Ukrainian Journal of Nephrology and Dialysis
Scientific and Practical, Medical Journal

**Founder:**
- National Kidney Foundation of Ukraine

**ISSN 2304-0238;**
**eISSN 2616-7352**

**Journal homepage: https://ukrjnd.com.ua**

## Original Papers

doi: 10.31450/ukrjnd.1(81).2024.05

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# The link between moderate COVID-19 and delayed manifestation of glomerulonephritis: Insights from cluster analysis of TGF-β1 and VEGF levels

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Citation: Zub LO, Horban BV, Kulachek VT. The link between moderate COVID-19 and delayed manifestation of glomerulonephritis: Insights from cluster analysis of TGF-β1 and VEGF levels. Ukr J Nephr Dial. 2024;1(81):35-41. doi: 10.31450/ukrjnd.1(81).2024.05.

**Abstract.** *Recent data reveal delayed pathological kidney changes occurring 8-12 months post-moderate COVID-19, often detected for the first time. While severe COVID-19 leads to interstitial and subsequent glomerular lesions, the predominant changes in moderate cases remain elusive. This study aimed to investigate the potential association between moderate COVID-19 and the manifestation of glomerulonephritis (GN).*

*Methods. This cross-sectional study included 25 patients with stage 1-2 GN and a history of moderate COVID-19 8-12 months before the onset of GN, 27 individuals without GN who experienced COVID-19, and 20 healthy subjects without a history of COVID-19. Transforming growth factor-β1 (TGF-β1) and vascular endothelial growth factor (VEGF) levels in blood and urine were measured using enzyme-linked immunosorbent assay. Cluster and classification data mining methods were utilized for these markers to assess potential relationships between moderate COVID-19 and GN manifestation.*

*Results. A significant increase in blood and urine TGF-β1 and VEGF levels was found in GN patients with a history of moderate COVID-19 ($p<0.05$), while elevated blood VEGF was observed in those without GN ($p<0.05$). Cluster analysis affirmed the correlation, emphasizing that urinary TGF-β1 within the range of 1.352 to 5.693 pg/ml and urinary VEGF < 214.12 pg/ml serve as classification rules for predicting GN.*

*Conclusions. The cluster and classification analysis method for TGF-β1 and VEGF levels can be utilized in clinical practice to predict the development of GN in the long-term post-COVID period.*

**Keywords:** *glomerulonephritis, COVID-19, transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor (VEGF).*

**Article history:**
*Received December 07, 2023*
*Received in revised form January 02, 2024*
*Accepted January 05, 2024*

**Conflict of interest.** The authors declare no conflict of interest.



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УДК: 616.611-002-02:616.98:578.834

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# Взаємозв'язок між помірним перебігом COVID-19 та відстроченим розвитком гломерулонефриту: дані кластерного аналізу рівнів TGF-β1 і VEGF

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**Резюме.** *Останні дані демонструють відстрочені патологічні зміни нирок, які виникають через 8-12 місяців після перенесеної COVID-19, навіть у пацієнтів з помірним перебігом хвороби. У той час як тяжкий перебіг COVID-19 призводить до інтерстиціального та подальшого ураження клубочків, переважні зміни у помірних випадках залишаються невловимими. Це дослідження мало на меті дослідити потенційний зв'язок між помірним перебігом COVID-19 і маніфестацією гломерулонефриту (ГН).*

*Методи. Це одномоментне дослідження включало 25 пацієнтів з ГН 1-2 стадії та перенесеним COVID-19 середньої тяжкості 8-12 місяців тому, 27 осіб без ГН, які перенесли COVID-19, і 20 здорових осіб без COVID-19 в анамнезі. Концентрації трансформуючого фактора росту-β1 (TGF-β1) і фактора росту ендотелію судин (VEGF) у крові та сечі визначали за допомогою твердофазного імуноферментного аналізу. Для оцінки потенційного зв'язку між перенесеним COVID-19 і ГН використовували кластерний та класифікаційний аналіз зазначених маркерів.*

*Результати. У пацієнтів з ГН та перенесеним середньотяжким COVID-19 спостерігалось значне підвищення рівнів TGF-β1 і VEGF у крові та сечі ($p<0,05$), тоді як підвищений рівень VEGF у крові без ГН ($p<0,05$). Кластерний аналіз підтвердив кореляцію, підкресливши, що TGF-β1 у сечі в межах від 1,352 до 5,693 пг/мл і VEGF у сечі < 214,12 пг/мл служать класифікаційними правилами для прогнозування ГН.*

*Висновки. Метод кластерного та класифікаційного аналізу для TGF-β1 і VEGF можна використовувати в клінічній практиці для прогнозування розвитку ГН у віддаленому посковідному періоді.*

**Key words:** *гломерулонефрит, COVID-19, трансформуючий фактор росту-β1 (TGF-β1), фактор росту ендотелію судин (VEGF).*

**Introduction.** Presently, it is established that the COVID-19 virus exerts cytotoxic effects, directly damaging the endothelium and tubular epithelium [1, 2]. Symptoms such as fever, vomiting, diarrhea, low blood pressure, and shock contribute to renal hypoperfusion, leading to acute kidney injury [1, 3]. Following such a renal catastrophe, the progression of pathological changes in the kidneys can be anticipated. Notably, heightened COVID activity plays a detrimental role in kidney damage, both through direct viral impact and indirectly via compromised immune mechanisms and cytokine storms [3, 4].

While the impact of severe COVID-19 on kidney function has been extensively studied [1, 3], the long-term effects of a moderate course of the disease on kidney outcomes have not been as thoroughly investigated. A meta-analysis of observational studies involving 6976 patients with COVID-19-associated acute kidney injury and 5223 COVID-19 patients without acute kidney injury found that patients with more comorbidities tend to have a higher renal non-recovery rate after COVID-19 [5]. For COVID-19 patients without acute kidney injury, a decrease in kidney function may occur during long-term follow-up [5]. This suggests that moderate COVID-19 can have a lasting impact on kidney health, which may become apparent months after the initial infection [5, 6]. The current understanding of the burden of chronic kidney disease (CKD) that develops after COVID-19 is still limited, highlighting the need for long-term follow-up and monitoring of patients who have recovered from COVID-19, regardless of the severity of their disease. In this context, the evaluation of novel prognostic factors based on cytokine response, such as transforming growth factor-beta 1 (TGF-β1) and vascular endothelial growth factor (VEGF), may provide valuable insights into the long-term kidney outcomes in patients with moderate COVID-19.

TGF-β plays a crucial role in maintaining vascular homeostasis and is involved in immune responses and inflammation, particularly in the context of high IL-6 activity [7]. It can stimulate immune responses and drive inflammation, contributing to tissue fibrosis. Increased TGF-β activity has been observed in COVID-19 patients, suggesting its potential role in disease progression and severity [7, 8]. VEGF is essential for vascular endothelial homeostasis and is present in numerous cells and tissues [8]. It plays a critical role in endothelial cell activation. Elevated levels of VEGF have been found in severe COVID-19 patients, and it has been hypothesized that high levels of VEGF are linked to a storm of blood

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clots in COVID-19 patients, which eventually leads to disease severity [8, 9]. Therefore, the evaluation of TGF-β1 and VEGF as prognostic factors could potentially enhance our understanding of the long-term kidney outcomes in patients with moderate COVID-19.

**The objective** of the study was to investigate the potential association between moderate COVID-19 and the manifestation of GN.

**Materials and Methods.** This cross-sectional study enrolled 25 patients diagnosed with stage 1-2 glomerulonephritis (GN) who had a history of moderate COVID-19 8-12 months prior to the onset of GN, 27 individuals who experienced COVID-19 without GN, and 20 healthy subjects with no history of COVID-19. The study adhered to the Declaration of Helsinki, and written informed consent was obtained from all participants.

Standard nephrological research methods, following adapted clinical guidelines and a unified protocol, were employed for patient evaluation.

TGF-β1 and VEGF levels in blood and urine were analyzed using enzyme-linked immunosorbent assay with a Stat Fax-303 analyzer. These markers were chosen based on previous studies demonstrating their relevance in the early detection of progressive chronic kidney disease (CKD).

To identify factors predicting the manifestation of GN in patients with moderate COVID-19 8-12 months ago, cluster and classification methods of Data Mining were employed. Objects were clustered into three groups based on the aforementioned sets of factors, and the cluster data content was determined. The first cluster represented healthy individuals without a history of COVID-19, the second cluster represented patients who had moderate COVID-19 8-12 months ago without GN, and the third cluster represented patients with chronic GN stage I-II who had moderate COVID-19 8-12 months ago. This classification analysis aimed to confirm the hypothesis and assess the significance of the factors for diagnosing GN.

Possible relationships between moderate COVID-19 and GN were explored using three different clustering methods grounded in distinct mathematical theories: the classical k-means method, Kohonen maps (neural networks), and fuzzy k-means (fuzzy logic).

**Results.** The levels of VEGF and TGF-β1 in both blood and urine were notably higher in patients with GN who had experienced moderate COVID-19 8-12 months prior, in comparison to both individuals without GN and the healthy control group (Table 1).

*Table 1*

**Blood and urine VEGF levels in studied groups (M±SD, n)**

| Indexes | Healthy with no history of COVID (n=20) | Without GN (n=27) | With chronic GN (n=25) |
|---|---|---|---|
| Blood VEGF (pg/ml) | 182.2±11.3 | 301±10.2* | 378.2±11,7*^ |
| | | p<0.05 | p<0.001 |
| Urinary VEGF (pg/ml) | 200.15±10.5 | 219.1±10.5 | 391.2±10.4*^ |
| | | p>0.05 | p<0.001 |
| Blood TGF-β1 (pg/ml) | 53.23±3.82 | 57.11±1.28 | 119.87±2.55*^ |
| | | p>0.05 | p<0.001 |
| Urinary TGF-β1 (pg/ml) | 1.59±0.08 | 1.60±0.02 | 4.82±0.07*^ |
| | | p>0.05 | p<0.05 |

Notes: * – reliability in comparison with the group of healthy individuals

^ – reliability in comparison with the group without GN

The results of the cluster analysis showed the following distribution of patients (Table 2).

*Table 2*

**Results of classification analysis by the neural network method**

| Groups of examined patients with real diagnoses | | | | |
|---|---|---|---|---|
| | Classified | | | |
| ACTUAL | Healthy | Without GN | With GN | Total |
| Healthy | 20 | | | 20 |
| Without GN | | 27 | | 27 |
| With GN | | | 25 | 25 |
| Total | 20 | 27 | 25 | 72 |

*Continuation of Table 1*

| Kohonen map | | | | |
|---|---|---|---|---|
| | Classified | | | |
| **ACTUAL** | **Healthy** | **Without GN** | **With GN** | **Total** |
| Healthy | 20 | | | 20 |
| Without GN | | 22 | | 22 |
| With GN | | | 30 | 30 |
| Total | 20 | 22 | 30 | 72 |
| k-means (fuzzy clustering) | | | | |
| | Classified | | | |
| **ACTUAL** | **Healthy** | **Without GN** | **With GN** | **Total** |
| Healthy | 20 | | | 20 |
| Without GN | | 21 | | 21 |
| With GN | | 1 | 29 | 30 |
| Total | 20 | 22 | 29 | 72 |

All healthy patients were placed in a separate cluster (cluster 0). We got the conclusions of all three clustering methods. But there were differences in the diagnosis of GN and the group without GN who had COVID.

When constructing the Kohonen map, patients diagnosed with GN were placed in a separate cluster (cluster 2). Our patients without GN who had COVID (27 people), according to the Kohonen maps, mostly fell into cluster "1" (22 patients). Some of them (5 patients) were classified as belonging to cluster "2". Thus, these individuals should have signs of EVD, although they have not been clinically diagnosed with EVD, but are only known to have moderate COVID. This means that such patients are at risk of developing GN. Such an analysis suggests that moderate COVID may have signs of a factor causing the manifestation of GN.

The results of clustering using k-means and fuzzy clustering showed the same results of belonging to clusters. However, the data from this method is slightly different from the previous methods. It is known that 27 people had moderate COVID, but they did not show signs of GN, but 5 were assigned to cluster "2". In the second cluster, there is also a discrepancy – 1 patient is assigned to cluster "1", i.e., patients without GN. This indicates that the boundary between the GN and non-GN (post COVID) clusters is somewhat blurred. This gives us the right to believe, to some extent, that moderate COVID can provoke the onset of GN. When analyzing the fuzzy clustering method, if for a new patient

$$\mu 2 = 0.02$$

the following data were obtained: $\mu 0 = 0.95$, $\mu 1 = 0.08$ - this correlates with cluster "0" by 95% and means that the subject is healthy.

In the case of $\mu 2 = 0.41 - \mu 0 = 0.01$, $\mu 1 = 0.55$, this correlates with belonging to GN (cluster "2"), even if the patient formally belongs to cluster "1". It means that in the future, this patient can be diagnosed with GN.

The clustering methodology allows us to confirm the link between moderate COVID and GN. However, this does not mean that this methodology determines the rules for correlating new patients and assigning them to certain clusters. This makes it impossible to conduct preliminary diagnostics without the introduction of information systems in which these clustering methods are programmed. Based on this, these rules can be constructed in the form of "decision trees" that can be obtained by means of classification analysis by neural networks. "Decision trees represent rules in a hierarchical structure that are sequential, where each object corresponds to only one node that provides a solution. For this purpose, we used the CART (Classification and Regression Tree) method. The CART method is an algorithm for building a binary decision tree (dichotomous classification model). In this algorithm, each node of the decision tree has two descendants. The rule formed in the node, according to each step of the tree construction, divides the given set of examples into two parts: the RIGHT descendant – the part in which the rule is executed and the LEFT descendant – the part in which the rule is not executed. To select the optimal rule, the partitioning quality evaluation function is used. Thus, neural networks implement this method.

The "input" parameters are the same factors, and the "output" is the cluster number. Thus, to establish the rules, we conducted a classification analysis for three output fields: the actual diagnosis, the cluster according to Kohonen maps, and the cluster according to k-means (fuzzy clustering). Table 3 shows that in the first 2 cases, the neural network can fully classify the subject's belonging to a particular cluster. In the case of fuzzy clustering, we see only one error of the neural network (assignment of a patient with GN to the first cluster).

With a notable level of accuracy in the results, it becomes feasible to construct a "decision tree" that elucidates the significance of factors in the diagnostic

process. In the case of classifying actual diagnoses, the pivotal factor is identified as Urinary TGF-β1. The detailed representation of this "decision tree" is provided in Table 3.

*Table 3*

**«The decision tree»**

| Diagnosis | Urinary TGF-β1, pg/ml |
|---|---|
| Healthy | Urinary TGF-β1 <1,351 |
| Without GN | Urinary TGF-β1 ≤ 1,351 |
| With GN | 1,352≤ Urinary TGF-β1 ≤ 5,693 |

After clustering using the Kohonen mapping method, the following classification rules were obtained (Table 4).

*Table 4*

**The classification rules**

| Diagnosis | Urinary TGF-β1, pg/ml, Urinary VEGF, pg/ml, Blood VEGF, pg/ml |
|---|---|
| Healthy | Urinary TGF-β1 <1,351 |
| Without GN | 185,721 ≤ Blood VEGF < 310,932<br>Urinary TGF-β1 <1,351<br>Urinary VEGF < 214,119 |
| With GN | Urinary VEGF n< 214,119<br>1,352≤ Urinary TGF-β1 ≤ 5,693 |

Key indicators of significance include Urinary TGF-β1, Urinary VEGF, and Blood VEGF. Notably, the criteria for characterizing a healthy patient remain consistent, with the distinction being evident in Blood VEGF. The application of fuzzy clustering yields a solution that deviates from previous approaches, as depicted in Table 5.

*Table 5*

**Fuzzy clustering**

| Diagnosis | Urinary TGF-β1, pg/ml, Urinary VEGF, pg/ml |
|---|---|
| Healthy | Urinary TGF-β1 <1,351 |
| Without GN | 205,111<Urinary VEGF < 214,119<br>Urinary TGF-β1 <1,351 |
| With GN | Urinary VEGF > 214,119<br>1,352≤ Urinary TGF-β1 ≤ 5,693 |

The primary determinant defining a patient's inclusion in the healthy cluster is urinary TGF-β1, with the threshold value remaining consistent. In contrast, the factor dictating the manifestation of glomerulonephritis is urinary VEGF.

Discussion. The worldwide COVID-19 pandemic has significantly influenced global health, highlighting the virus's impact on multiple organs [**2, 4, 10**]. Recent evidence indicates a variety of renal complications linked to the infection. The intricate relationship between COVID-19 and renal function is multifaceted, encompassing direct viral invasion, immune-mediated responses, and systemic effects on endothelial cells [**3, 6, 11**]. TGF-β1 and VEGF, recognized regulators of immune response and vascular function, respectively, are implicated in the pathophysiology of both COVID-19 and GN [**8, 9**].

TGF-β1 and VEGF are both implicated in the pathogenesis of severe COVID-19 [**8, 9**]. TGF-β1 is known to influence immune cells' development, differentiation, tolerance induction, and homeostasis. Elevated levels of TGF-β1 have been reported in COVID-19 patients, suggesting a potential link between the virus and fibrotic processes [**7, 8**]. VEGF, on the other hand, is known to promote endothelial cell proliferation, survival, migration, and permeability [**12**].

Our findings revealed a significant increase in blood and urine levels of TGF-β1 and VEGF in patients with GN who had a history of moderate COVID-19, compared to those who had moderate COVID-19 but did not develop GN. The study utilized cluster and classification data mining methods to understand the relationship between moderate COVID-19 and GN manifestation. The identified key indicators (Urinary TGF-β1,

Urinary VEGF, and Blood VEGF) contributed to the characterization of clusters, distinguishing healthy individuals, those with moderate COVID-19 but without GN, and those with post-moderate COVID-19 GN. The threshold values for urinary TGF-β1 remained consistent across clusters defining healthy individuals, while urinary VEGF was the factor primarily influencing the manifestation of GN. These findings are consistent with other studies that have shown kidney damage is common in COVID-19 patients, ranging from proteinuria, hematuria to acute kidney injury requiring kidney replacement therapy [2, 6]. Elevated concentrations of pro-fibrotic and inflammatory markers are consistent with research findings that indicate COVID-19 can induce profibrotic and inflammatory responses persisting for months and even years after the onset of infection [8, 13, 14].

While the study encompassed a diverse cohort, several limitations should be acknowledged. The cross-sectional nature and small sample size of our study limits the ability to establish causal relationships between COVID-19, TGF-β1, VEGF, and the manifestation of GN. Larger, multicenter studies are warranted to validate and extend our observations, ensuring robustness and generalizability. Furthermore, our study focused on a specific timeframe of 8-12 months post-moderate COVID-19. Longer-term follow-up assessments could provide a more comprehensive understanding of the trajectory of renal changes and their persistence over time. A more extended observational period would be particularly valuable in capturing delayed or progressive renal manifestations. The use of cluster and classification data mining methods provided a nuanced understanding of the relationship between moderate COVID-19 and GN manifestation. However, these methods are inherently exploratory, and while they generate hypotheses, they do not establish causation. Further experimental and longitudinal studies are necessary to establish a causal link and elucidate the underlying mechanisms. Finally, despite the identified indicators such as urinary TGF-β1, urinary VEGF, and blood VEGF contributing to the characterization of clusters, there is a need for standardized cut-off values. Determining universally applicable thresholds for these markers would enhance the clinical utility of our findings.

**Conclusions.** In conclusion, our investigation suggests a potential role of moderate COVID-19 in the delayed manifestation of GN. The identified indicators (urinary TGF-β1 and VEGF, blood VEGF) and clustering methods provide a foundation for future research and underscore the importance of considering renal implications in the aftermath of COVID-19 infections.

**Conflict of interest.** The authors declare no conflict of interest.

**Funding source.** The authors did not receive any financial support from any organization for the paper submitted.

**Data availability.** The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

**Authors' contribution.**

***L. Zub:*** conceptualization, manuscript reviewing;

***B. Horban:*** data collection, analysis, and statistical processing;

***V. Kulachek:*** statistical processing.

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