Detecting C4d in Liver Allografts: Immunohistochemistry and Immunofluorescence Show Equivalent Staining

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BACKGROUND

Endothelial deposition of C4d, detected by immunofluorescent staining (IF), is the gold standard for the diagnosis of antibody-mediated rejection (AMR) in renal allografts. In contrast, the reliability of C4d stains in liver allografts has yet to be definitively established. The aims of this study are:

- To compare the staining patterns of C4d stains in liver biopsies by IF and immunohistochemistry (IHC)
- To correlate endothelial deposition of C4d with the results of serologic tests for AMR

DESIGN

Our database was searched for biopsies of allograft and native livers for evaluation of rejection or chronic/recurrent hepatitis C (HCV); see Table 1. Serial allograft biopsies from individual patients were included. C4d stains were performed by IHC on formalin fixed tissue and by IF on frozen tissue where available. Positive staining was defined as strong linear endothelial (portal or sinusoidal) staining. C4d positivity was correlated with tests for donor serum antibodies (DSA), treatment regimens and clinical therapeutic response.

Table 1.

BIOPSY	CLINICAL INDICATION/BIOPSY DIAGNOSIS	PT	BX	DSA	IF
ALLOGRAFT	POSSIBLE AMR	10	19	19	17
ALLOGRAFT	ACUTE CELLULAR REJECTION	12	12	N/A	N/A
ALLOGRAFT	RECURRENT HCV	15	15	N/A	N/A
NATIVE	HCV STAGING	11	11	N/A	N/A

Table 1. Summary of liver biopsies included in the series

RESULTS

- As in prior studies, endothelial C4d staining was demonstrated in inflammatory conditions
  - 3/11 (27%) of native livers biopsied for HCV staging
  - 3/15 (20%) of allografts with recurrent HCV
  - 2/12 (16%) with acute cellular rejection
- Comparison of C4d staining by IF and IHC was done for 17 biopsies taken from 10 patients
  - 12/17 biopsies had concordance between C4d detection by IHC (portal or endothelial staining patterns) and IF; see Table 2 and Figure 1
- DSA tests were performed in 10 patients, with 19 corresponding biopsies; see Table 3

Table 2.

#	IF PORTAL	IHC PORTAL	IF SINUSOID	IHC SINUSOID
1	NEG	NEG	POS	NEG
2	NEG	NEG	NEG	NEG
3	NEG	NEG	NEG	NEG
4	NEG	NEG	NEG	NEG
5	POS	POS	POS	NEG
6	POS	NEG	POS	NEG
7	NEG	NEG	NEG	NEG
8	POS	NEG	POS	POS
14	NEG	NEG	NEG	NEG
15	NEG	NEG	NEG	NEG
9	NEG	POS	NEG	POS
10	NEG	POS	NEG	POS
11	NEG	NEG	POS	POS
12	NEG	NEG	POS	POS
13	POS	NEG	POS	POS
16	NEG	NEG	NEG	NEG
17	NEG	NEG	NEG	NEG

CONCORDANT C4d STAINING PATTERN

DISCORDANT C4d STAINING PATTERN

Table 2. Comparison of C4d immunostaining patterns by IF and IHC.

Figure 1.

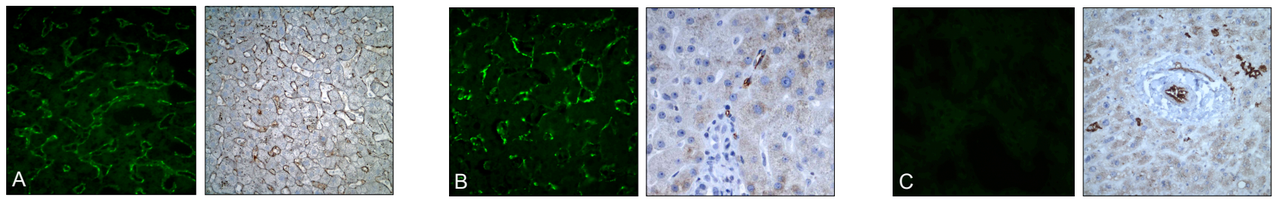

Figure 1. Comparison of C4d Immunostaining Patterns by IF and IHC. Concordant staining with strong linear endothelial sinusoidal positivity by both IF and IHC **(A)**. Concordant portal capillary positivity by both IF and IHC, while sinusoidal staining is discordant, with positive staining by IF and negative staining by IHC **(B)**. Discordant portal endothelial staining, with positivity by IHC and negative staining by IF **(C)**.

Table 3.

PT	DSA	IF	IHC	TREATED FOR	RESPONSE
1	POS	POS	-	AR	+
2	-	-	-	RAIH	+
2	-	-	-	RAIH	+
3	POS*	POS	POS	AMR	+
4	-	-	-	AR	+
5	POS	-	POS	AMR	+
5	POS	-	POS	AMR	+/-
5	POS	POS	POS	AMR	+
5	POS	POS	POS	DRUG INJURY	-
5	POS	POS	POS	AMR	+/-
5	POS	N/A	-	AMR	-
6	-	POS	-	AR	-
6	POS¶	-	-	AMR	-
6	-	-	-	AR	+
6	-	N/A	-	AR	-
7	POS	-	-	AMR	+/-
8	-	-	-	RHCV	+
9	WEAK POS	N/A	-	DRUG INJURY	+
10	-	N/A	-	AR	+
	% POS DSA	% STAINING IN POS DSA #			
	11/19 (55%)	5/10 (50%)	6/11 (55%)		

CONCORDANT C4d STAINING AND DSA RESULTS

DISCORDANT C4d STAINING AND DSA RESULTS

Table 3. Comparison of DSA results and C4d Immunostaining by IF and IHC and correlation with clinical parameters. *anti-B antibody, ¶anti-endothelial antibody, AR=acute cellular rejection, AMR=antibody mediated rejection, RAIH=recurrent autoimmune hepatitis, RHV=recurrent HCV, #in subset of biopsies with IF.

CONCLUSIONS

In his series, IHC and IF are equally sensitive methods for C4d when correlated with DSA. Staining was concordant with DSA in 15/19 biopsies, representing 8/10 patients, when both IHC and IF were employed. Discordance between DSA and C4d can occur with either staining method. Finally, a diagnosis of AMR, if made on the basis of either DSA or C4d tests, does not predict therapeutic response. Larger studies are needed to validate AMR diagnostic methods.