Pediatric Nephrology
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CLINICAL QUIZ

Gross hematuria, edema, and hypocomplementemia in a 9-year-old boy: Answers

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Answers

1. What is the differential diagnosis?

The patient presented with edema, gross hematuria, and nephrotic range proteinuria combined with hypocomplementemia. He showed clinical signs of glomerulonephritis (GN).

The first differential diagnosis was acute poststreptococcal glomerulonephritis (APGN) or viral-infection-related GN. He was screened for various infectious etiologies, including anti-streptolysin O (ASO), cytomegalovirus, Epstein-Barr virus, hepatitis B, hepatitis C, and human immunodeficiency virus. All of the tests were negative. Antibiotic treatment at the local hospital was not effective. This did not support a conclusion of infection-related GN.

The second differential diagnosis was IgA nephropathy, but hypocomplementemia is rare in IgA nephropathy. Accordingly, kidney pathology can help confirm the diagnosis.

The third differential diagnosis was immune complex–mediated GN. Given the negative double-stranded DNA (dsDNA), anti-neutrophil cytoplasmic antibodies (ANCA), and anti-glomerular basement membrane (anti-GBM), a process related to systemic lupus or ANCA-mediated vasculitis was deemed unlikely.

The fourth differential diagnosis was hereditary nephritis. The patient presented with gross hematuria and nephrotic range proteinuria. He had a history of cataracts, and his mother had a history of kidney problems (microscopic hematuria and mild proteinuria). Thus, Alport syndrome (AS) was suspected.

The patient had indications for renal pathology with signs of clinical syndrome and a family history of similar conditions.

2. What would you expect to see on kidney biopsy?

A kidney biopsy was performed to explore the patient's etiology. Light microscopy: 25 glomeruli showed moderate mesangial proliferation; moderate focal segmental proliferation was shown. Granular and vacuolar degenerations of renal tubular epithelial cells were shown with protein casts. An interstitial infiltrate containing lipid-laden foam cells was observed (Fig. 1a-c). There were no obvious abnormalities in the walls of the small arteries. Immunofluorescence (IF): IV collagen α2 (+), α5 (−) in the glomerular mesangial area, C3 + + +, IgM +, IgA, IgG, and C1q were all negative (Fig. 1d–f). Electron microscopy (EM) showed that some segments of the capillary basement membrane were thin (the thinnest part was observed to be approximately 180 nm thick) or the subendothelial gap was slightly widened, some segments were uneven in thickness, and the thick dense layer was torn and delaminated, showing a grid-like pattern. A few segments had mesangial insertion, and no

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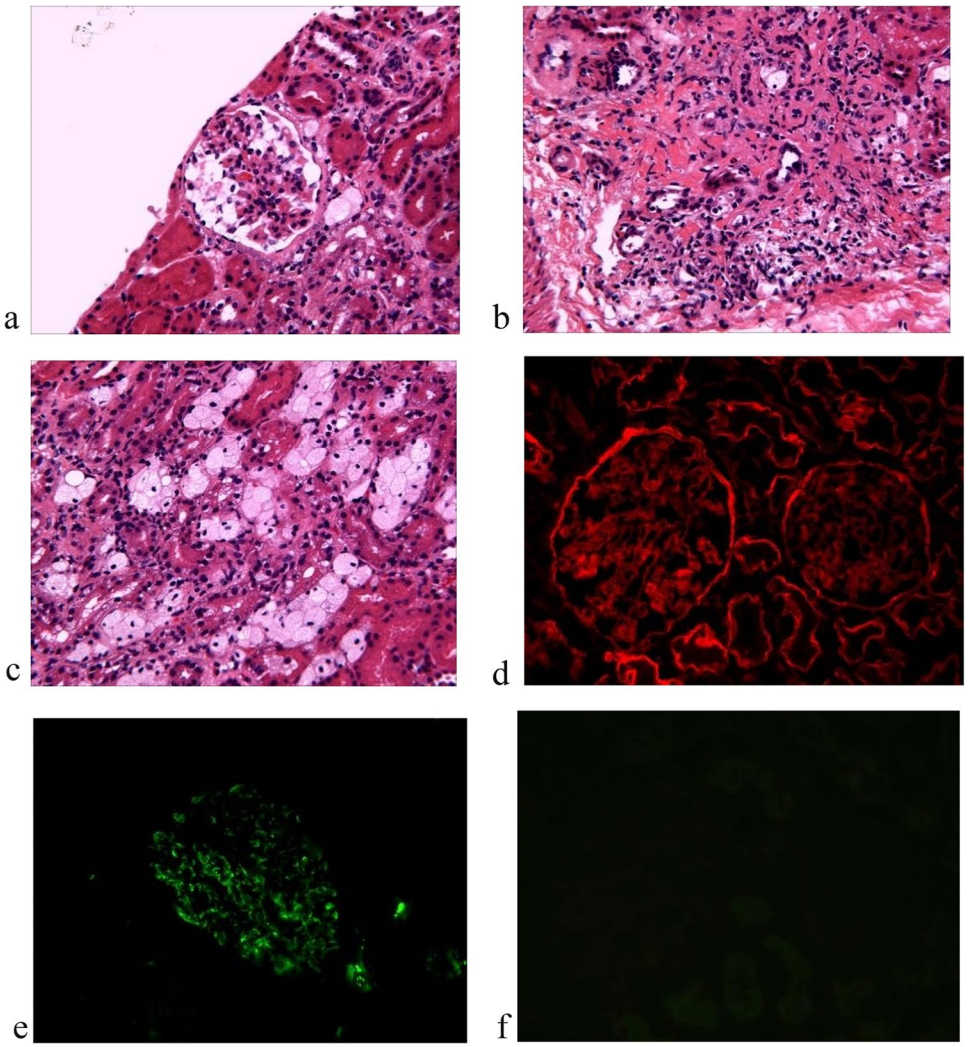

Fig. 1 **a–c** Moderate mesangial proliferation, focal segmental moderate hyperplasia. An interstitial infiltrate containing lipid-laden foam cell is visible. **d–f** Immunofluorescence (IF) in the glomerular mesangial area, C3 + + +, IV collagen α2 (+), and α5 (−)

electron-dense deposits were observed. Most of the foot processes were fused. The mesangial cells and mesangial matrix were slightly proliferated, and small pieces of electron-dense deposits were seen in some mesangial areas. Renal interstitial fibrosis was hyperplastic, and foam cells were visible (Fig. 2). No hump was observed in the ultrastructure.

The collagen α5 (IV) staining and basement membrane performance in EM supported the diagnosis of AS. However, C3 was visibly deposited in the mesangial area in IF, explaining the confluent electron-dense appearance in the mesangial area, instead of the intensely stained intramembranous osmiophilic ribbon-shaped deposits

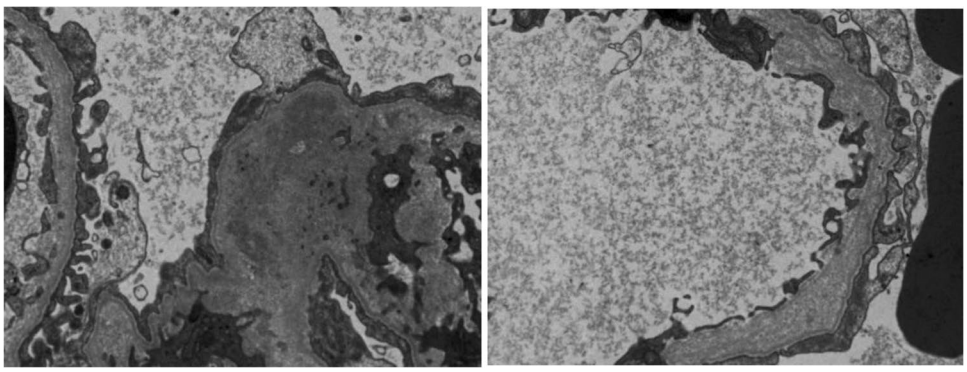

Fig. 2 Electron microscopy: Some segments of the capillary basement membrane were thin, and the subendothelial gap was slightly widened. Small electron-dense deposits were seen in some mesangial areas

pathognomonic for DDD. This allowed us to make a diagnosis of C3 glomerulonephritis (C3G).

3. Which additional diagnostic tests would you perform for the diagnosis?

Since C3G requires the evaluation of the alternative pathway of complement, further lab testing showed the titer of complement C3 nephritis factor (C3NeF) and human complement factors to be significantly increased; factor H antibody (Anti-FH) was slightly increased; human complement factor H (CFH) was slightly increased; and human complement factor I (CFI) was increased (Table 1).

Table 1 Laboratory results about complement factors

	Value	Range	Unit
C3NeF	949.62	95–538	ng/mL
CFI	631.50	42.5–288.5	ng/mL
CFH	979.56	210–452.5	ng/mL
Anti-FH	1345.33	262–1292.5	ng/mL

C3NeF complement C3 nephritis factor, *CFI* human complement factor I, *CFH* human complement factor H, *anti-FH* factor H antibody

Molecular genetic testing is necessary to establish a diagnosis in the cases of both AS and C3GN. We performed whole-exome sequencing for the patient. A hemizygous frameshift mutation (c.2014_2015 ins GCAGA) was found in exon 26 of *COL4A5* gene(NM_000495). The patient had inherited this mutation from his mother, who had a relevant kidney phenotype (microscopic hematuria and proteinuria) (Fig. 3). No mutation of genes related to the complement pathway was detected.

4. What is your diagnosis?

X-linked Alport syndrome (XLAS) combined with C3 glomerulonephritis (C3GN).

The patient was given fosinopril (0.3 mg/kg/day) and prednisone (2 mg/kg/day); proteinuria output was reduced in 7 days.

Discussion

AS is a hereditary disorder of glomerular, cochlear, and ocular basement membranes, resulting from mutations in the collagen IV genes *COL4A3*, *COL4A4*, and *COL4A5* [1–3]. The observation of a pathogenic *COL4A5* variant confirms the diagnosis of XLAS [4]. However, AS with

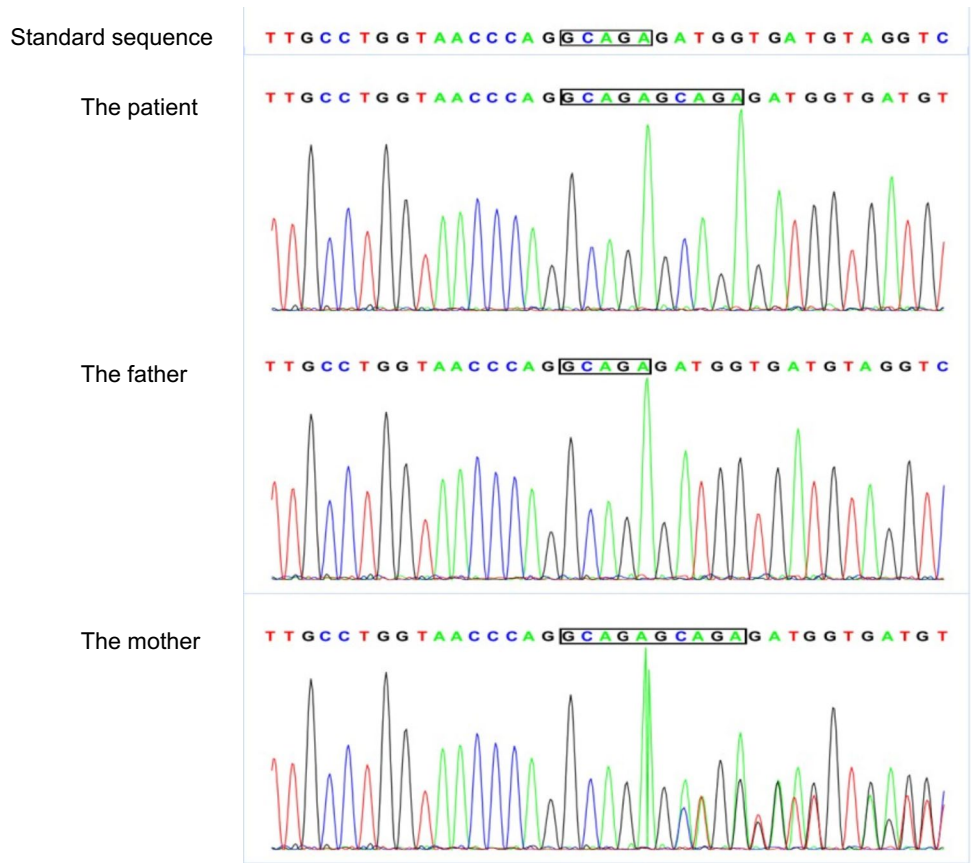

Fig. 3 Sanger sequencing chromatogram. The proband appeared hemizygous. c.2014 (exon26) c.2015 (exon26) insGCAGA of *COL4A5*; his mother as heterozygous, and his father as normal

the co-occurrence of another nephritis is rare. AS in C3GN spontaneous remissions has only been reported once in the literature [5].

In XLAS, there is often a family history of hematuria (with or without proteinuria) or kidney failure [6, 7]. On kidney biopsy, AS produces no specific light microscopic findings [8]. Immunohistochemical analyses typically show the complete absence of immunostaining for the collagen α5(IV) chain in XLAS [9]. EM findings show irregular thickening and thinning of the GBM, lamellation, and splitting of the dense lamina. These findings are specific to AS; EM findings are indispensable for the pathological diagnosis of this condition [8, 10]. In our case, the patient's collagen α5(IV) chain was negative, and we observed a mix of thin and lamellated GBM, which are the characteristic pathological manifestations of AS. With the development of genetic testing, Next-generation sequencing (NGS) has become another means of diagnosis. Expert guidelines currently state that individuals with hematuria and a lamellated GBM or hearing loss, lenticonus, or fleck retinopathy are likely to have AS and should be offered genetic testing for mutations in all three Alport genes (*COL4A5, COL4A3*, and *COL4A4*) [11]. NGS detected a frameshift mutation in *COL4A5* in the patient, and genetic analysis showed the same heterozygosity as his mother. The variant shows low frequency in the general population (1000 genomes: minor allele frequency [MAF] <0.005%). Frameshift mutation leads to possible loss of protein function, and according to ACMG guidelines [12], this mutation is pathogenic. For this reason, the diagnosis of XLAS in this patient and his mother can be made clear.

Notably, the child presented with hypocomplementemia, C3 + + + deposited in mesangium under IF, and electron-dense objects deposited in the mesangial tissue, visible under EM. No hump was observed in the ultrastructure. These pathological findings suggested C3 glomerulopathy (C3G).

C3G is a rare entity defined by C3 dominant GN and a proliferative histologic lesion with C3 deposition at least two orders of magnitude greater than any other immune reactant visible on kidney biopsy IF [13]. The category includes both C3GN and C3 dense deposit disease (C3DDD). EM performance is the main point of identification [14, 15]. In C3GN, discrete C3 deposits are located in the mesangium and subendothelial tissue, whereas DDD is characterized by electron-dense intramembranous deposits that form a unique ribbon-shaped band [15].

Alternative complement pathway (AP) dysregulation leads to C3 deposition in the glomerulus. This is the pathophysiological mechanism underlying C3G [16]. This unrestrained complement activation may be driven by autoantibodies against complement components [17] or abnormalities in complement genes [18–20]. C3 nephritic factor (C3NeF) is an autoantibody that stabilizes C3 convertase (C3bBb) and causes AP dysregulation [18]. Furthermore, 40–50% of patients with C3GN are positive for C3NeF [14, 21]. In our patient, the low C3 levels and the presence of C3NeF in the serum further supported the diagnosis of C3GN.

Ding et al. reported a case describing the co-existence of AS and C3GN. The mechanism underlying C3GN was an uncommon heterozygous variation in *CFHR5*, which regulates the complement cascade [5], but there is no existing report on the association between AS and C3GN, which requires further study.

In addition, there is currently no radical therapy for AS. The goal of treatment is to delay the progression to kidney failure. Recently published expert guidelines recommend that male patients initiate treatment with ACEIs at the time of diagnosis with AS [22].

Treatment for C3GN is based on the inhibition of factors that activate the complement pathways. This is done by administering such drugs as corticosteroids and antiproliferative drugs (mycophenolate mofetil) [23] or complement inhibitors (eculizumab) [24]. In our case, we treated the patient with ACEI and prednisone, and proteinuria decreased in 7 days. During the follow-up, C3NeF and complement C3 levels returned to normal within 1 month. The child received 2 mg/kg of prednisone for 6 weeks, after which the dose was gradually reduced. The total course of prednisone was 8 months. At present, the child has microscopic hematuria with mild proteinuria.

Conclusion

The clinical course of our case is rare. Alport syndrome may be associated with other glomerular diseases. The pathogenic mechanism is unknown, and further study is required.

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Habib R, Gubler MC, Hinglais N, Noel LH, Droz D, Levy M, Mahieu P, Foidart JM, Perrin D, Bois E, Grunfeld JP (1982) Alport's syndrome: experience at Hopital Necker. Kidney Int Suppl 11:S20–S28
2. Kleppel MM, Kashtan CE, Butkowski RJ, Fish AJ, Michael AF (1987) Alport familial nephritis. Absence of 28 kilodalton noncollagenous monomers of type IV collagen in glomerular basement membrane. J Clin Invest 80:263–266. https://doi.org/10.1172/JCI113057
3. Feingold J, Bois E, Chompret A, Broyer M, Gubler MC, Grunfeld JP (1985) Genetic heterogeneity of Alport syndrome. Kidney Int 27:672–677. https://doi.org/10.1038/ki.1985.63

4. Beicht S, Strobl-Wildemann G, Rath S, Wachter O, Alberer M, Kaminsky E, Weber LT, Hinrichsen T, Klein HG, Hoefele J (2013) Next generation sequencing as a useful tool in the diagnostics of mosaicism in Alport syndrome. Gene 526:474–477. https://doi.org/10.1016/j.gene.2013.05.045
5. Ding Y, Tang X, Du Y, Chen H, Yu D, Zhu B, Yuan B (2021) Coexistence of Alport syndrome and C3 glomerulonephritis in a proband with family history. Eur J Med Res 26:71–75. https://doi.org/10.1186/s40001-021-00543-5
6. Jais JP, Knebelmann B, Giatras I, De Marchi M, Rizzoni G, Renieri A, Weber M, Gross O, Netzer KO, Flinter F, Pirson Y, Verellen C, Wieslander J, Persson U, Tryggvason K, Martin P, Hertz JM, Schröder C, Sanak M, Krejcova S, Carvalho MF, Saus J, Antignac C, Smeets H, Gubler MC (2000) X-linked Alport syndrome: natural history in 195 families and genotype-phenotype correlations in males. J Am Soc Nephrol 11:649–657. https://doi.org/10.1681/ASN.V114649
7. Bekheirnia MR, Reed B, Gregory MC, McFann K, Shamshirsaz AA, Masoumi A, Schrier RW (2010) Genotype-phenotype correlation in X-linked Alport syndrome. J Am Soc Nephrol 21:876–883. https://doi.org/10.1681/ASN.2009070784
8. Rumpelt HJ, Langer KH, Schärer K, Straub E, Thoenes W (1974) Split and extremely thin glomerular basement membranes in hereditary nephropathy (Alport's syndrome). Virchows Arch A Pathol Anat Histol 364:225–233. https://doi.org/10.1007/BF00433075
9. Cosgrove D, Liu S (2017) Collagen IV diseases: a focus on the glomerular basement membrane in Alport syndrome. Matrix Biol 57–58:45–54. https://doi.org/10.1016/j.matbio.2016.08.005
10. Nozu K, Nakanishi K, Abe Y, Udagawa T, Okada S, Okamoto T, Kaito H, Kanemoto K, Kobayashi A, Tanaka E, Tanaka K, Hama T, Fujimaru R, Miwa S, Yamamura T, Yamamura N, Horinouchi T, Minamikawa S, Nagata M, Iijima K (2019) A review of clinical characteristics and genetic backgrounds in Alport syndrome. Clin Exp Nephrol 23:158–168. https://doi.org/10.1007/s10157-018-1629-4
11. Savige J, Ariani F, Mari F, Bruttini M, Renieri A, Gross O, Deltas C, Flinter F, Ding J, Gale DP, Nagel M, Yau M, Shagam L, Torra R, Ars E, Hoefele J, Garosi G, Storey H (2019) Expert consensus guidelines for the genetic diagnosis of Alport syndrome. Pediatr Nephrol 34:1175–1189. https://doi.org/10.1007/s00467-018-3985-4
12. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL, Laboratory Quality Assurance Committee ACMG (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17:405–424. https://doi.org/10.1038/gim.2015.30
13. Fakhouri F, Frémeaux-Bacchi V, Noël LH, Cook HT, Pickering MC (2010) C3 glomerulopathy: a new classification. Nat Rev Nephrol 6:494–499. https://doi.org/10.1038/nrneph.2010.85
14. Pickering MC, D'Agati VD, Nester CM, Smith RJ, Haas M, Appel GB, Alpers CE, Bajema IM, Bedrosian C, Braun M, Doyle M, Fakhouri F, Fervenza FC, Fogo AB, Fremeaux-Bacchi V, Gale DP, Goicoechea De Jorge E, Griffin G, Harris CL, Holers VM, Johnson S, Lavin PJ, Medjeral-Thomas N, Paul Morgan B, Nast CC, Noel LH, Peters DK, Rodriguez De Cordoba S, Servais A, Sethi S, Song WC, Tamburini P, Thurman JM, Zavros M, Cook HT (2013) C3 glomerulopathy: consensus report. Kidney Int 84:1079–1089. https://doi.org/10.1038/ki.2013.377
15. Cook HT, Pickering MC (2015) Histopathology of MPGN and C3 glomerulopathies. Nat Rev Nephrol 11:14–22. https://doi.org/10.1038/nrneph.2014.217
16. Sethi S, Gamez JD, Vrana JA, Theis JD, Bergen HR 3rd, Zipfel PF, Dogan A, Smith RJH (2009) Glomeruli of dense deposit disease contain components of the alternative and terminal complement pathway. Kidney Int 75:952–960. https://doi.org/10.1038/ki.2008.657
17. Noris M, Donadelli R, Remuzzi G (2019) Autoimmune abnormalities of the alternative complement pathway in membranoproliferative glomerulonephritis and C3 glomerulopathy. Pediatr Nephrol 34:1311–1323. https://doi.org/10.1007/s00467-018-3989-0
18. Paixao-Cavalcante D, Lopez-Trascasa M, Skattum L, Giclas PC, Goodship TH, de Cordoba SR, Truedsson L, Morgan BP, Harris CL (2012) Sensitive and specific assays for C3 nephritic factors clarify mechanisms underlying complement dysregulation. Kidney Int 82:1084–1092. https://doi.org/10.1038/ki.2012.250
19. Licht C, Heinen S, Jozsi M, Loschmann I, Saunders RE, Perkins SJ, Waldherr R, Skerka C, Kirschfink M, Hoppe B, Zipfel PF (2006) Deletion of Lys224 in regulatory domain 4 of factor H reveals a novel pathomechanism for dense deposit disease (MPGN II). Kidney Int 70:42–50. https://doi.org/10.1038/sj.ki.5000269
20. Iatropoulos P, Noris M, Mele C, Piras R, Valoti E, Bresin E, Curreri M, Mondo E, Zito A, Gamba S, Bettoni S, Murer L, Fremeaux-Bacchi V, Vivarelli M, Emma F, Daina E, Remuzzi G (2016) Complement gene variants determine the risk of immunoglobulin-associated MPGN and C3 glomerulopathy and predict long-term renal outcome. Mol Immunol 71:131–142. https://doi.org/10.1016/j.molimm.2016.01.010
21. Prohászka Z, Nilsson B, Frazer-Abel A, Kirschfink M (2016) Complement analysis 2016: clinical indications, laboratory diagnostics and quality control. Immunobiology 221:1247–1258. https://doi.org/10.1016/j.imbio.2016.06.008
22. Savige J, Gregory M, Gross O, Kashtan C, Ding J, Flinter F (2013) Expert guidelines for the management of Alport syndrome and thin basement membrane nephropathy. J Am Soc Nephrol 24:364–375. https://doi.org/10.1681/ASN.2012020148
23. Rabasco C, Cavero T, Román E, Rojas-Rivera J, Olea T, Espinosa M, Cabello V, FernándezJuarez G, González F, Ávila A, Baltar JM, Díaz M, Alegre R, Elías S, Antón M, Frutos MA, Pobes A, Blasco M, Martín F, Bernis C, Macías M, Barroso S, de Lorenzo A, Ariceta G, López-Mendoza M, Rivas B, LópezRevuelta K, Campistol JM, Mendizábal S, Rodríguez de Córdoba S, Praga M; Spanish Group for the Study of Glomerular Diseases (GLOSEN) (2015) Effectiveness of mycophenolate mofetil in C3 glomerulonephritis. Kidney Int 88:1153-1160.https://doi.org/10.1038/ki.2015.227
24. Bomback AS, Smith RJ, Barile GR, Zhang Y, Heher EC, Herlitz L, Stokes MB, Markowitz GS, D'Agati VD, Canetta PA, Radhakrishnan J, Appel GB (2012) Eculizumab for dense deposit disease and C3 glomerulonephritis. Clin J Am Soc Nephrol 7:748–756. https://doi.org/10.2215/CJN.12901211