New perspectives in proliferative glomerulonephritis

PhD Theses

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1. INTRODUCTION

1.1 **Membranoproliferative glomerulonephritis**

Membranoproliferative glomerulonephritis (MPGN) is a histological entity, characterized by mesangial hypercellularity, endocapillary proliferation and capillary wall thickening with double contour formation, a pathological pattern detected by light microscopy on kidney biopsies. Historically MPGN was used as a diagnosis and based on the light microscopy findings classified into three different subgroups. Recent improvements in our understanding of the disease pathogenesis, with the recognition of the pivotal role of complement alternative pathway (AP), led to the description of complement-mediated (C3-glomerulopathy, C3G), and immunecomplex-mediated (IC-MPGN) forms.

In the background of the immunecomplex-mediated MPGN infections (HBV, HCV EBV), autoimmunity (SLE, rheumatoid arthritis) or malignant diseases can be identified. In contrast, in C3G the dysregulation of the AP is crucial in the pathogenesis.

1.2. C3 glomerulopathy

C3G is a very rare disease with a prevalence of 1-2 patients/million/year. The disease onset is in childhood or in young adults. C3G is presented with asymptomatic hematuria, proteinuria, high blood pressure and renal failure.

In older patients secondary causes have to be excluded in the background of the disease. Nearly 50 % of the patients progressed to end-stage renal disease (ESRD). In several cases trigger events such as upper respiratory tract infection can be identified in anamnestic data. Usually C3G is presented with low C3 level along with normal C4 level. The complement activation marker levels (C3a, sC5b-9) are usually elevated and besides of that, pathogenic factors can be identified. The diagnosis is based on renal biopsy and membranoproliferative pattern can be seen on light microscopy. Dominant (minimum two-order magnitude stronger C3 staining than any other immunoreactant) C3 staining is characteristic on immunofluorescence microscopy. C3G is classified according to electron microscopy, where C3 glomerulonephritis (C3GN) has usually less dense mesangial, subendothelial and subepithelial deposits, whereas dense deposit disease (DDD) is characterized by electron dense intramembranous deposits along the glomerular, tubular basement membrane, mesangium and Bowman-capsule.

A rare genetic, hereditary, endemic form was named CFHR5-nephropathy in Cyprus, which is caused by an internal duplication of exons 2 and 3 of the *CFHR5* gene.

1.2.1 Pathogenesis

C3G is caused by the dysregulation of the AP due to the presence of variations of complement genes, or by autoantibodies against complement proteins.

The latter include several different autoantibodies that can be detected in the patients' sera such as anti-Factor H, anti-C3b, anti-Factor B and C3-, C4- or C5 nephritic factors which are presented in 40-80% of patients.

C3 nephritic factor (C3NeF) can stabilize the AP C3 convertase. With the prolongation of the half-life of the AP C3-convertase enzyme complex, C3NeFs can maintain and prolong the complement activation.

C4 nephritic factor (C4NeF) is analogous to C3NeF, this autoantibody can stabilize the C3-convertase (C4bC2a) shared by the classical and by the lectin pathways, in a dose-dependent manner. In MPGN patients, it was shown that it could be present with or without C3NeF.

Anti-Factor H, anti-C3b, anti-Factor B can be detected around in 10% of the patients diagnosed with MPGN.

Mutations in the genes encoding the regulators or components of the complement system also play a role in disease pathogenesis. The variations can be loss-of-function or gain-of-function mutations which led to the overactivation of the complement AP.

Despite the improved understanding of the disease pathomechanism, in more than 30% of the patients, etiologic factor could not be identified with the traditional complement and genetic analysis.

However, there are new candidates which may broaden the group of pathogenic factors. One potential candidate is the complement Factor H-related protein (FHR) family, that contains 5 different proteins. FHR proteins are highly homologous to Factor H but their regulator domain is missing.

FHR-5 was first described as a pathogenic factor in a subtype of C3G in 2010 by Gale at al. This hereditary, endemic form in Cyprus was named CFHR5-nephropathy, which is caused by an internal duplication of exons 2 and 3 of the *CFHR5* gene. FHR-5 can bind to heparin, C-reactive protein, pentraxin-3 and the extracellular matrix and it can also inhibit the C3- and C5-convertases based on previous studies however its deregulator activity is still a question.

1.3. New approach in the classification of membranoproliferative glomerulonephritis

Despite improved understanding of disease pathogenesis, several questions still remain unanswered. The differentiation between immunecomplex- and complement-mediated forms is not clear in many cases. One can observe deviation between pathological and clinical presentations, and there is a hampered differentiation between C3GN and DDD (sometimes with IC-MPGN) or even changes in the characteristic patterns if repeated biopsies are done.

A large portion of patients with IC-MPGN may have positive signs of AP dysregulation such as C3NeF, or show decreased C3 levels with normal C4. Nowadays it seems likely that C3G and IC-MPGN are not two different entities, rather the two ends of a disease spectrum.

Taking all these limitations and considerations into account, Iatropoulos et al. recently explored the potential utility of unsupervised cluster analysis to generate clinically meaningful subgroups of C3G/IC-MPGN patients. In their study 4 distinct clusters were generated based on clinical, histological, complement and genetic parameters.

Patients in cluster 1 were younger, had elevated terminal pathway activation marker (sC5b-9) levels with low C3 concentrations, with the high prevalence of likely pathogenic variations (LPV) or C3NeF.

Patients in cluster 2 had also high sC5b-9 levels with higher Ig staining on immunofluorescent microscopy, whereas patients with dense deposits, decreased C3 but only moderately elevated sC5b-9 fell into cluster 3.

Cluster 4 was characterized by lower complement activation, higher C3 level, and the presentation of LPV and C3NeF was less prevalent. Older patients were classified into this cluster with more sclerotic glomeruli.

Based on these results this analysis separated patients with fluid-phase complement activation (clusters 1–3) from patients with solid-phase complement activation (cluster 4).

Notably, reclassification of C3GN, DDD and IC-MPGN patients by cluster analysis proved to be better at predicting renal survival. In cluster 4, where solid-phase complement activation was hypothesized, patients had worse renal survival compared to the other clusters.

However, as such unsupervised mathematical approaches are completely dependent on the studied cohort and on the variable set, validation in independent cohorts is essential.

2. AIMS

The background of membranoproliferative glomerulonephritis is not fully known. There are several cases where there is no strict border between immunecomplex- and complement-mediated forms.
Our aim was to identify potential novel pathogenic factors and to get new insights into the disease pathogenesis with a different approach.

2.1. Investigating the role of Factor H-related protein 5 in C3G/IC-MPGN patients

FHR5 shows high homology with Factor H, therefore it could have a pathogenic role in complement-mediated renal diseases.
We have the following questions:

1. Is there any difference between patients and healthy controls according to their FHR-5 serum levels?

2. Is there any difference in FHR-5 levels between patients with or without *CFHR5* variations?

3. Is there any correlation with FHR-5 serum levels and the patients' clinical, laboratory, genetic and complement data?

4. Is there any correlation between *CFHR5* variations, FHR-5 levels and the patients' renal survival?

2.2. Investigating the level of C4 nephritic factor in C3G/IC-MPGN patients

Our first aim was to examine the presence of C4NeF along with its connection with clinical features, and with other pathogenic factors (autoantibodies and genetic variants) in a large cohort of IC-MPGN/C3G patients.
We examined the following aspects:

1. Is there any connection between the presence of C4NeF and the histology-based classification?

2. Is there any correlation with the presence of C4NeF and the patients' clinical, laboratory, genetic and complement data?

5. Is there any correlation between C4NeF and the patients' renal survival?

2.3. Repetition and validation of the hypothesis-free, data-driven histology-independent cluster analysis based on the patients' clinical, laboratory, genetic and complement data

1. Are our results comparable with the originally characterized clusters with similar consequences?

2. Is there any difference in renal survival between the clusters? Do the clusters have better prognostic significance compared to the histology-based groups?

3. Are there further complement parameters which can characterize the clusters? Are there any differences in the presence of C4NeF, in levels of FHR-5 or in the occurrence of the identified *CFHR5* variations between the clusters?

3. METHODS

3.1 Patients and controls

Samples of 206 patients were sent to our Research Laboratory from Central-European clinical centres (n=34) with the suspicion of complement-mediated renal disease for complement investigations, and for them genetic analysis was also carried out. 86 patients were excluded because of alternative diagnosis or secondary MPGN. 120 patients with the diagnosis of IC-MPGN/C3G were enrolled in the study. Forty-one patients had C3GN (34.1%), 12 (10%) had DDD and 67 (55.8%) had IC-MPGN.

In one patient we could not determine C4NeF, therefore we enrolled 119 patients in this study.

92 patients were enrolled into cluster analysis, who had a full dataset for this statistical approach.

Eighty-five subjects formed the control group (68 adults, 17 children). All of them were referred for routine medical examination and none of them had any known disease at time of blood sampling.

3.2. Methods

3.2.1. Determinations of complement parameters

C3 and C4 concentrations were measured by turbidimetry (*Beckman Coulter, Brea, CA).*

AP activation was measured by Wieslab AP ELISA kit (Eurodiagnostica), total classical pathway activity was measured by a home-made hemolytic titration test based on Mayer's method.

Radial immunodiffusion was performed to measure the antigenic concentrations of Factor I and Factor B, using specific antibodies (Quidel).

Levels of Factor H, C1q and antibodies against Factor H, C1q, C3 and Factor B were measured with in-house ELISA methods (reagents: anti-Factor H, anti-C1q: Binding site; Factor H: Merck; C1q, C3, Factor B: Quidel).

C3NeF titer was determined based on a hemolytic method.

Further complement components, activation markers and split products, such as Factor D, sC5b-9, C3a, Bb and C4d were detected with commercially available ELISA kits (Hycult Complement Factor D, MicroVue C3a-desArgEIA, MicroVue C4d EIA, MicroVue sC5b-9 Plus EIA, MicroVue Bb Plus EIA).

3.2.2. ELISA for measuring the serum level of FHR-5

FHR-5 serum levels were measured with a newly developed in-house ELISA method. Microtiter ELISA plates were coated with 1μg/mL commercially available monoclonal mouse anti-human-FHR-5 (R&D System) in PBS overnight, followed by blocking with PBS and 2% BSA. Diluted serum was added to the plate and incubated for 1 hour at RT. FHR-5 binding was detected using polyclonal goat anti-human-FHR-5 IgG (R&D System). The concentrations of the samples were determined based on the standard curve of the two-fold dilution series of recombinant human FHR-5 protein.

3.2.3. Determination of C4 nephritic factor

The C4NeF hemolytic test was performed based on the protocol of Zhang et al.
In brief, sheep erythrocytes (EA) in Alsever solution were used, which were sensitized with hemolysin and washed several times in gelatine-veronal buffer (GVB) containing calcium and triethylenetetramine-N,N,N',N'',N''',N'''-hexaacetic acid (CaTTHA). NHS (pooled serum from healthy controls) was added to the solution to prepare EA+C1+C4. The cells were washed several times in GVB containing Ca^{2+} and were incubated in the buffer. After the incubation, GVB containing Ca^{2+} and Mg^{2+} was used for washing which enabled that the EA+C1+C4 cells bind human complement C2 protein (Calbiochem). Next, the patients' serum samples were added to the cells and after incubation and washing procedures rat serum was added to the cells as the source of complement components C5-C9. After incubation, the hemolytic reaction was stopped by adding cold EDTA-GVB buffer. The ODs of the supernatants were measured and the hemolysis in the patient's samples was given in % of total lysis of sheep erythrocytes. The threshold of positivity was set based on 48 healthy controls, and determined as 18%.

3.2.4. Genetic analysis

In order to screen for mutations, rare variations or risk polymorphisms in the coding regions of complement Factor H (*CFH*), Factor I (*CFI*), membrane cofactor protein (*CD46*), thrombomodulin (*THBD*), Factor B (*CFB*) and C3 (*C3*), the samples were analyzed by direct bidirectional DNA sequencing following PCR amplification.
Previously identified and functionally characterized missense, as well as nonsense and splice site mutations were categorized as likely pathogenic variants. In case of novel missense variations, they were regarded as LPVs if they were not found

or found with a minor allele frequency of <0.1% in international databases (dbSNP, Exome Variant Server and 1000Genomes Project) and had CADD score ≥10. Those missense, nonsense or splice site mutations that were previously identified were categorized as LPVs. The possible functional effect of novel variations was predicted *in silico* using the following online prediction tools, such as PolyPhen, SIFT, PROVEAN, Human Splicing Finder and Mutation-Taster.
Multiplex ligation-dependent probe amplification (MLPA) was performed to study the presence of copy-number alterations in the chromosomal regions of the *CFHR1*, *CFHR2*, *CFHR3* and *CFHR5* genes, with the SALSA MLPA probemix.

3.2.5. Statistical analysis

The continuous variables showed skewed distribution according to the results of Shapiro-Wilk's test. Therefore, for descriptive purposes, the values are given as median and 25^{th}–75^{th} percentiles. Categorical variables were shown as numbers and percent.
The comparative analysis between the resulting clusters was performed using Kruskal-Wallis test and Dunn's post-hoc test in case of continuous variables and Pearson's Chi-square test was applied for categorical variables.

For cluster analysis we used the same method and variable set that was applied in the study of Iatropoulos et al. The cluster analysis was performed with hierarchical clustering using Ward's method with squared Euclidean distance using IBM SPSS 20 software.

For analyzing the difference in renal survival between the different clusters Kaplan-Meier analysis was used.

4. RESULTS

4.1 Investigating the role of Factor H-related protein 5 in C3G/IC-MPGN patients

4.1.1. Investigating the FHR-5 serum levels of patients and healthy controls

Serum FHR-5 levels were significantly lower in patients (median: 1.8 mg/L, interquartile ranges: 1.4-2.3) compared to healthy controls (median: 2.1 mg/L, interquartile ranges: 1.8-2.5) (p=0.004), independently from variation carrier status (median: 1.54 mg/L in patients with *CFHR5* variation; median: 1.82 mg/L in patients without *CFHR5* variation).

4.1.2. Identified *CFHR5* variations

CFHR5 genetic analysis was performed in 111 patients. Altogether 8 different, rare heterozygous *CFHR5* variations were identified by Sanger-sequencing in 14 patients including 2 frame-shift mutations (c.479_480insAA; c.479_480insA) and 6 missense variations (P46S, V110A, K144N, C208R, G278S, R356H). Remarkably, the identified missense and frame-shift variations affect the short consensus repeat (SCR) domains 1-6 in the FHR-5 protein.

We identified 3 patients with *CFHR* hybrid genes by MLPA analysis and these patients had elevated FHR-5 levels. Because of the special genetic background we excluded them from further analysis.

7 patients with *CFHR5* variations did not carry any additional known etiological components in the examined factors causative for AP dysregulation.

We considered 5 variations as likely pathogenic, because of 1) variation was previously reported in C3G patients and/or is a recognized pathogenic factor of the disease (p.E163Kfs*10, p.E163Rfs*35) or 2) based on literature data showing segregation with the disease (in case of P46S) or 3) based on results of the functional analysis (for G278S and R356H).

There was no remarkable association of the FHR-5 protein concentrations with the localization of the variations in various FHR-5 domains.

4.1.3. Functional characterization of *CFHR5* variations

To determine whether *CFHR5* variations influence FHR-5 functions, we measured serum FHR-5 binding to C3b. FHR-5 binding was significantly lower in patients carrying the G278S mutation compared with those expressing only wild-type FHR-5, or carrying mutations in the dimerization domains SCR1-2

(P46S, V110A, K144N) or carrying the R356H mutation in the predicted ligand binding region SCR6.

4.1.4. Laboratory and clinical associations of FHR-5 levels

In order to have a homogeneous group of MPGN patients with wild-type FHR-5, we stratified the patients carrying *CFHR5* variations into a separate group, to facilitate better understanding of the association and relevance of FHR-5 protein levels with clinical features.

There was a positive correlation between FHR-5 serum levels and the presence of sclerotic glomeruli on light microscopy.

Significant positive correlation was also seen between C3, C4 levels, AP and classical pathway activity and Factor H levels.

4.1.5. Association between FHR-5 serum levels, *CFHR5* genetic variations and renal survival

Based on the successfully followed patients' (n=101 among whom 93 had genetic analysis) FHR-5 levels and data on development of ESRD, groups with high or low FHR-5 concentrations were made, where the cut-off point (1.565 mg/L) was defined by ROC-analysis. None of the patients with *CFHR5* variation(s) progressed to ESRD during follow-up. Patients with higher FHR-5 levels (median: 2.16 mg/L; 1.87-2.85) had the worst renal survival, when compared to patients with low FHR-5 concentrations (median: 1.34 mg/L; 1.12-1.46) (p=0.034).

4.2. Investigating the titer of C4 nephritic factor in C3G/IC-MPGN patients

4.2.1. The presence of C4NeF in patients with C3G/IC-MPGN

We determined C4NeF activity in 119 patients' sera with histologically diagnosed C3G/IC-MPGN with hemolytic test. C4NeF positivity was detected in 17 patients (14.3%) among whom 7 (17.5%) had IC-MPGN, 1 (8.3%) had DDD and 9 (16.4%) had C3G.

4.2.2. Relationship between the presence of C4NeF with the clinical and complement profile

We examined whether there are any differences between the C4NeF positive and C4NeF negative patients' clinical and complement parameters. No difference was observed regarding the patients' clinical parameters, however, renal impairment was less frequent at disease onset in patients with C4NeF. By exploring the C4NeF positive or negative patients' complement profile the activity of the classical or the alternative pathway was decreased in patients with C4NeF.

Because the prevalence of C3NeF was tendentiously higher in patients with C4NeF ($p=0.063$), we further analyzed 4 groups based on the joint presence or absence of C3NeF and/or C4NeF, in order to better understand their associations with the disease. This classification identified 20 patients, who were positive only for C3NeF, 10 patients who were positive only for C4NeF, 7 patients with double positivity and 82 patients with double negativity for both of these autoantibodies. Double positive patients were younger compared to antibody negative patients. Renal impairment was less prevalent in patients with only C4NeF.

The double positive group was characterized by lower C3 levels. In line with these results, the concentration of the terminal complement complex (sC5b-9) was significantly higher in the double positive group and it was decreased but still above the reference range in the group of patients positive for only C4NeF.

The presence of other complement autoantibodies such as anti-C1q and anti-C3 antibody was the highest in the double positive group.

4.2.3. Association between C4NeF and renal survival

We examined whether C4NeF positivity has any influence on the patients' renal survival. Of the 119 patients, we followed 103 subjects successfully for a median follow-up of 1.52 years (range: 0.05-18.18 years). During the follow-up period 17 patients progressed to, or stayed in ESRD with the need of renal replacement therapy. 14 from these 17 patients belong to the C3NeF/C4NeF negative group

whereas 3 patients were positive only for C3NeF. There was no significant difference in renal survival according to nephritic factor status. Remarkably, none of the patients with C4NeF progressed to ESRD.

4.3. Repetition and validation of the hypothesis-free data-driven histology-independent cluster analysis based on the patients' clinical, laboratory, genetic and complement data

4.3.1. Characterization of the clusters and comparison with the Italian study

With hierarchical cluster analysis four distinct clusters were generated.

Cluster 1 was characterized by low C3 levels (median: 0.5 g/L), very high sC5b-9 levels (median: 540 ng/mL) and high prevalence of LPVs and C3NeF. The median age of onset was 13 years. Renal impairment and creatinine levels were low in that cluster at the time of onset. In light microscopy no sclerotic glomeruli or crescents were seen, the strong C3 staining was not isolated, and Ig staining was also seen in cluster 1 on immunofluorescense microscopy, in concordance with the original study of Iatropoulos et al.

Cluster 2, due to the low case number, was excluded from further analysis.

In cluster 3 the lowest sC5b-9 levels (median: 250 ng/mL) were observed, compared to other clusters, along with moderately decreased C3 concentration (median: 0.77 g/L) and with high prevalence of LPVs. On light microscopy the highest degree of interstitial fibrosis, interstitial inflammation and the highest prevalence of sclerotic glomeruli were seen with low prevalence of crescents, compared to the other clusters.

In cluster 4 the median age of onset (39.5 years) was the highest compared to the other clusters and this cluster was also characterized by near-normal C3 levels and with lower prevalence of intramembranous deposits.

4.3.2. Association between clusters and renal survival

Ultimately, 10 out of the 79 successfully followed patients progressed to ESRD. Renal outcome was found to be worse in cluster 3 and cluster 4 compared to cluster 1 with Kaplan–Meier analysis ($p<0.05$ for clusters 3 vs. 1 and clusters 4 vs. 1), indicating that patients with higher age at diagnosis, more prevalent glomerular sclerosis at diagnosis, and lower prevalence of C3NeF and/or LPVs have inferior renal outcome. There was no difference in renal survival according to the histology groups.

4.3.3. Further complement parameters in association with clusters

Significantly lower classical and alternative pathway activity, along with decreased Factor D concentrations were observed in cluster 1, where C1q levels were also the lowest. These results together with decreased C3 levels indicate overactivation and consumption of CP/AP in cluster 1.

We have examined whether there is any relationship between C4NeF and the different clusters. The increased prevalence of C4NeF in cluster 1 was statistically significant ($p=0.028$) compared to the other clusters along with a higher prevalence of C3NeF and other complement autoantibodies.

We have examined the distribution of FHR-5 levels and *CFHR5* genetic variations in the different clusters. We included patients with wild-type FHR-5 protein and we observed that the distribution on FHR-5 levels are not random among the different clusters ($p=0.0003$). Patients in cluster 3 (median: 2.35 mg/L, IQ-range 1.77-3.16) and cluster 4 (median: 1.96 mg/L, IQ-range 1.48-2.23) had significantly higher FHR-5 levels when compared to cluster 1 (median: 1.47 mg/L, 1.25-1.98, $p<0.05$, Dunn's post hoc test, $p=0.0003$, ANOVA). The presence of patients with rare variations or LPVs in *CFHR5* was the highest in cluster 1 (10/49; 20.4%) when compared to the other clusters (4/58; 6.9%) ($p=0.047$, $\chi 2$-test).

5. CONCLUSIONS

The main findings of the thesis are the following:

1. Significantly lower FHR-5 levels were detected in patients compared to healthy controls with in-house ELISA method.

2. We have identified *CFHR5* variations in 11.7% of our patients in the scr1-6 domains. 6 missense and 2 frame-shifts variations were detected. There was no obvious relation between the presence of *CFHR5* variations and the patients' FHR-5 serum levels.

3. FHR-5 serum levels correlated with complement levels.

4. Patients with higher FHR-5 levels had worse renal survival compared to patients with lower levels of FHR-5. During the follow-up period none of the patients carrying *CFHR5* variation progressed to ESRD.

5. 14.3% of the patients were positive for C4NeF. The prevalence of C4NeF did not differ between the histology-based groups (IC-MPGN/C3GN/DDD).

6. The prevalence of C4NeF was higher in patients with C3NeF. Patients positive for both autoantibodies had higher degree of complement activation with lower C3 levels and higher terminal pathway activation marker levels. The presence of renal failure at time of diagnosis was lower in patients positive only for C4NeF.

7. There was no significant association between the presence of nephritic factors and patients' renal survival. Remarkably none of the C4NeF positive patients progressed to ESRD.

8. We validated the existence of clinically relevant clusters in IC-MPGN and C3G. Based on the patients' clinical, laboratory, complement, genetic and histological data 4 distinct clusters were generated. The role of the complement system was identified in the background of the different clusters. Cluster 1 was characterized by younger age, increased complement activation and higher prevalence of alternative pathway abnormality. In cluster 2, Ig staining was higher. Cluster 3 was characterized with lower C3 and terminal pathway

activation marker levels, with high prevalence of etiologic factors. In contrast in cluster 4 older patients were classified with normal C3 levels.

9. Clusters show significant association with patients' renal survival. There was no such association between the histology-based groups. Clusters 3 and 4 had worse renal survival compared to clusters 1 and 2.

10. We successfully identified further pathogenic factors in the clusters. The prevalence of C4NeF was higher in cluster 1 together with other complement autoantibodies (C3NeF, anti-C3, anti-Factor B, anti-Factor H, anti-C1q).
In cluster 1 lower FHR-5 serum levels were detected compared to clusters 3 and 4. The prevalence of *CFHR5* genetic variations was also the highest in cluster 1.

6. List of the candidate's publications

6.1. Publications related to the PhD thesis

1) Garam N, Prohaszka Z, Szilágyi Á, Aigner C, Schmidt A, Gaggl M, et al.
Validation of distinct pathogenic patterns in a cohort of membranoproliferative glomerulonephritis patients by cluster analysis.
Clinical Kidney Journal. 2019:1-10. IF: 2.975 (2018)

2) Garam N, Prohaszka Z, Szilágyi Á, Aigner C, Schmidt A, Gaggl M, et al.
C4 nephritic factor in patients with immune-complex-mediated membranoproliferative glomerulonephritis and C3-glomerulopathy.
Orphanet Journal of Rare Diseases. 2019;14(247). IF: 3.687 (2018)

1) Garam N, Prohaszka Z, Szilágyi Á, Aigner C, Schmidt A, Gaggl M, et al.
CFHR5 genetic variations and serum levels in patients with immune-complex-mediated membranoproliferative glomerulonephritis and C3-glomerulopathy.
(under publication)

Cumulative impact factor of the publications related to the thesis: 6.662.

6.2. Publications not related to the PhD thesis

1) Garam N, Maláti É, Sinkovits G, Gombos T, Szederjesi A, Barabás L, Gráf L, Kocsis J, Prohászka Z.
Platelet Count, ADAMTS13 Activity, von Willebrand Factor Level and Survival in Patients with Colorectal Cancer: 5-Year Follow-up Study.
Thromb Haemost. 2018 Jan;118(1):123-131 IF: 4.662 (2018)

2) Jubran R, Kocsis J, Garam N, Maláti É, Gombos T, Barabás L, Gráf L, Prohászka Z, Fishelson Z.
Circulating mitochondrial stress 70 protein/mortalin and cytosolic Hsp70 in blood: Risk indicators in colorectal cancer.
Int J Cancer. 2017 Dec 1;141(11):2329-2335 IF: 4.982 (2018)

3) Gráf L, Barabás L, Madaras B, Garam N, Maláti É, Horváth L, Prohászka Z, Horváth Z, Kocsis J.
High serum Hsp70 level predicts poor survival in colorectal cancer: Results obtained in an independent validation cohort.
Cancer Biomark. 2018;23(4):539-547 IF: 2.859 (2018)

4) Trojnar E, Józsi M, Szabó Z, Réti M, Farkas P, Kelen K, Reusz GS, Szabó AJ, Garam N, Mikes B, Sinkovits G, Mező B, Csuka D, Prohászka Z
Elevated Systemic Pentraxin-3 Is Associated With Complement Consumption in the Acute Phase of Thrombotic Microangiopathies.
Front Immunol. 2019 Feb 25;10:240 IF: 4.716 (2018)

Cumulative impact factor of the publications not related to the thesis: 17.219.

Total impact factor of the indicated publications: 23.881.