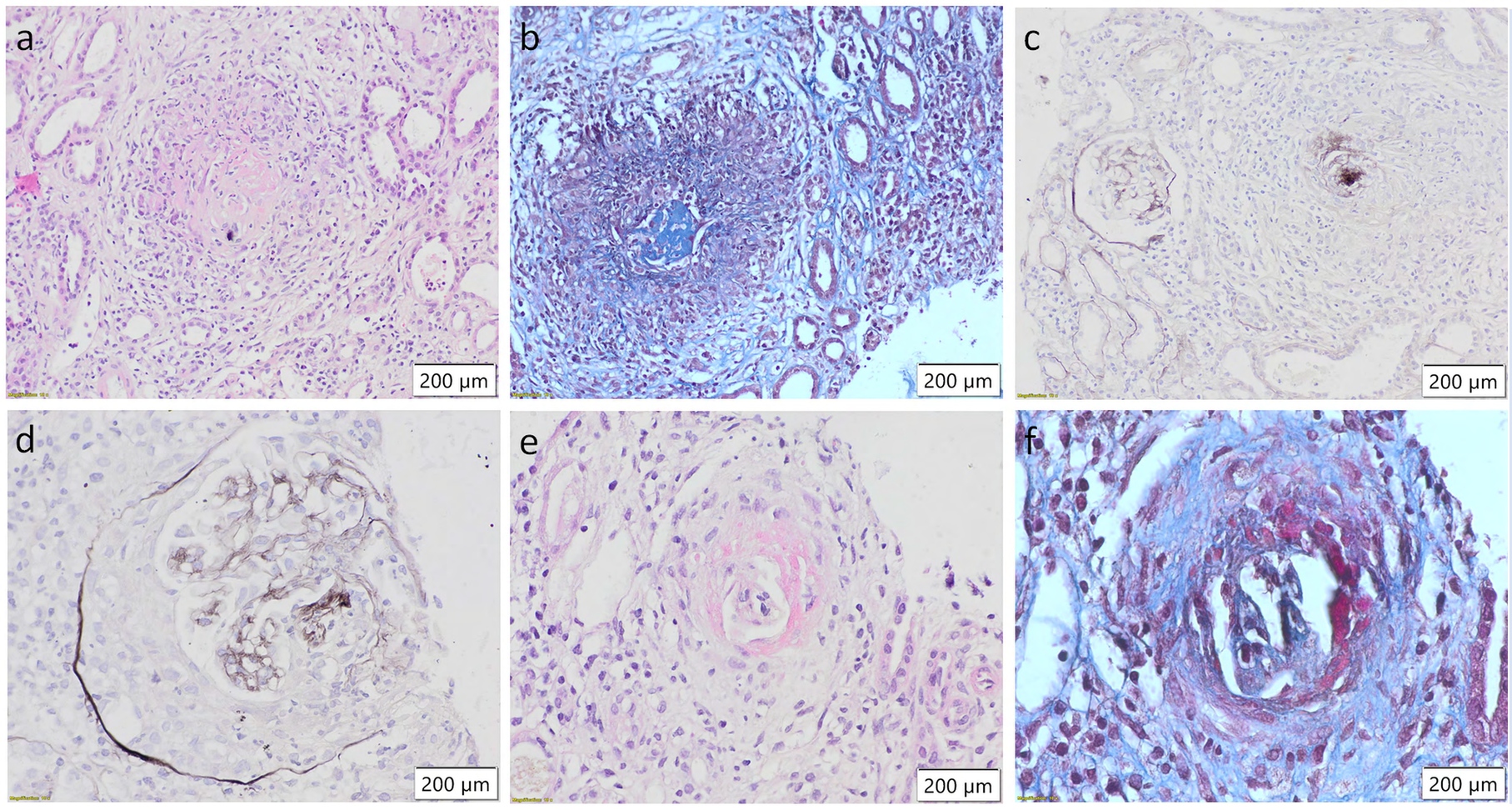

Figure 2. Interesting case of GPA (Wegener granulomathosis). A) fibrinoid necrosis with granulomatous inflammation, visible on HE slide; B) the same area stained with MTS and with C) methenamine silver stain; D) fibrinoid necrosis of GBM with crescent formation and partial distruption of Bowman capsule; E) fibrinoid necrosis of medium sized blood vessles on HE and F) MTS staining.

RENAL TISSUE EXPRESSION OF microRNAs IN ANCA-ASSOCIATED VASCULITIS

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OUTLINE & AIM

In ANCA-associated vasculitis (AAV), the accurate characterization of disease-specific microRNAs (miRNAs) is lacking despite mounting evidence of their implication in the pathogenesis of AAV.

Renal tissue miRNA expression profile in AAV has not been comprehensively studied, especially compared to both healthy adults and patients with various glomerulonephrites other than AAV. To identify a potential AAV-specific miRNA expression profile, we have compared pooled tissue samples of treatment-naive AAV to both healthy controls and controls with other glomerulonephrites.

Disease- and tissue-specific alterations of miRNAs thus characterized could serve as non-invasive biomarkers of disease activity and of the underlying AAV-related renal inflammatory involvement. Additionally, this knowledge could contribute to better understanding of the multifactorial etiopathogenesis of AAV by addressing its epigenetic aspect.

METHODS

Pooled RNA isolates from formalin-fixed, paraffin-embedded renal biopsy material of 26 treatment-naive MPO^+ and $PR3^+$ AAV patients with florid renal involvement and 26 control patients (10 without pathohistological change on light microscopy, ie. CTRL and 16 with various glomerulonephrites other than AAV, ie. GN) were included for the comprehensive miRNA expression profiling of the 752 panel-included miRNAs by qPCR.

↓

Differences in individual miRNA expression values ($\Delta\Delta$Ct), normalized to global mean average, were tested for statistical significance between the pooled samples.

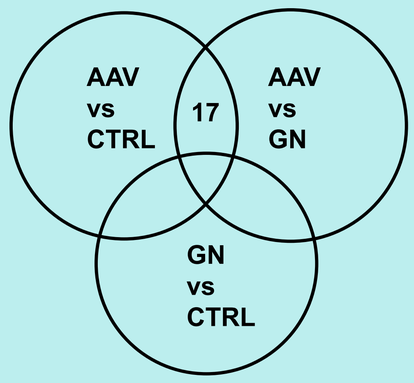
↓

The miRNAs demonstrating statistical significance in expression difference between AAV and control group pools were then annotated to signalling pathways and to their targets.

Age, gender, eGFR, daily proteinuria values and the degree of interstitial fibrosis/tubular atrophy at kidney biopsy were recorded for each included case and every effort was made to match AAV patients and controls by age, gender, and IF/TA to the maximum extent possible.

RESULTS

17 individual miRNAs differentiated AAV from control pools with statistical significance.
A considerable subset were implicated in processes considered important in AAV pathogenesis such as monocyte and macrophage polarization, T-cell activation/differentiation, renal fibrogenesis, endothelial injury and in cytokines such as IL-6 and B-cell activating factor.



The screening process also identified 7 miRNAs that have not yet been affiliated with the pathogenesis of any non-neoplastic renal disease.

MicroRNA	MicroRNA FAMILY	KNOWN IMPLICATIONS
hsa-miR-21-3p	no	TGF-β/Smad3 signaling (fibrosis)
hsa-miR-24-2-5p	miR-24	/
hsa-miR-30a-3p	miR-30	BAFF, Notch1, p53 signalling (podocyte injury)
hsa-miR-30b-5p	miR-30	IFN-α signalling (mesangial proliferation in LN), Notch1, p53 signalling (podocyte injury)
hsa-miR-30c-5p	miR-30	Notch1, p53 signaling (podocyte injury)
hsa-miR-96-5p	no	/
hsa-miR-130b-5p	miR-130b	/
hsa-miR-142-5p	no	SOCS1/STAT6 signalling (macrophage polarization)
hsa-miR-150-5p	no	PU.1 transcription factor (macrophage polarization)
hsa-miR-181a-5p	no	SHP2/STAT3 signalling (macrophage polarization)
hsa-miR-204-5p	miR-204/211	IL-6 receptor (chemokine generation in renal tubular epithelium)
hsa-miR-376a-5p	miR-376	/
hsa-miR-508-3p	miR-606	/
hsa-miR-542-5p	no	TGF-β signaling (Th17 and T_{reg} differentiation)
hsa-miR-582-5p	no	FOXO1 (monocyte apoptosis)
hsa-miR-769-5p	no	/
hsa-let-7a-5p	let-7	CD11b signaling (macrophage polarization)

CONCLUSION

Altered expression of miRNAs in AAV-affected renal tissue and their (potential) tissue-serum concordance could and should form the basis for determining AAV-related renal involvement noninvasively. The fact that a substatial proportion of hitherto identified miRNAs relate to established biological processes that are considered to be important in AAV pathogenesis lends credence to the results. However, a validation study of these candidate differentiatially expressed miRNAs in an independent and larger cohort is mandatory to establish their diagnostic and/or prognostic utility.

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