CD68 immunostaining of tubular epithelium is an excellent indicator of Calcineurin inhibitor toxicity in kidney transplant patient

Hussam Abu-Farsakh, MD\(^1\), Hisham Abu Farsakh\(^2\), Nidal Badran, MD\(^3\)

C\(\text{alcineurin inhibitor [CNI], discovered in late 1970s, with cyclosporine followed by tacrolimus (1-3). The nephrotoxicity of cyclosporine was reported in the first publications on the clinical use of cyclosporine in humans after renal transplantation, followed by other publications on its long-term use with irreversible renal functional deterioration (4,5).}

\(\text{CNI toxicity can be divided into acute and chronic CNI nephrotoxicity. The acute changes were reported in different structures: arteries, tubules and endothelial cells by causing acute arteriolopathy without histologic changes, tubular isometric vacuolization and thrombotic microangiopathy (6-12). Chronic CNI nephrotoxicity, on the other hand, includes the following reported changes (9,10,12-16): Interstitial fibrosis and tubular atrophy \text{[typically striped]}, medial arterial hyalinosis, glomerular capsular fibrosis, global glomerulosclerosis, focal segmental glomerulosclerosis \text{[FSGS]}, juxtaglomerular apparatus hyperplasia, and/or tubular microcalcifications. The Pathogenesis of CNI nephrotoxicity is attributed to at least the following means: Its vasoconstrictive effect on the arteriolar arteries, by an increase in endothelin and thromboxane and activation of the renin-angiotensin system as well as a reduction of vasodilator factors like prostacyclin, prostaglandin E\(2\), and nitric oxide (5,17). It also directly activates apoptosis genes and increase apoptosis in tubular and intersititital cells, thereby inducing tubular atrophy (5,18,19). The third possible way is its direct toxic effect on the tubules (20).}

\(\text{CNI and rejections are two major important diagnostic dilemmas that face the nephrologist and the nephropathologist in the post-transplant period. The diagnosis has to be timely and accurate. Unfortunately, the treatment of each category is in the opposite direction of the other. Any mistakes in this field can results in loss of the patient’s kidney and revert him to dialysis soon. The aim of this study is to diagnose changes of CNI by an objective tool, like immunohistochemistry and confirm them as soon as possible.}

\(\text{There are several immunohistochemical markers that are useful for rejections, CD4, CD8 and CD68 (21, 22). So far, there is no objective stain that can diagnose reliably CNI toxicity. Trials to detect CNI toxicity was attempted by Meehan et al. by performing immunohistochemistry to CD64, a platelets marker (23). CD61 stained arterioles in cases with CNI toxicity, especially in cases with thrombotic Microangiopathy [TMA] (23). But, in their study, it was not helpful when no TMA is present. CD68 immunohistochemical marker of histiocytes. It is useful in confirming rejection by staining the histiocytes in renal biopsies (21,22). We noticed that C4d stains tubular epithelium cells in cases with CNI toxicity, but it does not stain them in cases of rejection. This observation made us to retrospectively study all cases of renal transplant biopsies that were submitted to our nephropathology department, by using CD68 to sort cases that were positive in the tubular epithelium vs. those that are positive in the histiocytes.}

\(\text{DESIGN}
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A retrospective study on 162 cases of kidney transplant core biopsies were reviewed over the last 4 years in our Nephropathology Department. The diagnosis of CNI was rendered in 54 cases [33%]; while rejection was rendered in 78 cases [48%]. In all cases, C4d and CD68 were performed by immunohistochemistry on formalin fixed, paraffin embedded tissue. The pattern of CD68 positivity was then analyzed into two patterns: histioctye staining pattern and tubular epithelium granular pattern.

\(\text{RESULTS}
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C4d was positive in 44 out of 162 cases [27%]. CD68 histiocyte staining pattern and tubular epithelium granular pattern. The pattern of CD68 positivity was then analyzed into two patterns: histiocyte staining pattern in 82 out of 162 cases [51%], and tubular epithelium granular pattern in 53 out of 162 cases [33%]. In the remaining 27 cases [16%], both patterns are seen within the same case. 53 [out of 54 cases: 98%] with CNI showed tubular epithelium granular pattern at least focally. On the other hand, only one case with rejection out of 78 cases [1%] showed granular tubular epithelium pattern.

\(\text{CONCLUSION}
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CD68 immunostaining in tubular granular pattern in biopsies from kidney transplant patient is an excellent marker for diagnosing CNI in kidney transplant patients.

\(\text{Key Words: CD68; C4d; Calcineurin inhibitor nephrotoxicity; Macrophage protein; Kidney transplant}
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Abbreviations: CNI: Calcineurin Inhibitor Toxicity; TMA: Thrombotic Microangiopathy

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1\text{Department of Pathology, First Medical Lab, Amman, Jordan; 2King Abdullah University Hospital, Amman, Jordan; 3Department of Nephrology, Istituti Hospital, Amman, Jordan.}

Correspondence: Dr. Hussam Abu-Farsakh, Department of Pathology, First Medical Lab, Amman, Jordan. Telephone +96265931310, email f1lab@yahoo.com

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Figure 1) Isometric vacuolization of the proximal tubules, arrows, (Trichome stain, 400X).

Figure 2) A: Immunostaining for C4d on the peritubular capillaries (PTC) and as an internal control in the glomerular basement membrane (400X). B: Immunostaining for C4d in PTC (arrows) away from the glomeruli (400X).
Figure 3) CD68 positivity in histiocytes infiltrating the tubular epithelium (arrows) (400X)

Figure 4) CD68 positivity in granular pattern in different parts of the tubular cytoplasm, arrows: A and B: Low power: 200X, C and D: high power 400X
DISCUSSION AND CONCLUSION

CD68 is an immunostaining of protein called: macrosialin. The gene for macrosialin protein encodes a 110kD transmembrane glycoprotein that is highly expressed by human monocytes and tissue macrophages. It is a member of the lysosomal/endosomal-associated membrane glycoprotein [LAMP] family (24, 25). The protein primarily localizes to lysosomes and endosomes with a smaller fraction circulating to the cell surface (24, 25). It is a type I integral membrane protein with a heavily glycosylated extracellular domain and binds to tissue- and organ-specific lectins or selectins (24, 25). The protein is also a member of the scavenger receptor family. Scavenger receptors typically function to clear cellular debris, promote phagocytosis, and mediate the recruitment and activation of macrophages. Alternative splicing results in multiple transcripts encoding different isoforms (24, 25). Tubular epithelium has a prominent endoplasmic reticulum that upon tubular toxic effect become more prominent. Calcineum inhibitors exert toxic effect on tubules by enlargement of the endoplasmic reticulum and increased lysosomes (26). Histologically, this effect manifests as isometric vacuolization of the tubular cytoplasm. Isometric tubular vacuolization could be the consequence of relative ischemia caused by afferent arteriolar vasoconstriction, or direct toxic tubulopathy (27, 28). The other toxic changes in the tubules, is inclusion bodies in the tubular epithelium cytoplasm. Ultrastructurally, these inclusion bodies represent giant mitochondria and autolysosomes (29, 30). Another toxic effect has been attributed to inhibition of the cell cycle through cyclosporine-induced accumulation of p53 (31-33).

The authors propose that Macrosialin protein becomes more prominent in the tubular cytoplasm as a reaction to toxic effect of CNI. Thus, it becomes easily stainable by CD68. This may detect subclinical cases of CNI toxicity effect on the tubular cytoplasm and help in the early management of this problem. Our study proves that by performing a relatively a cheap immunohistochemical marker [CD68], nephropathologists can sort out cases of rejection [by strong staining of histiocytes] vs. CNI toxicity [by staining of tubular epithelium]. The cases that show mixed pattern of staining, the nephropathologists has to use, in addition, the clinical status, the other histological criteria for rejection or CNI toxicity and the amount of histiocytes that are stained. In our opinion, the wisely interpretation of CD68 immunostainings is very helpful in cases of renal transplant management. We advocate the use of this marker routinely in all cases of renal transplant.

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