Abstract. Membranoproliferative glomerulonephritis (MPGN) is a heterogeneous disease characterized by a morphological injury pattern that can be seen under various disease conditions that share common pathogenic mechanisms. In this study, we analyzed clinical features, pathological findings, long-term kidney outcomes according to the new pathohistological classification of MPGN.

Methods. This retrospective study included 20 CKD patients with biopsy-proven MPGN that had been diagnosed between 2011 and 2019. We reclassified the patterns of MPGN as immune-complexes mediated (ICM) and complement-mediated (CM) according to the new classification.

Results. The level of daily proteinuria was lower in the ICM MPGN than the CM MPGN group but was not statistically significant at the end of the study. Histopathologically, the difference in C3 staining was found between the patients with ICM and CM MPGN. At the end of the follow-up period, no patients developed end-stage renal disease, and no death occurred in response to treatment in the ICM MPGN group. In the CM MPGN group, 2 patients evolved to end-stage renal disease and 1 of them had renal transplantation.

Conclusion. Larger sample size and longer follow-up may change the relationship between histological factors, treatment strategies, and kidney outcomes. We believe that the use of the new diagnostic approach that applies to the ICM MPGN and CM MPGN will help nephrologists to improve treatment options and renal outcomes for patients with MPGN.

Key words: membranoproliferative glomerulonephritis, classification, treatment, kidney outcomes.

Conflict of interest statement. The authors declare no competing interest.
Переоцінка ниркової прогнозу пацієнтів з мембранопроліферативним гломерулонефритом на основі нової патоморфологічної класифікації

Університет наук про здоров’я, навчальний госпіталь ім. Карталя Люфті Кірдара, Стамбул, Туреччина

Резюме. У цьому дослідженні ми проаналізували клінічні особливості, патоморфологічні дані та віддалені результати лікування хворих на мембранопроліферативний гломерулонефрит (МПГН) відповідно до нової класифікації.

Методи. Нами проведено ретроспективне, одноцентрове дослідження із залученням медичної документації 20 пацієнтів з морфологічно верифікованим діагнозом МПГН, який був встановлений у період між 2011 та 2019 рр. Ми перекласифікували патоморфологічні патерни як імунно-комплексний (ІК) МПГН та комплемент-асоційований (КА) МПГН відповідно до нової класифікації.

Результати. Рівень добової протеїнурії був нижчим у хворих на ІК МПГН у порівнянні з пацієнтами з КА МПГН, але не статистично значно не відрізнявся на час закінчення дослідження. Підігнозологічно визначено статистично значну різницю у С3 між пацієнтами обох груп. У групі ІК МПГН у жодного пацієнта не розглядалася термінальна стадія хронічної хвороби нирок, одному з яких виконана трансплантація нирок. Гістопатологічно визначено асоційовані (КА) МПГН відповідно до нової класифікації.

Висновок. Більша вибірка пацієнтів та триваліший період спостереження можуть змінити взаємозв’язок між патоморфологічними характеристиками МПГН, стратегіями лікування та нирковим прогнозом. Ми вважаємо, що використання нового діагностичного підходу, який застосовується до ІК та КА МПГН, допоможе нефрологам поліпшити можливості лікування та нирковий прогноз для пацієнтів з МПГН.

Ключові слова: мембранопроліферативний гломерулонефрит, класифікація, лікування, прогноз.
Arteriosclerotic changes were also evaluated using a similar grading scale: grade 0, no abnormality; grade 1, present. The recorded IF microscopy findings included staining force for IgG, IgA, IgM, C3, C1q, kappa, lambda. The intensity of glomerular mesangial proliferation was graded as follows: 1, mild; 2, moderate; 3, severe (graded 0–3 +).

Cases of MPGN clinically known to be related to systemic lupus erythematosus (or other connective tissue disorders), infections including hepatitis B, C, paraproteinemia or B cell lymphoma or plasma cell dyscrasia were excluded from the study cohort. The clinicopathological findings and prognosis of patients were compared between ICM- and CM MPGN.

Clinical and laboratory data during the initial kidney biopsy were obtained manually from the patient’s medical records and included age, gender, blood pressure, hematuria, proteinuria, serum creatinine, serum albumin, serum C3, and treatment. The following laboratory tests were performed according to standard hospital procedures: measurement of 24-h urinary protein excretion (UP g/day); urinary red blood cell count per high power field (U-RBC/HPF); measurement of serum creatinine (sCr, mg/dl); estimated glomerular filtration rate (eGFR, ml/min); serum albumin (Alb, g/dl); C3 (mg/dl). Renal status was recorded at the last clinical follow-up. Microscopic hematuria was defined as > 5 red cells per HPF in microscopic examination. The patients with nephrotic syndrome (UP ≥ 3.5 g/day) were given immunosuppressive therapy.

Statistical analysis was performed using the SPSS package program. For the statistical analysis, we used the Student’s t-test and the data expressed as mean and standard deviation (M ± SD). Categorical variables were expressed as proportions. The Chi-square tests (χ2) were used to compare 2 groups.

Results. The clinical and laboratory characteristics of the included patients are summarized in Table 1.

|                  | ICM MPGN (n = 14) | CM MPGN (n = 6) | P-value |
|------------------|-------------------|----------------|---------|
| **Age**          | 46.93±14.31       | 36.50±8.45     | 0.09    |
| **Gender (F/M)** | 6/8               | 3/3            | 0.77    |
| **Creatinine (first) (mg/dl)** | 2.00±2.70       | 1.21±0.76       | 0.68    |
| **Creatinine (end) (mg/dl)** | 1.09±0.44       | 1.99±2.39       | 0.90    |
| **Proteinuria (first) (g/day)** | 5.30±3.4        | 4.1±2.9         | 0.93    |
| **Proteinuria (end) (g/day)** | 2.2±1.4         | 2.7±1.7         | 0.13    |
| **Hematuria (present/not)** | 13/1          | 6/0             | 0.51    |
| **Serum albumin (first) (g/dl)** | 3.11±0.54       | 2.90±0.48       | 0.47    |
| **Serum albumin (end) (g/dl)** | 3.96±0.52       | 3.66±0.41       | 0.21    |
| **C3 (first)**   | 1.40±0.30         | 1.40±0.30       | 0.91    |
| **C3 (end)**     | 1.46±0.26         | 1.61±0.16       | 0.15    |
| **Hypertension (present/not)** | 13/1           | 5/1             | 0.52    |
| **Follow-up period (month)** | 66.42±33.32     | 55.0±24.71      | 0.54    |

Although there was no statistically significant difference in clinical and laboratory data between the patients of ICM and CM groups, the creatinine value decreased in the ICM MPGN group, while the creatinine value increased in the CM MPGN group at the end of the study period. The PU level was also lower in the patients of ICM MPGN compared with the patients of the CM MPGN group, but it was not statistically significant at the end of the study.

As presented in Table 2, a statistically significant difference in C3 staining was found between the groups.
Table 2

|                          | ICM MPGN (n = 14) | CM MPGN (n = 6) | P-value |
|--------------------------|-------------------|-----------------|---------|
| **Global sclerosis**     |                   |                 |         |
| Absent                   | 11                | 2               | 0.06    |
| Present                  | 3                 | 4               |         |
| **Crescent**             |                   |                 | 0.52    |
| Absent                   | 13                | 5               |         |
| Present                  | 1                 | 1               |         |
| **Mesengial proliferation(%)** |           |                 |         |
| Mild                     | 3                 | 0               | 0.21    |
| Moderate                 | 8                 | 5               |         |
| Severe                   | 3                 | 1               |         |
| **Interstitial fibrosis(%)** |               |                 | 0.09    |
| None                     | 12                | 3               |         |
| 1-25                     | 2                 | 3               |         |
| 26-50                    | 0                 | 3               |         |
| >50                      | 0                 | 3               |         |
| **Arteriolar sclerosis** |                   |                 | 0.09    |
| Absent                   | 12                | 3               |         |
| Present                  | 2                 | 3               |         |
| **IgG**                  | 14                | 0               |         |
| **IgA**                  | 8                 | 0               |         |
| **IgM**                  | 8                 | 0               |         |
| **C3**                   | 6                 | 6               | 0.02    |
| **C1q**                  | 4                 | 0               |         |
| **Kappa**                | 2                 | 0               |         |
| **Lambda**               | 3                 | 0               |         |

RAA as a treatment option was used in 13 (92.9%) patients in ICM MPGN, while RAA was prescribed for all patients in the CM MPGN group. Methylprednisolone in a dose 1 g per 3 days was used in both groups. 13 (92.9%) patients of the CM MPGN group used methylprednisone and all the patients of the CM MPGN group. Methylprednisone treatment was started in dose 0.8 mg/ kg/day and decreased at the end of 2 months and discontinued in 6 months. Cyclophosphamide was applied to 10 (71.4%) patients in the ICM MPGN group and 5 (83.3%) patients of the CM MPGN group. Finally, in the ICM MPGN group, none of the patients neither developed end-stage renal failure nor died in response to the treatment. In the CM MPGN group, 2 patients evolved to end-stage renal disease and 1 of them underwent kidney transplantation.

Discussion. Several recent studies have greatly improved our understanding of MPGN’s pathophysiology and emphasized frequent association with complement activation or lymphoproliferative disease disorders [10]. Due to these findings, a new classification divides MPGN into cases that are CM or ICM MPGN. This study provides a detailed explanation of the differences in clinical and histopathological features observed in ICM and CM MPGN patients. Viswanathan et al calculated the CM MPGN incidence in all renal biopsies of 0.7% [11]. Indian study conducted by Himamani showed an incidence of 14% among all MPGN cases [12]. In our study, the percentage of CM MPGN covers 30% of all MPGN patients. Servais et al. reported that 71% of CP-mediated glomerulonephritis patients had genetic disorders and/or autoantibodies. Genetic abnormalities such as variants of complement factor H (CFH), complement factor I (CFI), membrane cofactor protein (MCP), and autoantibodies, such as anti-C3-Nef (C3 nephritic factor) and anti-factor H antibodies, stabilize C3 convertase (C3bBb) and amplify conversion from C3 to the active molecule C3b in...
the fluid phase of complement activation [13]. Genetic studies of complement factors could not be performed due to the technical inadequacy of CM MPGN.

Serum C3 was significantly lower in the CM MPGN group than in the ICM MPGN group. This fact is evidence of a more active alternative complement pathway in the fluid phase [14]. In our study, there was no difference in serum C3 levels between IC MPGN and CM MPGN at the time of diagnosis and after the treatment. The small sample size might result in this conclusion.

In addition, there were no differences in sclerosis or necrotic glomeruli, crescent presence, degree of mesangial proliferation, interstitial fibrosis percentage, and other pathological findings in terms of arteriolar sclerosis. These results suggest that there is no significant difference in complement activation of the solid phase in local tissue, and therefore no equivalence in other pathological findings and kidney prognosis [15]. In our study, light microscopy findings had similar characteristics in both groups.

In patients with ICM MPGN, targeting the treatment of the underlying disease (hepatitis, lupus, dysproteinaemia) is effective. Otherwise, immunosuppressive agents are widely used in the treatment of MPGN [16, 17]. The secondary MPGN cases were not included in our study population.

Steroids are the cornerstone therapy for MPGN even when other immunosuppressive agents are used together. In terms of immunosuppressive agents, only a few reports are showing that cyclophosphamide (CYC) is effective for patients with MPGN other than steroids [18, 19]. The mechanism of conventional therapy for solid-phase complement activation remains unclear. AP irregularity also occurs in CM MPGN, and the establishment of treatment for complement activation in the solid phase is expected in recent years [20].

Conclusions. In conclusion, we believe that the complement-mediated type is important because complement-targeting therapies can be developed as potential disease-modifying agents. Eculizumab is one of the available anti-complement treatments. Bomback et al found promising results using eculizumab in c3 glomerulopathies in natural and allograft biopsies [21, 22]. In our study, most of the patients in both groups received steroid and cyclophosphamide therapy as immunosuppressive therapy. Although there was no statistical difference in response to treatment, at the end of the study, ICM MPGN cases were found to have lower creatinine and proteinuria values compared to CM MPGN. Considering this difference, it may be thought that giving specific agents effective to the alternative complement pathway to the CM MPGN group may affect the treatment results. Eculizumab treatment was given to a patient who developed posttransplant CM MPGN, and improvement in proteinuria and kidney function tests of the patient was observed during the following treatment. Nephrologists should consider checking alternative complementary pathway defects in patients with CM MPGN in IF.

Limitations. It was a small sample size retrospective study performed in a single-center, and, thus, our results will need to be confirmed in full-scale studies. There was no study on the evaluation of alternative path abnormalities. We also believe that the choice of alternative pathway-specific agents can select the treatment selection and outcomes of CM MPGN relationships. Larger sample size and longer follow-up may change the relationship between histological factors, treatment strategies, and kidney outcomes.

Conclusions. In conclusion, we believe that the use of the new diagnostic approach that applies to the ICM MPGN and CM MPGN will help nephrologists to improve treatment options and renal outcomes for patients with MPGN.

References:

1. Hohenstein B. C3-Glomerulopathie und MPGN – aktuelle Klassifikationen [C3 glomerulopathy and MPGN - current classification]. Disch Med Wochenschr. 2020;145(4):232-239. doi:10.1055/a-0974-8418.
2. Andrighetto S, Leventhal J, Zaza G, Cravedi P. Complement and Complement Targeting Therapies in Glomerular Diseases. Int J Mol Sci. 2019;20(24):6336. doi: 10.3390/ijms20246336.
3. Sethi S, Fervenza FC. Membranoproliferative glomerulonephritis—a new look at an old entity. N Engl J Med. 2012; 366: 1119-31. doi: 10.1056/NEJMra1108178.
4. Sethi S, Fervenza FC. Membranoproliferative glomerulonephritis: pathogenetic heterogeneity and proposal for a new classification. Semin Nephrol. 2011; 31: 341-348. doi: 10.1016/j.sente.2011.06.005.
5. Bomback AS, Appel GB. Pathogenesis of the C3 glomerulopathies and reclassification of MPGN. Nat Rev Nephrol. 2012;8(11):634–42. doi: 10.1038/nrneph.2012.213.
6. Sethi S, Nester CM, Smith RJ. Membranoproliferative glomerulonephritis and C3 glomerulopathy: resolving the confusion. Kidney Int. 2012; 81: 434-41. doi: 10.1038/ki.2011.399.
7. D’Agati VD, Bomback AS. C3 glomerulopathy: what’s in a name? Kidney Int. 2012; 82: 379-81. doi: 10.1038/ki.2012.80.
8. Cook HT, Pickering MC. Clusters not classifications: making sense of complement-mediated kidney injury. J Am Soc Nephrol. 2018;29(1):9-12. doi: 10.1681/ASN.2017111183.
9. Santos JE, Fiel D, Santos R, et al. Rituximab use in adult glomerulopathies and its rationale. J Bras
10. Sethi S, Fervenza FC, Zhang Y, Zand L, Meyer NC, Borsa N, Naras SH, Smith RJ. Atypical postinfectious glomerulonephritis is associated with abnormalities in the alternative pathway of complement. Kidney Int. 2013; 83: 293-299. doi: 10.1038/ki.2012.384.

11. Viswanathan GK, Nada R, Kumar A, et al. Clinico-pathologic spectrum of C3 glomerulopathy — an Indian experience. Diagn Pathol. 2015;10:6. doi: 10.1186/s13000-015-0233-0.

12. Himamani S. Membranoproliferative glomerulonephritis common glomerular disease — changing pattern of biopsy proven renal disease in a Tertiary Care Hospital. Int J Sci Stud.2016;3(11):193-6. Available from: https://www.semanticscholar.org/paper/Membranoproliferative-Glomerulonephritis-Common-of-Himamani/a89017f20e4cb4e7afed56652a3d74317f15a720#paper-header.

13. Servais A, Noël LH, Roumenina LT, Le Quintrec M, Ngo S, et al. Acquired and genetic complement abnormalities play a critical role in dense deposit disease and other C3 glomerulopathies. Kidney Int. 2012;82(4):454–64. doi: 10.1038/ki.2012.63.

14. Bomback AS, Santoriello D, Avasare RS, Regunathan-Shenk R, et al. C3 glomerulonephritis and dense deposit disease share a similar disease course in a large United States cohort of patients with C3 glomerulopathy. Kidney Int. 2018;93(4):977–85. doi: 10.1016/j.kint.2017.10.022.

15. Nakano M, Karasawa K, et al. Characteristics of membranoproliferative glomerulonephritis based on a new classification at a single center. Clin Exp Nephrol. 2019 Jun;23(6):852-58. doi: 10.1007/s10157-019-01716-7.

16. Morales JM, Kamar N, Rostaing L. Hepatitis C and renal disease: epidemiology, diagnosis, pathogenesis and therapy. Contrib Nephrol. 2012;176:10-23. doi: 10.1159/000333772.

17. Nargund P, Kambham N, et al. Clinico-pathological features of membranoproliferative glomerulonephritis under a new classification. Clinical Nephrology, 2015; Vol. 84, No. 6:323-30. doi: 10.5414/CN108619.

18. Corvillo F, Okrój M, Nozal P, Melgosa M, Sánchez-Corrall P, López-Trascasa M. Nephritic Factors: An Overview of Classification, Diagnostic Tools and Clinical Associations. Front Immunol. 2019;10:886. doi: 10.3389/fimmu.2019.00886.

19. Imtiaz S, Dhrolia MF, Nasir K, Salman B, Ahmad A. Type of immune and complement deposits and response of immunosuppressive treatment on membranoproliferative glomerulonephritis - a single centre experience. J Pak Med Assoc.2015;65(9):995-1000. Available from: https://pubmed.ncbi.nlm.nih.gov/26338748/.

20. Iatropoulos P, Daina E, Curreri M, Piras R, Valoti E, et al. Registry of membranoproliferative glomerulonephritis/C3 glomerulopathy; Nastasi. Cluster analysis identifies distinct pathogenetic patterns in C3 glomerulopathies/immune complex-mediated membranoproliferative GN. J Am Soc Nephrol. 2018;29(1):283–94. doi: 10.1681/ASN.2017030258.

21. Deshpande N, Tewari C, Badwal C, et al. Evaluation of cases of membranoproliferative glomerulonephritis according to newer classification: A retrospective record-based study. Med J Armed Forces India. 2018;74: 264-7. doi: 10.1016/j.mjafi.2017.01.008.

22. Bomback AS, Smith RJ, Barile GR, et al. Eculizumab for dense deposit disease and C3 glomerulonephritis. Clin J Am Soc Nephrol. 2012;7:748-56. doi: 10.2215/CJN.12901211.