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# Kidney Histopathology of Patients with Hepatitis C Infection and Diabetes Mellitus before and after Availability of Direct-Acting Antiviral Therapy

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**Keywords**
Hepatitis C · Diabetes · Direct-acting antiviral agent · Immune complex · Glomerulonephritis

**Abstract**

***Introduction:*** Type 2 diabetes mellitus (DM) and diabetic kidney disease are increasing. Hepatitis C infection (HCV) occurs in 1% of the world population and can induce several kidney diseases. DM prevalence is increased in individuals with HCV; however, kidney diseases in those with both DM and HCV have not been assessed. Direct-acting antiviral agents (DAAs) became available for HCV treatment in 2014; it is unknown if DAAs altered the spectrum of kidney disease in patients with DM and HCV. ***Methods:*** Case review identifying patients with kidney biopsy and clinical history of DM and HCV between 2009–2013 (pre-DAA) and 2016–2020 (post-DAA), excluding kidney transplant, hepatitis B, HIV, and inadequate biopsy, identified 245 biopsies. Biopsies were evaluated for diabetic glomerulosclerosis (DGS) class, global and focal segmental glomerulosclerosis (FSGS), other glomerular diseases, interstitial fibrosis/tubular atrophy (IFTA), interstitial nephritis, acute tubular injury and degree of arterial and arteriolar sclerosis. Kidney disease differences in pre-DAA versus post-DAA eras and in mild versus severe DGS were assessed by $\chi^2$ and Fisher's exact tests. ***Results:*** The most common non-DGS lesions were non-collapsing FSGS (41%), HCV-related IgM dominant immune complex glomerulonephritis (IgM-ICGN, 18%), IgA nephropathy (9%), and membranoproliferative glomerulonephritis (MPGN, 7%). Collapsing FSGS was more common pre-DAA versus post-DAA (8% vs. 1%, $p = 0.03$). Biopsies from patients with HCV and DM were reduced in post-DAA (0.7%) versus pre-DAA (1.3%) ($p < 0.0001$). Post-DAA there were less MPGN (2% vs. 10%, $p = 0.02$) and more advanced DGS (85% vs. 61%, $p = 0.0002$), non-collapsing FSGS (57% vs. 31%, $p < 0.0001$), IFTA (2.0 vs. 1.6, $p = 0.0002$), and vascular sclerosis (2.1 vs. 1.6, $p < 0.0001$). ***Conclusion:*** Post-DAA there were reduced biopsies and MPGN, with more severe DGS class, non-collapsing FSGS, IFTA, and chronic vascular changes. This suggests a modulating effect of DAAs on HCV-related kidney pathology with DM and chronic changes driving indications for kidney biopsy.



## Introduction

Type 2 diabetes (DM) and Hepatitis C virus (HCV) infection are both widespread public health problems. The global prevalence of DM has reached epidemic proportions fueled by rising obesity and population

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aging, among other factors. In US adults, the prevalence of DM is 15%, and globally it is 9% and predicted to increase to 10% by 2040 [1–3]. The prevalence of diabetic kidney disease, clinically characterized by impaired kidney function and/or albuminuria in patients with DM, varies by geographic location, ranging from 27% to 83%, and is overall thought to be at least 40% [1, 4, 5]. In medical kidney biopsies, the frequency of diabetic glomerulosclerosis (DGS) has dramatically increased from 6% in biopsies performed from 1986–1995 to 19% in biopsies performed from 2006–2015 [6]. HCV, a small, enveloped, single-stranded RNA virus in the family *Flaviviridae*, is transmitted by exposure to infectious blood which, in the USA, most commonly occurs due to intravenous drug use, and more rarely is transmitted by sexual contact, unscreened transfusion, or transplacentally. An estimated 2.4 million people in the USA and 71 million people worldwide (1% of the population) are infected with HCV [7, 8].

DM and HCV infection are commonly coincident. For incompletely understood reasons, possibly related to HCV interfering with the insulin-signaling pathways by up-regulating inflammatory cytokines and gluconeogenic genes in individuals at high risk for DM [3], HCV infection increases the risk of developing DM by 11-fold [9] and augments the risk of developing chronic kidney disease by 57% [10]. Indeed, DM is more common in patients with HCV infection compared to those without HCV [11]. In a cohort of 100,518 US veterans infected with HCV, there was a 21% prevalence of diabetes [12], and in a study of 400,000 patients in the Medicare Coverage Database with HCV infection, 38% were also diabetic [13]. Similarly, in Europe, there is a 13–25% prevalence of diabetes in patients infected with HCV [14].

For both DM and HCV infection, the kidneys are a primary organ site manifesting pathology. Studies of kidney biopsies from diabetic patients have shown a spectrum of findings including diabetic nephropathy (DN) alone in 28–52%, only nondiabetic kidney disease in 28–53%, and DN with nondiabetic kidney disease in 6–33%, likely varying due to geographic location and biopsy practice [15–20]. HCV-infected patients also may develop diverse kidney pathologies, including immune complex-mediated glomerulonephritis (ICGN) and nonimmune glomerular diseases as well as tubulointerstitial and vascular lesions [10, 21–26]. Despite the co-occurrence of DM and HCV, to date there are no large studies analyzing the range of lesions found in kidney biopsies, specifically DGS, HCV-related lesions, and other forms of kidney injury, from patients with these two disease processes.

While the arsenal of anti-hyperglycemic agents for the treatment of DM has continually evolved over the last decades, the treatment of HCV was singularly revolutionized by the advent of direct-acting antiviral agents (DAAs), which were first approved for stand-alone treatment of HCV infection by the US Food and Drug Administration in 2014 [27]. Prior to DAAs, HCV treatment consisted of interferon and ribavirin, which are poorly tolerated, associated with severe adverse effects and produce only 30–40% sustained virologic response rates [28, 29]. In contrast, DAAs, are well tolerated and achieve >90% sustained virologic response rates [30, 31]. In this study, we report the spectrum of kidney biopsy findings in patients with concurrent DM and HCV, and examine changes in these findings following the availability of DAA treatment.

## Materials and Methods

*Case Selection and Clinical Data*

In this retrospective study, the departmental records at Cedars-Sinai Medical Center were searched for kidney biopsies from patients with a clinical history of diabetes and reported clinical history of HCV infection from years 2009 to 2013 (pre-DAA) and 2016 to 2020 (post-DAA). In the US, DAAs were approved as stand-alone treatment for HCV in 2014 and first entered practice guidelines in 2015 [32, 33]; therefore, the years 2014 and 2015 were excluded to ensure the cohorts studied reflected biopsies performed in the pre- and post-DAA time frames. All kidney transplant biopsies, inadequate biopsies (defined as those with fewer than 5 glomeruli or missing light, immunofluorescence, or electron microscopy), and biopsies from patients with HIV or hepatitis B infection were excluded. Clinical data were abstracted from the electronic health records including age, sex, CMS "race"/ethnicity designations, serum creatinine, and proteinuria (as measured urine protein to creatinine ratio or 24-h urine protein collection) at the time of kidney biopsy.

*Pathologic Evaluation*

All qualifying kidney biopsy specimens were processed by standard light microscopy, immunofluorescence, and electron microscopy and were reviewed for this study for confirmation of the diagnoses in the pathology report. Kidney biopsies were evaluated for the class of DGS using published criteria [34]. In brief, class I is thickened glomerular basement membranes, class IIa is mild mesangial expansion, class IIb is moderate to severe mesangial expansion (expanded mesangial area is > mean capillary lumen area in >25% of all mesangial regions), class III is nodular glomerulosclerosis, and class IV is any class I–III with >50% global glomerulosclerosis. The extent of interstitial fibrosis/tubular atrophy (IFTA) was determined as the percentage of the total involved area of the tubulointerstitium as follows: 0 = absence of interstitial fibrosis, 1 = <25%, 2 = 25–50%, and 3 = >50% [35]. Vascular lesions were scored based on the most severely affected vessel. Arteriosclerosis was scored in both large (arcuate) and small (interlobular) arteries, when present, as follows: 0 = no intimal thickening, 1 = intimal fibrosis <25% of

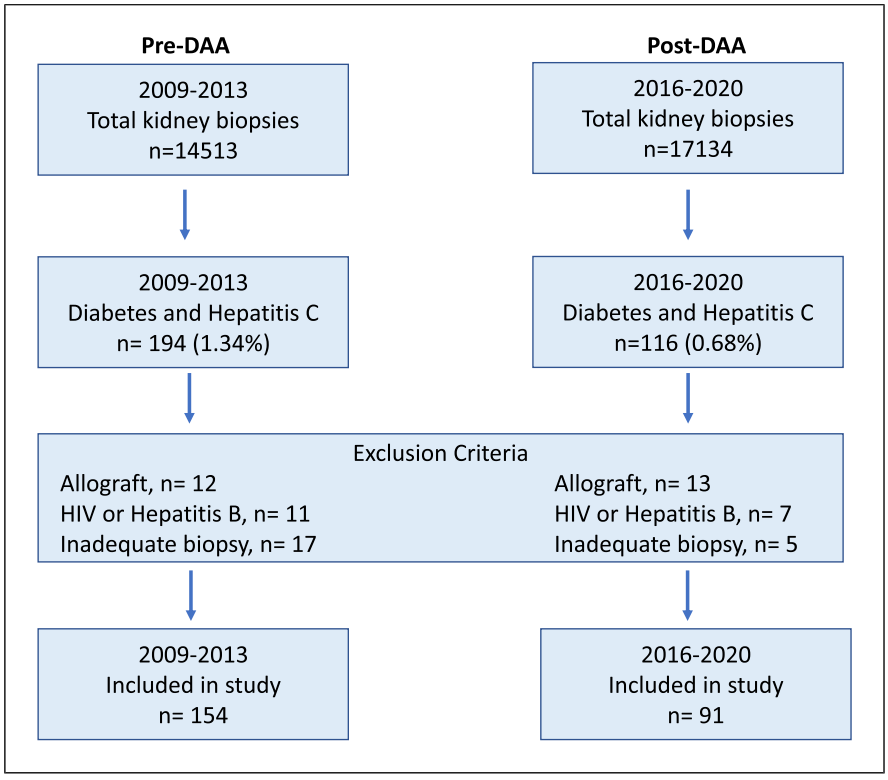


**Fig. 1.** Study case selection.

the medial thickness, 2 = intimal fibrosis 25–50% of medial thickness, 3 = intimal fibrosis >50% of medial thickness. Arteriolosclerosis was scored as 0 = no hyalinosis, 1 = hyalinosis with no wall thickening, 2 = hyalinosis with wall thickening but patent lumen, 3 = hyalinosis with luminal narrowing. In the setting of severe diffuse or nodular DGS, a diagnosis of focal and segmental glomerulosclerosis (FSGS) required segmental obliteration of the tuft with podocyte/visceral epithelial cell hypertrophy and/or hyperplasia, or halo formation with or without other features of FSGS, while insudates, lipid, foam cells, and adhesions alone were considered insufficient for a diagnosis of FSGS. FSGS variants were determined according to the Columbia FSGS Classification System [36]. Biopsies with a diagnosis of fibrillary glomerulonephritis were stained for DNAJB9 by immunohistochemistry.

*Statistical Methods*

For categorical variables, $\chi^2$ test was performed when $n > 10$ and Fisher's exact test was performed when $n < 10$. For continuous variables, unpaired $t$ tests were performed. For all statistical analyses, values of $p < 0.05$ (2-tailed) were considered significant. All analyses were performed using GraphPad Prism version 10.0.0 for Windows (GraphPad Software, Boston, Massachusetts USA). Univariate and stepwise multivariate logistic regression modeling were performed to evaluate the association of potentially relevant covariates (global glomerulosclerosis, non-collapsing FSGS, IFTA, ATI/ATN) to both DGS severity and DAA era. Having $p < 0.2$ for individual covariates was the threshold for subsequent inclusion in a multivariable model. Supervised backwards elimination was used to produce the final parsimonious multivariable model and a $p$ value <0.05 was considered statistically significant. Logistic regression modeling was performed using SAS version 9.2 (SAS, Cary, NC, USA).

## Results

*Kidney Biopsies of Patients with DM and HCV Decreased in the Post-DAA Era*

A total of 310 kidney biopsies from patients with DM and HCV were identified (Fig. 1). This represents 1.34% (194/14,513) of all kidney biopsies in the pre-DAA era compared with 0.68% (116/17,134) of post-DAA era biopsies ($p < 0.0001$). 245 biopsies (154 pre-DAA, 91 post-DAA) met inclusion criteria.

*Biopsied Patients Were Predominantly Older, "White" or "Hispanic" Males with Heavy Proteinuria*

Overall patient clinical features/demographics as well as clinical features in the pre-DAA versus post-DAA eras and in individuals with mild (classes 0-IIa) versus severe (classes IIb-IV) DGS are presented in Table 1, and online supplementary Tables 1 and 2 (for all online suppl. material, see https://doi.org/10.1159/000537977). The average age of patients was 59 years (median 58 years,

**Table 1.** Demographics and clinical features of biopsied patients with HCV and DM

| | All cases ($n$ = 245) | Pre-DAA ($n$ = 154) | Post-DAA ($n$ = 91) | $p$ value | DGS class 0-IIa ($n$ = 74) | DGS class IIb-IV ($n$ = 171) | $p$ value |
|---|---|---|---|---|---|---|---|
| Demographics | | | | | | | |
| Age, mean±SD, years | 59±9 | 58±9 | 60±9 | 0.0941 | 61±9 | 58±9 | 0.0174* |
| Sex (M:F) | 2.5 | 2.1 | 3.6 | 0.1074 | 2.2 | 2.6 | 0.5494 |
| Individuals with "race"/ ethnicity designation, $n$ (%) | 84 (206) | 79 (121) | 93 (85) | | 82 (61) | 85 (145) | |
| "White" % | 37 | 37 | 38 | 0.9468 | 38 | 37 | 0.9499 |
| "Black" % | 19 | 25 | 12 | 0.0199* | 28 | 16 | 0.0467* |
| "Asian" % | 8 | 7 | 8 | 0.8333 | 11 | 6 | 0.1971 |
| "North American Native" % | 1 | 1 | 0 | 0.4008 | 2 | 0 | 0.1222 |
| "Hispanic" % | 35 | 30 | 42 | 0.0619 | 2 | 41 | <0.0001**** |
| Clinical features | | | | | | | |
| Cases with serum creatinine quantified, $n$ (%) | 87 (214) | 87 (134) | 88 (80) | | 84 (62) | 89 (152) | |
| Serum creatinine, mg/dL, mean±SD | 3.0±2.2 | 2.9±1.9 | 3.2±2.7 | 0.3425 | 3.1±2.3 | 2.9±2.2 | 0.5522 |
| Cases with proteinuria quantified, $n$ (%) | 36 (88) | 34 (53) | 38 (35) | | 37 (27) | 36 (61) | |
| Proteinuria 0–1 g/24 h, % | 5 | 2 | 9 | 0.2968 | 11 | 2 | 0.0840 |
| Proteinuria 1–3 g/24 h, % | 18 | 23 | 11 | 0.2605 | 30 | 13 | 0.0777 |
| Proteinuria >3 g/24 h, % | 77 | 75 | 80 | 0.7957 | 59 | 85 | 0.0122* |

*$p < 0.05$, ****$p < 0.0001$.

interquartile range 53–65 years) with an overall male to female ratio of 2.5. Of the 84% of patients for whom "race"/ethnicity was designated, the majority were "White" (37%) or "Hispanic" (35%). "Hispanic" patients had more severe compared to mild DGS (41% vs. 2%, $p < 0.0001$). In contrast, patients identified as "Black" had less severe DGS ($p < 0.05$). There were no significant patient demographic differences between the pre- and post-DAA eras with the exception of "Black" patients, who were significantly less represented in the post-DAA compared with pre-DAA biopsies (12% vs. 25%, $p = 0.02$). The serum creatinine level at the time of biopsy was similarly elevated irrespective of DAA era and DGS severity. Where quantified (36% of cases, $n = 88$), 77% of patients had nephrotic range (>3 g/24 h) proteinuria, which was unaffected by DAA era but more common in patients with severe versus mild DGS (85% vs. 59%, $p = 0.01$).

### *DAA Era Impacts the Prevalence of Glomerular Disease and DGS Severity*

Only nondiabetic glomerular pathology was present in 9.4% (23/245) of cases, and only DGS was present in 24.5% (60/245) of cases. Most (70%) patients had severe DGS, and the proportion of biopsies with severe DGS increased from 61% in the pre-DAA era to 85% in the post-DAA era ($p = 0.0002$). There were no correlations between a specific DGS class and any one glomerular lesion (online suppl. Table 3). However, when grouped as mild and severe DGS, both non-immune complex and immune complex-mediated lesions were more common in biopsies with mild DGS. Similarly, both nonimmune complex and immune complex-mediated lesions were more common in pre-DAA biopsies (Table 2; Fig. 2).

A glomerular lesion in addition to DGS was identified in the majority of biopsies (66.1%, 162/245, online suppl. Table 3). The most common non-DGS lesion was FSGS, present in 46% (113/245) of biopsies, of which 43% (49/113) showed FSGS plus another non-DGS glomerular lesion. Most (96%) of the non-collapsing FSGS were the not otherwise specified (NOS) variant and appeared to be secondary or adaptive, although histology alone is not sufficient to determine the etiology of FSGS. This is supported by the observations that the average podocyte foot process effacement (FPE) in cases with NOS FSGS (excluding cases with an underlying immune complex glomerular lesion and glomeruli for electron microscopy showing severe ischemia or podocyte detachment) was 63% ± 16% (mean± standard deviation) and the median FPE was 65%. There was somewhat more extensive FPE with respect to DGS class, with 52% ± 10% FPE in mild DGS (classes 0–IIa) and 66% ± 16% FPE in

**Table 2.** Biopsy findings in patients with HCV and DM

| | All cases ($n$ = 245) | Pre-DAA ($n$ = 154) | Post-DAA ($n$ = 91) | $p$ value | DGS Class 0-IIa ($n$ = 74) | DGS Class IIb-IV ($n$ = 171) | $p$ value |
|---|---|---|---|---|---|---|---|
| Chronic glomerular lesions | | | | | | | |
| Global GS, %, mean±SD | 28±20 | 25±19 | 33±21 | 0.0025** | 21±16 | 31±21 | 0.0003*** |
| Non-collapsing FSGS, % ($n$ = 100) | 41 | 31 | 57 | <0.0001**** | 34 | 43 | 0.1645 |
| Nonimmune complex mediated, % ($n$ = 33) | 13 | 18 | 7 | 0.0154* | 26 | 6 | 0.0002*** |
| Collapsing FSGS, % ($n$ = 13) | 5 | 8 | 1 | 0.0349* | 9 | 4 | 0.0674 |
| MCD, % ($n$ = 7) | 3 | 3 | 3 | 1.0000 | 5 | 2 | 0.2032 |
| Hepatic GS, % ($n$ = 6) | 2 | 4 | 0 | 0.0872 | 3 | 2 | 1.0000 |
| AA, % ($n$ = 3) | 1 | 1 | 1 | 1.0000 | 4 | 0 | 0.0268* |
| PICGN, % ($n$ = 3) | 1 | 1 | 1 | 1.0000 | 3 | 1 | 1.0000 |
| TBMN, % ($n$ = 1) | 0.4 | 1 | 0 | 1.0000 | 1 | 0 | 0.3020 |
| Immune complex mediated, % ($n$ = 97) | 40 | 45 | 31 | 0.0300* | 51 | 35 | 0.0133* |
| IgM-ICGN, % ($n$ = 44) | 18 | 19 | 13 | 0.2066 | 23 | 15 | 0.1112 |
| IgAN, % ($n$ = 23) | 9 | 10 | 9 | 0.8056 | 9 | 9 | 0.9798 |
| MPGN, % ($n$ = 18) | 7 | 10 | 2 | 0.0208* | 11 | 6 | 0.1877 |
| FGN, % ($n$ = 7) | 3 | 3 | 2 | 1.0000 | 7 | 1 | 0.0278* |
| PIGN, % ($n$ = 4) | 2 | 2 | 1 | 1.0000 | 1 | 2 | 1.0000 |
| MN, % ($n$ = 1) | 0.4 | 0 | 1 | 0.3714 | 0 | 1 | 1.0000 |
| DGS | | | | | | | |
| DGS class 0, % ($n$ = 23) | 9 | 12 | 5 | 0.1191 | | | |
| DGS class I, % ($n$ = 24) | 10 | 13 | 4 | 0.0432* | | | |
| DGS class IIa, % ($n$ = 27) | 11 | 14 | 6 | 0.0359* | | | |
| DGS class IIb, % ($n$ = 12) | 5 | 3 | 8 | 0.1347 | | | |
| DGS class III, % ($n$ = 126) | 51 | 47 | 59 | 0.0646 | | | |
| DGS class IV, % ($n$ = 33) | 14 | 11 | 18 | 0.1759 | | | |
| DGS class 0-IIa, % ($n$ = 74) | 30 | 39 | 14 | 0.0002*** | | | |
| DGS class IIb-IV, % ($n$ = 171) | 70 | 61 | 85 | 0.0002*** | | | |
| Tubulointersitial lesion | | | | | | | |
| IFTA (0–3, mean±SD) | 1.8±0.8 | 1.6±0.8 | 2.0±0.8 | 0.0002*** | 1.4±0.7 | 1.9±0.8 | 0.0001*** |
| ATI/ATN, % | 49 | 41 | 64 | 0.0006*** | 54 | 51 | 0.3236 |
| AIN, % | 21 | 25 | 16 | 0.1324 | 26 | 22 | 0.9978 |
| CIN, % | 9 | 8 | 10 | 0.5709 | 8 | 10 | 0.4234 |
| Vascular sclerosis | | | | | | | |
| Overall vascular sclerosis (0–3, mean±SD) | 1.7±0.8 | 1.6±0.8 | 2.1±0.9 | <0.0001**** | 1.3±0.9 | 1.9±0.8 | 0.0001*** |
| Large artery intimal fibrosis (0–3, mean±SD) | 1.9±0.8 | 1.8±0.8 | 2.2±0.8 | 0.0002*** | 1.7±1.0 | 2.0±0.8 | 0.0133* |
| Small artery intimal fibrosis (0–3, mean±SD) | 0.9±0.9 | 0.7±0.8 | 1.0±1.0 | 0.0105* | 0.7±0.9 | 0.9±0.9 | 0.1116 |
| Arteriolar intimal hyalinosis (0–3, mean±SD) | 1.6±1.0 | 1.4±1.0 | 1.9±1.0 | 0.0002*** | 0.9±1.0 | 1.8±1.0 | <0.0001**** |

AA, amyloid A amyloidosis; AIN, acute interstitial nephritis; ATI/ATN, acute tubular injury/necrosis; CIN, chronic interstitial nephritis; FGN, fibrillary glomerulonephritis; FSGS, focal and segmental glomerulosclerosis; GS, global glomerulosclerosis; Hepatic GS, hepatic glomerulosclerosis; IFTA, interstitial fibrosis and tubular atrophy; IgAN, IgA nephropathy; IgM-ICGN, IgM dominant immune complex-mediated glomerulonephritis; MCD, minimal change disease; MN, membranous nephropathy; MPGN, membranoproliferative glomerulonephritis; PICGN, pauci-immune crescentic glomerulonephritis; PIGN, post-infectious/infection-related glomerulonephritis; TBMN, thin glomerular basement membrane nephropathy. *$p$ < 0.05, ** $p$ < 0.01, ***$p$ < 0.001, ****$p$ < 0.0001.

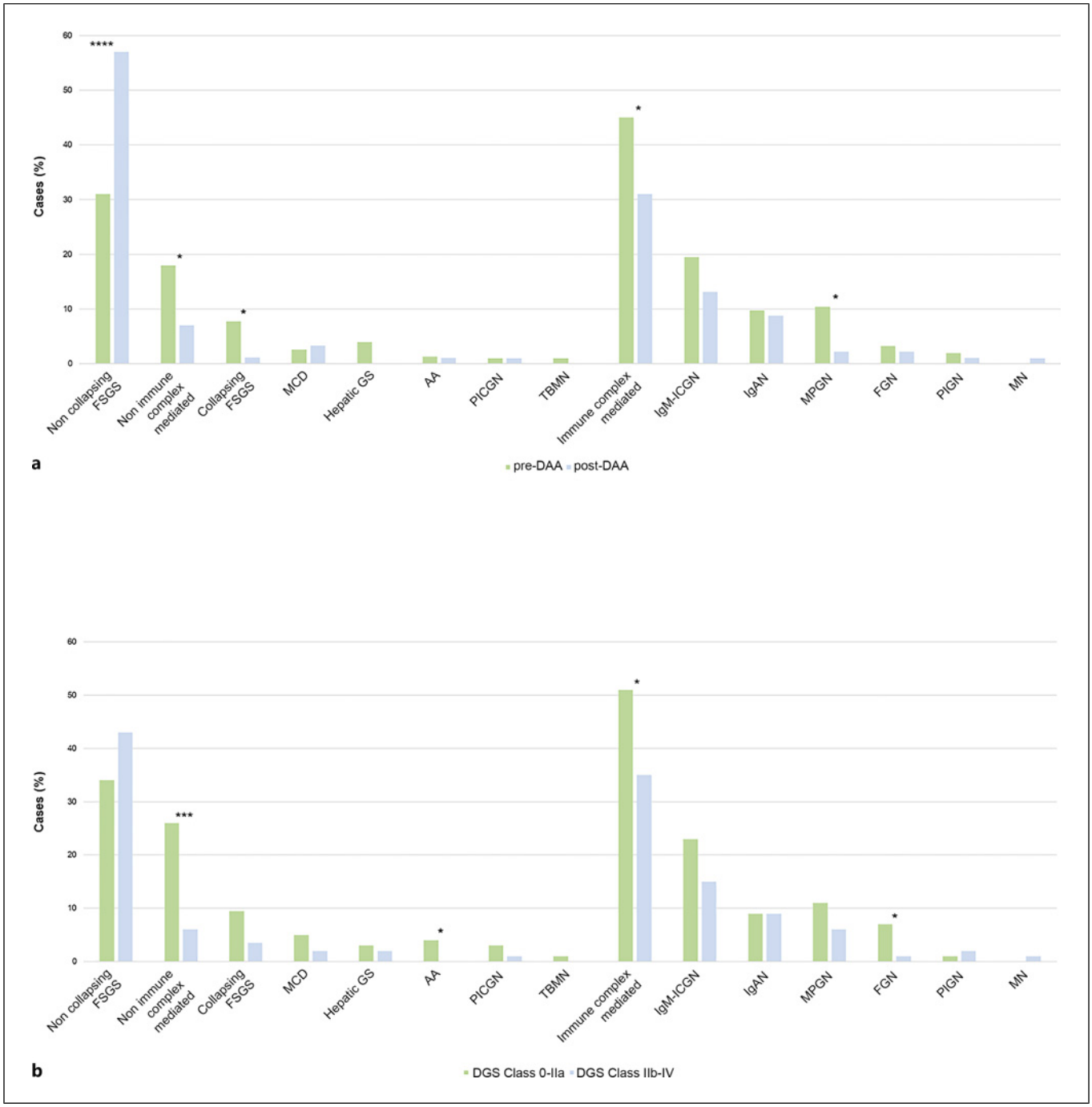


**Fig. 2.** Both nonimmune complex and immune complex-mediated lesions are more common in pre-DAA (**a**) biopsies and in biopsies with less severe DGS (**b**). Nonimmune complex lesions include collapsing FSGS, minimal change disease (MCD), hepatic glomerulosclerosis (GS), amyloid A amyloidosis (AA), pauci-immune crescentic glomerulonephritis (PICGN), and thin basement membrane nephropathy (TBMN). Immune complex-mediated lesions include IgM dominant immune complex-mediated glomerulonephritis (IgM-ICGN), IgA nephropathy (IgAN), membranoproliferative glomerulonephritis (MPGN), fibrillary glomerulonephritis (FGN), post-infectious/infection-related glomerulonephritis (PIGN), and membranous nephropathy (MN). Non-collapsing FSGS is more common in post-DAA biopsies. Corresponding data are presented in Table 2. *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$.

severe (classes IIb–IV) DGS. FPE >80% was seen in 10% of cases without nondiabetic lesions, all of which were associated with nodular DGS (class 3). In the post-DAA era, the prevalence of non-collapsing FSGS increased (57% post-DAA vs. 31% pre-DAA, $p < 0.0001$), while collapsing FSGS decreased (1% post-DAA vs. 8% pre-DAA, $p = 0.03$) (Table 2). Five of the 13 patients with collapsing FSGS were known to have been treated with interferon. Aside from FSGS, nonimmune complex glomerular pathologies were rare, consisting of minimal change disease (3%, 7/245), hepatic glomerulosclerosis (2%, 6/245), AA amyloidosis (1%, 3/245), pauci-immune crescentic glomerulonephritis (1%, 3/245), and thin basement membrane nephropathy (0.4%, 1/245). None of these significantly changed in prevalence between the pre- and post-DAA eras, although AA amyloidosis was significantly more prevalent in biopsies with mild versus severe DGS ($p = 0.03$).

More commonly identified were immune complex glomerular pathologies, the most prevalent of which was IgM-dominant/co-dominant ICGN (IgM-ICGN) (18%, 44/245), most of which were mesangial proliferative, none of which had crescents, and which had a similar prevalence of associated segmental sclerosis pre- versus post-DAA (30% vs. 27%, $p = 0.7$). Other immune complex glomerulonephropathies included IgA nephropathy (9%, 23/245); membranoproliferative glomerulonephritis (MPGN) which was predominantly IgM dominant or IgM-IgG codominant (7%, 18/245); fibrillary glomerulonephritis (3%, 7/245); postinfectious glomerulonephritis (2%, 4/245); and membranous nephropathy (0.4%, 1/245). Of these, only the prevalence of immune complex-mediated MPGN was significantly affected by DAA availability, decreasing from 10% pre-DAA to 2% post-DAA ($p = 0.02$) (Table 2). Cryoglobulin type deposits were seen by electron microscopy in 56% (10/18) of the immune complex-mediated MPGN cases and were not different in the pre- versus post-DAA eras ($p = 0.90$). Fibrillary glomerulonephritis, confirmed with positive staining for DNAJB9 in the five cases with sufficient tissue for staining, was identified more frequently with mild versus severe DGS ($p = 0.03$).

*Chronic Glomerular, Tubulointerstitial, and Vascular Lesions Correlate with DGS Severity and Post-DAA Era*

On logistic regression analysis, >28% global glomerulosclerosis, presence of non-collapsing FSGS, IFTA >1.8, overall vascular sclerosis >1.7, and arteriolar intimal hyalinosis >1.6 were all significantly associated with severe DGS (odds ratio [OR]: 13.1, $p = 0.02$, 95% CI: 1.4–121; OR: 6, $p = 0.03$, 95% CI: 1.2–29; OR: 7, $p = 0.02$, 95% CI: 1.4–10; OR: 10, $p = 0.01$, 95% CI: 1.4–101, OR: 3.2, $p = 0.01$, 95% CI: 1.3–7.4, respectively). These also correlated with post-DAA versus pre-DAA eras, demonstrating more severe DGS in post-DAA biopsies. Interestingly, acute tubular injury also was more frequent in the post-DAA biopsies (post-DAA 62% vs. pre-DAA 41%, $p = 0.0002$). Other variables, including presence or absence of each of the observed nondiabetic glomerular lesions, sex, age, reported "race"/ethnicity, proteinuria >3 g/24 h, and serum creatinine >3.0 mg/dL, were not significantly associated with global or segmental glomerulosclerosis, tubulointerstitial scarring, or vascular sclerosis.

## Discussion

In this study, we describe the spectrum of renal lesions in patients with the widespread and frequently coincident diseases of diabetes and HCV infection both before and after the advent of DAAs. We demonstrated changes in the prevalence of HCV-related and nondiabetic glomerular lesions, and the severity of DGS and tubulointerstitial and vascular sclerosis, related to the availability of DAAs in patients with concomitant DM and HCV infection. In our cohort of HCV-infected DM patients, likely due to the presence of HCV-related glomerular lesions, the prevalence of nondiabetic glomerular lesions concurrent with DGS (66%) was higher than that previously reported in patients with DM (19–33%) [15, 16, 19, 20]. In the post-DAA era, there was an overall reduction of biopsies from patients with DM and HCV, possibly due to a decrease in HCV-associated ICGN and nonimmune complex lesions. FSGS was the most common nondiabetic glomerular lesion, found in 46% of all biopsies and more prevalent in the post-DAA era, suggesting proteinuria from this lesion supplanted that of HCV-associated glomerulopathies. It is possible that DAA treatment itself may have been responsible, in part, for the increase in FSGS post-DAA [37]. In terms of ICGN, in contrast to other studies where IgA nephropathy was the most common ICGN identified in patients with DM [15, 16, 38], in this study IgM-ICGN was observed most often followed by IgA nephropathy. IgM-ICGN was also the most common ICGN lesion identified in a study by Guo et al. [39] describing renal lesions in HCV patients, irrespective of a diagnosis of diabetes. In our study, there was no significant decrease in IgM-ICGN in the post-DAA era. It is possible some ICGNs began during HCV infection and did not resolve, or even occurred following successful DAA treatment [40, 41]. In contrast, MPGN was observed predominantly in the pre-DAA era, suggesting an effect of HCV eradication following availability of DAAs.

The observed reduction in collapsing FSGS in the post-DAA era may reflect DAA efficacy and changes in clinical practice away from use of interferons, and/or changing patient demographics over time. If, as some have postulated, HCV, like HIV, directly induces podocyte injury resulting in collapsing glomerulopathy [42, 43], then HCV eradication by DAAs should eliminate HCV-induced collapsing glomerulopathy. However, the association of collapsing glomerulopathy with HCV more likely is indirect, possibly due to viral treatment with interferon. In our cohort, 5 of the 13 patients with collapsing glomerulopathy received interferon-based therapy, which is known to induce collapsing glomerulopathy [44] and was the mainstay of HCV therapy in the pre-DAA era. Thus, replacement of interferons by DAAs is likely, at least in part, to underlie the decline in collapsing glomerulopathy. Collapsing glomerulopathy is also strongly associated with particular variants of the apolipoprotein L1 (*APOL1*) gene, which are more common in individuals of African descent [45]. While *APOL1* variant testing was not performed in any of our patients with collapsing glomerulopathy, there was a higher proportion of "Black" patients with collapsing glomerulopathy than the overall proportion of "Black" patients in our study (50% vs. 19%, $p = 0.002$, online suppl. Table 1). There was, however, also an overall reduction in the proportion of "Black" patients in the post-DAA era compared to the pre-DAA era (decrease from 25% to 12%, $p = 0.02$). It is unknown if the reduction in collapsing glomerulopathy may have accounted for this demographic change or vice versa.

There were more severe DGS, vascular sclerosis, and FSGS post-DAA. There also was more acute tubular injury in the post-DAA era, although this was unrelated to the severity of DGS. These findings suggest that, with a likely reduction in HCV-related kidney injury, diabetic, and hypertensive-related chronic kidney injury as well as acute kidney injury is driving symptoms resulting in kidney biopsy. Interestingly, the number of patients with nephrotic range proteinuria significantly correlated with severe DGS but not with pre- versus post-DAA eras, although this might be due to the limited data availability. The above observations suggest that the clinical threshold for kidney biopsy may be lower when there is active HCV infection in diabetic patients. There was no difference in the serum creatinine level relative to the severity of DGS or DAA era. It is not clear why this is the case, and may be related to biopsy practice as well as a reflection of the inaccuracies of serum creatinine as a marker of renal function.

Limitations of this study include the non-standardized and incomplete reporting of HCV treatment status and modalities used. However, by excluding years 2014 through 2015 when DAAs were approved as stand-alone treatment for HCV and first entered practice guidelines [32, 33], our pre-DAA (2009–2013) and post-DAA (2016–2020) study eras were selected such that many patients with HCV likely were treated with DAAs in the post-DAA era. Nevertheless, the prevalence of untreated patients, viral loads, results of rheumatoid factor and cryoglobulin testing, and the proportion of patients achieving sustained virologic response remain unexamined in our study and may confound study interpretation. There was incomplete data reporting, including clinical, laboratory, and urinary findings including quantitated proteinuria, limiting further analyses in these areas. Lastly, the last year of data collection (2020) overlapped with the SARS-CoV-2 pandemic, which may have impacted kidney biopsy practices. However, it is unlikely this change would have impacted the overall findings as cases that met inclusion criteria included 14 in 2018, 9 in 2019, and 12 in 2020.

In summary, since the advent of DAAs, patients with DM and HCV are less likely to get a kidney biopsy. In those who are biopsied, the prevalence of MPGN and collapsing FSGS has decreased while the severity of DGS, IFTA, and vascular sclerosis, and the prevalence of non-collapsing FSGS have increased. Thus, the reduction of active HCV may delay performance of kidney biopsy, resulting in more advanced disease unrelated to HCV infection. Earlier biopsy in these patients may provide a larger window for therapeutic intervention [46]. Future studies incorporating clinical data, especially duration of HCV infection prior to clearance and HCV sustained virologic response status, are needed to better understand the impact on progression of DGS and the mechanism(s) by which DAA-mediated HCV eradication affect the pathogenesis of IgM-ICGN and MPGN.

## Statement of Ethics

This study protocol was reviewed, the need for written informed consent was waived, and the study was approved by Institutional Review Board at Cedars-Sinai Medical Center, approval number STUDYCR00000611.

## Conflict of Interest Statement

V.L.K. and G.G. have no conflicts of interest to declare. C.C.N. is a consultant for BioCryst Pharmaceuticals.

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## Author Contributions

V.L.K. and C.C.N. conceived and designed the study. V.L.K., G.G., and C.C.N. contributed to data collection, analysis, and manuscript writing and revisions.

## Data Availability Statement

The data that support the findings of this study are not publicly available due to privacy reasons but are available from the corresponding author upon reasonable request.

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