Calcineurin Inhibitor Induced Toxicity in Renal Allograft.
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Calcineurin inhibiting drugs (CID), such as cyclosporine A (CsA) and tacrolimus (FK506), have significantly improved outcomes in organ transplantation. Since the introduction of the CsA in the 1980s, one year survival increased from 60% up to 82% by 1993 (UNOS). Both drugs differ structurally and isolated from different fungus species. However, CsA and FK506 drugs have similar mechanisms of action, therapeutic and adverse effects.

Calcineurin dephosphorylates intracytoplasmic nuclear regulatory proteins in lymphocytes and facilitates their translocation into the nucleus with consecutive activation as intranuclear factors for various mediators (TNF-alpha, interferon gamma, IL-4, IL-2, etc). Calcineurin typically promotes T-cell activation. Cyclosporine and tacrolimus bind to the intracytoplasmic receptor proteins, the immunophilins. The immunophilin/tacrolimus or CsA complexes bind to and inhibit Calcineurin (phosphatase). In therapeutic doses, tacrolimus and CsA block approximately 50% of Calcineurin activity. Some studies seem to demonstrate equal efficacy of both drugs in suppressing acute rejection episodes and improving long-term graft outcomes under current, optimized dosing regimens.

Unfortunately Calcineurin inhibitor therapy is associated with nephrotoxic side effects, which can present in two major forms: 1) functional toxicity (vasospasm without morphological changes) and 2) structural toxicity (earlier or late histological alterations, associated with functional toxicity). Side effects are predominantly dose dependent and seen at high trough levels. However, in some individuals they can also occur with normal therapeutic levels. Calcineurin inhibitors demonstrate identical structural changes primarily involving renal tubules, arterioles, and glomeruli in native and transplanted kidneys referred to as “Calcineurin inhibitor toxicity (CNIT)”

**Functional toxicity:** CID treated patients commonly present with decreased glomerular filtration rate (GFR) due to arteriolar vasospasm, and the most pronounced cases of “functional CNIT” clinically present with acute renal failure and oliguria. On the tubular level, there is a notable decrease in Mg+ reabsorption and K+ and H+ secretion. By definition, morphologically allograft biopsies are normal in appearance.

**Structural toxicity:** CNIT changes seen in tubules, arterioles and glomeruli (Fig-1). “Stripped” interstitial fibrosis, often seen in CNIT, is a nonspecific finding reflecting nephron loss.

**Tubules:** CNIT toxicity predominantly affects the proximal tubules spearing the ducts in the medulla. The morphological changes include: 1) isometric vacuolization (Fig.-2a & b), 2) tubular calcifications, and 3) giant mitochondria. Isometric clear vacuolization, defined as tubular epithelial cells filled with uniformly sized small vacuoles, sometimes associated with loss of tubular brush border. This change reported is allocated to the straight portion of the proximal tubule; however, involvement of the convoluted portion and parietal epithelial cells lining of the Bowman’s capsule has also being reported. The vacuoles are smaller than the nucleus, contain clear fluid, and correspond to the dilated portions of the endoplasmic reticulum, detected by electron microscopy. The giant mitochondria, seen on electron microscopy and small, egg-shell-shaped dystrophic tubular calcifications are also described in association with CNIT. The isometric vacuolization and giant mitochondria are considered reversible, while calcifications may persist.

**Blood vessels:** CNIT involves small vessels, arterioles, and glomerular capillaries. The structural changes resemble different patterns of thrombotic microangiopathy (TMA)-like toxic changes, ranging from mild variants (hyaline arteriopathy or glomerulopathy) to rare, fully developed forms of hemolytic uremic syndrome leading to graft failure.
CNIT most characteristically involves afferent arterioles, but the arteriolar lesions can extent downstream into the glomerulus and upstream into the small arteries with up to two layers of smooth muscle cells. Arteriolar smooth muscle cells undergo swelling, which occasionally may be marked, “balooning” (Fig. 2c). The ballooning of the many of the smooth muscle cells may lead to narrowing and occlusion of the vascular lumen. The “balooning” changes may progress into the classical CNIT “hyaline” arteriolopathy, where nodular protein deposits (hyaline deposits) replace individual necrotic smooth muscle cells of the media. Multiple consecutive hyaline nodules show pearl-string-like pattern. Hyaline nodules can be most pronounced along the adventitial layer and best demonstrated with the PAS stain (Fig. 2d). The more advanced hyaline arteriolopathy may show segmental or circumferential, transmural hyalinosis, complete loss of medial smooth muscle cells, and complete arteriolar stenosis. The hyaline arteriolopathy reportedly can be detected as early as 15
days after surgery with only minority of the arterioles are affected (2), and with progressive increase of the percentage of arteriolar involvement from 5% at six month to 9% at one year, and 12% at two years (3). Of note, the hypertension in transplant patients may also contribute to arteriolar damage leading to “late” lesions of “arteriolopathies of the mixed conventional hypertensive and CNIT types” (4, 5, 6, 7).

**Glomeruli:** 1) Glomerular fibrin thrombi and endothelial cells swelling are early minor and major changes, respectively. These changes may be associated with arteriolar change and mesangiolysis. 2) Glomerular basement membrane (GBM) duplication (CNIT glomerulopathy) presents as a late change and reflects “TMA-like” glomerular remodelling with widening of the lamina rara interna and subendothelial new basement membrane formation (duplication of the capillary wall) (Fig. 3a). CNIT glomerulopathies are associated with arteriolopathies in up to 65% in some series (7). FSGS often develops as a secondary change, with possible progression to global sclerosis.

**Interstitial fibrosis and tubular scarring:** These changes, in part, result from CNIT. However, the patchy/striped fibrotic interstitial changes are nonspecific and indicate nephron loss (Fig. 3b).

![Fig. 3 a – duplication of GBM (2), b-“strype” fibrosis.](image)

To assess degree of severity of morphologic changes induced by CNIT and evaluate clinical relevance of these changes, a semi quantitative, clinically correlated calcineurin inhibitor toxicity score (CNIT score) was developed by N. Kambham ...M. Sarwal (CAJSN 2007) (8). The score includes evaluation of tubular isometric vacuoles (0-3), arteriolar medial hyalinosis (0-3), mesangial matrix increase (0-3), glomerulosclerosis (0-3), interstitial fibrosis (0-3), and tubular atrophy (0-3); with a maximum CNIT score of 18. The pathologists in our center routinely assess CNIT score as a part of the allograft evaluation.

We recently utilized this morphologic scoring system to evaluate CNIT on surveillance kidney biopsies performed at 6, 12 and 24 months on a subset of our kidney transplant patients who were managed by a steroid-free maintenance protocol SFI (9).

Steroids have remained an integral component of immunosuppression in solid-organ transplantation for the last five decades. However, there have been concerns about numerous adverse effects associated with their usage that has resulted in an impetus for withdrawing, minimizing or avoiding steroids in transplant immunosuppression protocols. With the availability of newer immunosuppressive agents, rapid elimination of steroid therapy (steroid withdrawal) and complete steroid avoidance protocols have been successfully used, both in adults and children. Despite the efficacy of steroid-free maintenance immunosuppression (77% of the patients managed with SFI at our center continued to remain off steroids by the end of 1st year), their role in preventing long-term CNIT remains unknown.

Not only did the CNIT score increase overtime in the group as a whole, but there was a statistically significant increase (1.75 ± 1.8 at 6 months to 2.58 ± 1.9 at 12 months; 9 = 0.02) even in patients who had not experienced any episode of rejection. Interestingly, the sub-group of patients who received steroids for the initial few days (steroid withdrawals) had lower CNIT scores at both time points (Fig. 4 &5). Additional studies are needed in determining the role of steroids in the prevention of progressive histological damage due to CNIT.
References: