CASE OF THE MONTH JUNE 2023

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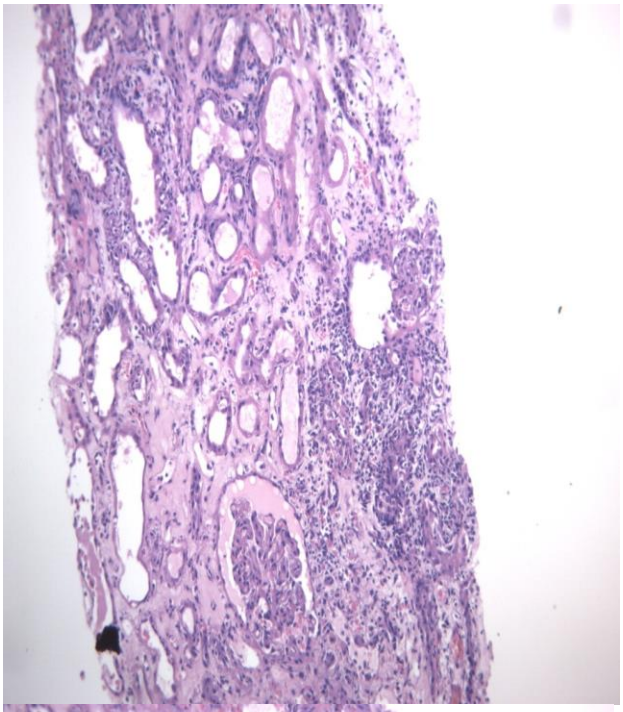
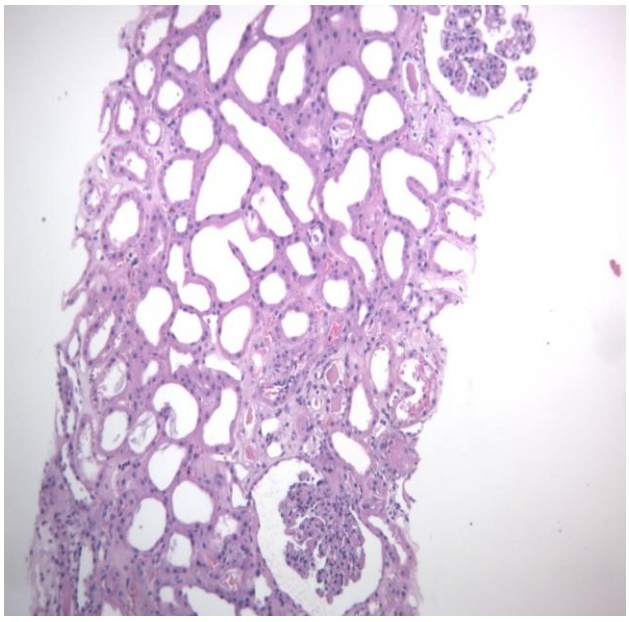
- **24 yrs/M,**
- **CKD with severe hypertension. On maintainance hemodialysis.**
- **Live related renal Transplant done 6 months back,**
- **Father donor**
- **3 Vials of ATG induction given.**
- **Post- operative day 3 [POD3] : Serum creatinine increased from 4.4 to 5.6 mg/dl.**

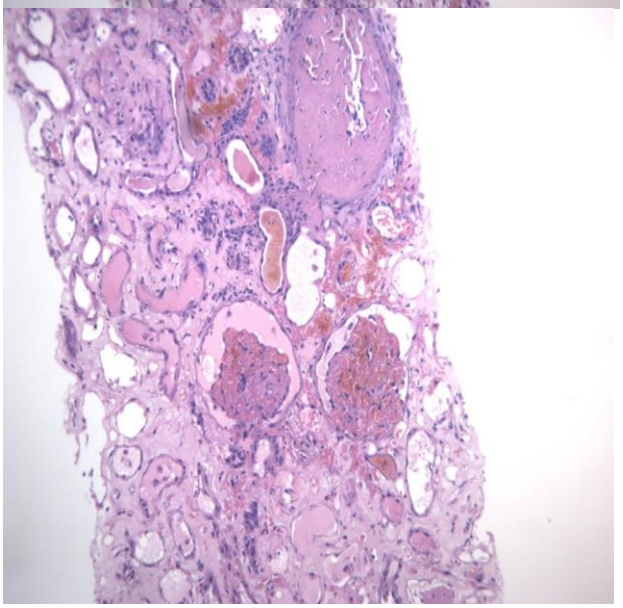
Also had severe hypertension requiring three antihypertensives.

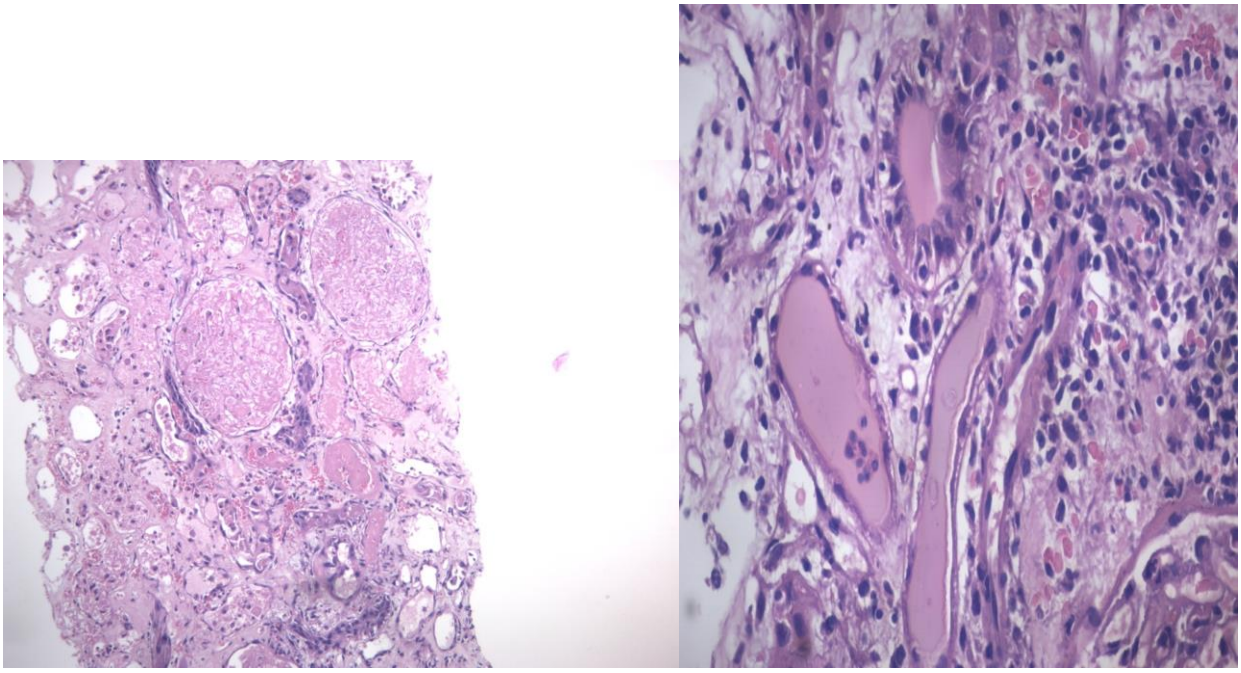
- **Tacrolimus level-20**
- **On USG graft-Renal artery showed low RI value (0.5 to 0.6)**
- **Clinical diagnosis: ? ATN ? ABMR**
- **During subsequent course 3 post transplant graft renal biopsies were performed as follows:**
- **Graft biopsy 1: POD 3 , Graft biopsy 2: POD7, Graft biopsy 3: POD 14**
- **Native kidney biopsy had been performed 7 months prior to transplant and reported as diffuse global glomerulosclerosis with Thrombotic microangiopathy (TMA). ANA was 2+ positive. IF study showed full house pattern and ANA profile was advised to rule out SLE and lupus nephritis. Slides, blocks or images not available for review.**
- **IF in all post –transplant biopsies was negative. C4d study done in all three post transplant biopies were negative.**

EM study also done in biopsy of POD 7th day-No evidence of TMA or Immune complex mediated disease









<u>Morphological diagnosis: Post transplant biopsy (POD3 and POD7) are those of thrombotic Microangiopathy (TMA) with cortical necrosis and acute tubular necrosis in viable area</u>.

<u>Points favouring diagnosis</u>: Infarction of tubules and glomeruli with fibrin thrombi. Blood vessels with fibrointimal hyperplasia, intimal mucoid oedema and fibrinoid necrosis near completely obliterating vascular lumina.

Most common causes of TMA in post transplant patients are Antibody mediated rejection (ABMR), Calcineurin inhibitor toxicity (CNI toxicity), infection.

Work up for etiology of TMA done as follows

1) **ABMR** was ruled out as C4d & DSA were negative**.**
2) **CNI toxicity :**Tacrolimus dose was reduced but, owing to reduction in Immunosuppression, the patient developed acute T cell mediated rejection (as seen in 14th POD biopsy) and

biopsy changes of TMA persisted. Tacrolimus replaced by Cyclosporine.

3) **There was** no obvious infective etiology.(bacterial, viral**)**
4) **Possibility of** classical Shigatoxin related hemolytic uremic syndrome was ruled out.

Native kidney biopsy also had TMA & had demonstrated a full house IF pattern &pre-transplant ANA had been positive led to concern about possibility of recurrence of SLE in the graft biopsy. However, post transplant ANA was negative, IF on all post-transplant biopsies were negative & on electron microscopy there was no evidence of immune complex glomerulonephritis.

Other etiologies common in native kidney (Hemolytic uremic syndrome, TTP)

were than considered.

1) **ADAMTS 13,** to rule out TTP-was in normal range**.**
2) **Antifactor H antibody** was negative by ELISA
3) **Mutation analysis of complement system to rule out atypical HUS-**The results showed CFHR5, Exon 7 heterozygous deletion of unknown significance (VUS-c.993>A(p.Cys331Ter) found with Autosomal dominant inheritance

Final diagnosis: Post transplant recurrent CFHR5 mutation related atypical Hemolytic uremic syndrome (Familial aHUS)

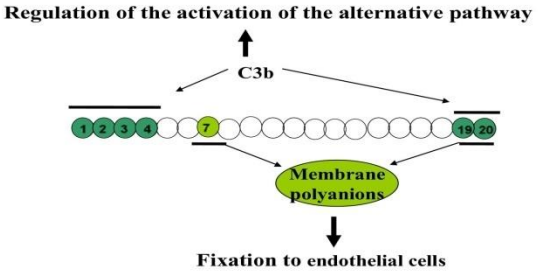
Some salient features of aHUS:

It is an extremely rare disease which differs from typical HUS which is usually associated with Shiga toxin– producing *E. coli*. Typical HUS usually resolves with supportive care (e.g., fluid rehydration, red cell transfusions) & subsides on removal of underlying cause in contrast to aHUS which has a relapsing course & may result in permanent renal impairment.

Pathogenesis and genetics

As is well known, the complement system is a complex group of proteins that work together to fight infection in the body. Complement proteins respond to bacteria, viruses or other foreign substances in the body and ultimately produce a large multi-protein complex (C3 convertase) that directly attacks these foreign invaders. Other complement proteins regulate the formation of this attack complex in order to protect the body's own cells from being damaged. Individuals withaHUS have a mutation in one or more of the genes that encode the complement regulatory proteins.

About 30% of the time, aHUS is associated with malfunctions in the gene (CFH) responsible for the production of a blood protein known as factor H which is one of the regulatory proteins of the complement system. CFHR5 is located in the regulator of complement activation gene cluster on chromosome 1.and constituted by 20 short consensus repeats (SCR). The two binding sites for C3b are in SCR 1-4 and 19-20. The binding sites for polyanions of cell surface (vascular endothelium) are in SCR 7 and 19-20. SCR 1-4 are involved in the binding of CFH to circulating C3b i.e. the regulation of complement alternative pathway activation in the fluid phase. SCR 7 and 19-20 are involved in the binding of CFH to polyanionic surface-bound C3b i.e. the regulation of complement alternative pathway activation at the endothelial cell surface as shown below.



Complement regulatory proteins may be impaired in their action by loss-of-function mutations (*CFH, CFI, CD46*, and *THBD*) or acquired antibodies (specifically to complement factor H). Conversely, potentiation may be augmented by gain-of-function mutations in *CFB* or *C3.* Anti-factor H autoantibodies have been reported in 6-10% of cases, mainly children. Less often, autoantibodies that target other complement proteins have been identified. In approximately 30%-50% of individuals with aHUS, no mutation in a complement gene and no autoantibodies can be detected. These individuals may be referred to as having idiopathic aHUS.

CFH and CFH related proteinsdownregulates the alternative complement pathway by (i) competitively binding C3b to prevent C3 convertase activity and (ii) acting as a cofactor for the proteolytic inactivation of C3b by complement factor I.

Inheritance:

The genetic mutations in complement genes that predispose individuals to aHUS usually occur sporadically, meaning that there is no previous family history of the disorder. The disorder has run in families only about 20% of the time. In such instances, these mutations are transmitted (inherited) as an autosomal dominant trait or, less often, as an autosomal recessive trait.

The penetrance of the disease is low, as less than half of family members carrying the same mutation as the patient with atypical HUS will be affected with the disease. The risk is the same for males and females.

Prognosis: Frequent recurrences and if untreated becomes chronic with severe hypertension and end stage renal disease.

Treatment:

Patients with aHUS are treated with plasmapheresis and Eculizumab[an expensive drug] which is a humanized immunoglobulin G2 monoclonal antibody against complement C5.Prior to the availability of Eculizumab, 50% of aHUS patients relapsed after kidney transplant resulting in a graft failure rate of 80% to 90% after relapses. Currently, aHUS patients who undergo kidney transplant require rigorous risk assessment and preparation including vaccinations and administration of eculizumab to be started prophylactically prior to and continued aftertransplant. The length of prophylactic therapy with eculizumab after kidneytransplant depends on the risk of recurrence in the recipients. Patients with a high risk of recurrence (FH, C3, and FB mutations) need life-long prophylactic Eculizumab therapy.

Conclusion and take home message: Pretransplant diagnosis of aHUS with more robust genetic tests and functional complement assays, with quicker turnaround are needed to facilitate the diagnosis and monitoring of the therapeutic response of aHUS patients. Living donor transplant (particularly from members of the same family) was contraindicated in aHUS patients due to the concerns about the possibility of recurrence aHUS in the transplanted kidney.

Answers to questions.

Q1] What is the morphological diagnosis?

Ans: Thrombotic microangiopathy (TMA) with cortical necrosis

Q2) What are possible etiologies of TMA in post transplant patient?

Ans: Acute antibody mediated rejection (ABMR), CNI toxicity, typical HUS, atypical HUS (See Table)

Q3) What Further work up required?

Ans :C4d study, DSA level, Drug level, antifactor H study, Molecular test for abnormal complements.

4) How is patient with this diagnosis managed?

Ans: Plasmapheresis and Eculizumab

References:

1. Ng MS, McClymont K, McCallum N, Dua R, Holman K, Bennetts B, Ho G, Patel C, Mallett AJ. CFHR5 nephropathy in a Greek-Cypriot Australian family: ancestry-informed precision medicine. Kidney International Reports. 2018 Sep 1;3(5):1222-8.

2. Ávila A, Gavela E, Sancho A. Thrombotic microangiopathy after kidney transplantation: an underdiagnosed and potentially reversible entity. Frontiers in Medicine. 2021 Apr 8;8:642864.

3.Enjeti AK, de Malmanche T, Chapman K, Ziolkowski A. Genomic investigation of inherited thrombotic microangiopathy—aHUS and TTP. International Journal of Laboratory Hematology. 2020 Jun;42:33-40.