Clinical and functional consequences of anti-properdin autoantibodies in patients with lupus nephritis

Maria Radanova[1], Galya Mihaylova[1], Desislava Ivanova[1], Marie Daugan[2], Valetin Lazarov[3], Lubka Roumenina[2], and Vasil Vasilev[3]

[1]Medical University Varna Prof Dr Paraskev Stoyanov
[2]INSERM
[3]Medical University of Sofia

May 5, 2020

Abstract

Properdin is the only one positive regulator of the complement system. In this study, we characterize the prevalence, functional consequences and disease associations of autoantibodies against properdin in a cohort of patients with autoimmune disease systemic lupus erythematosus (SLE), suffering from lupus nephritis (LN). We detected autoantibodies against properdin in plasma of 22.5% of the LN patients (16/71) by ELISA. The binding of these autoantibodies to properdin was dose-dependent and was validated by surface plasmon resonance. Higher levels of anti-properdin were related to high levels of anti-dsDNA and ANA and to low concentrations of C3 and C4 in patients and also with histological signs of LN activity and chronicity. The high negative predictive value (NPV) of anti-properdin and anti-dsDNA combination suggested that patients who are both negative for anti-properdin and anti-dsDNA will not have severe nephritis. IgG from anti- properdin positive patients' plasma increased the C3b deposition on late apoptotic cells by flow cytometry. Nevertheless, these IgGs did not modify substantially the binding of properdin to C3b, the C3 convertase C3bBb and the pro-convertase C3bB, evaluated by surface plasmon resonance. In conclusion, anti- properdin autoantibodies exist in LN patients. They have weak but relevant functional consequences, which could have pathological significance.

Introduction

Properdin is a plasma glycoprotein, which is the only known positive regulator of the complement by stabilizing C3 (C3bBb) and C5 ($(C3b)_2$-nBb) convertases of the alternative pathway [1]. Under physiological conditions it is found to form cyclic dimers (P2), trimers (P3) and tetramers (P4) as the convertase-stabilizing activity of the tetramer is greater than the trimer [2-4].

Along with its stabilizing role for the C3 convertase, it has been shown that properdin could act as a pattern recognition molecule. Several authors have reported that properdin can recognize structures, independent of C3, like glycosaminoglycans on tubular cells leading to complement activation [5]; microbial surfaces, apoptotic and necrotic cells, providing a platform for C3 convertase assembly [6-9]. The pattern recognition role remains controversial since other authors have reported that properdin was only able to bind structures in C3-dependent manner [10].

The role of properdin in complement-mediated diseases still is not clear. Properdin deficiency contributes to infectious and non-infectious diseases in various models [11-13]. Moreover, properdin is detected in kidney biopsies and in serum/plasma/urinary samples from patients with various complement-mediated renal diseases [14]. For example, in patients with membranoproliferative glomerulonephritis and lupus nephritis (LN) were detected low serum levels of properdin but properdin depositions in glomeruli, implying that low properdin levels may be due to hypercatabolism [15]. SLE patients with low plasma levels of C3 have

also low plasma levels of properdin [16]. Few studies report isolated cases of anti-properdin autoantibodies in different pathological contexts. Józsi et al., 2014 demonstrated weak antibody positivity to properdin, C3b, and Factor B – to all components of the convertase – in patients with dense deposit disease (DDD) [17]. Anti-properdin antibodies were found also in a patient with LN, carrying heterozygous C3 mutation, together with autoantibodies against others complement alternative pathway proteins – Factor I, Factor B, and C3 [18]. Functional assays showed that all these autoantibodies cause alternative pathway activation, which could contribute to the tissue damage in kidney of the patient. Tanuma, et al., 1990 found in sera from patients with membranoproliferative glometulonephritis (MPGN) and Dense Deposit Disease (DDD), C3 Nephritic Factor (C3NeF:P), which displayed the properties of properdin and IgG. The authors consider that C3Nef:P is an immune complex of IgG autoantibody against properdin and properdin [19].

Since LN affects the course of the disease, quality of patient's life and the prognosis of SLE [20-22], there is an unmet need of more efficient biomarkers for early diagnosis, to more precisely evaluate the disease activity, the degree of disease severity and the response of therapy. Here we show that autoantibodies against properdin exist in about 20% of the LN patients, potentiating its activity. Although likely not a driver of the disease, these autoantibodies may be a contributing factor with pathological relevance for LN.

Materials and methods

Cohort description

Seventy one clinically diagnosed SLE patients, according to the American College of Rheumatology (ACR) criteria with biopsy-proven LN, all from Nephrology Clinics of University Hospital "Tzaritza Ioanna – ISUL", Medical University of Sofia were included in the study.

LN activity was defined according to the British Islet Lupus Assessment Group (BILAG) renal score [23, 24]. All patients were divided into four BILAG categories as follows: 23 patients (31.08%) with category A LN, 24 patients (32.43%) with category B LN, 8 patients (10.81%) with category C LN and 19 patients (25.68%) with category D LN. There were no patients with category E LN in our cohort.

The patients with biopsy-proven LN were also distributed according to the LN classification of the International Society of Nephrology (ISN) and the Renal Pathology Society (RPS) [25, 26] as follows: 4 patients (5.63%) had LN Class I, 23 patients (32.39%) had LN Class II, 7 patients (9.86%) had LN Class III, 25 patients (35.21%) had LN Class IV, 11 patients (15.49%) had LN Class V, 1 patient (1.41%) had LN Class VI.

The presences of antinuclear antibodies (ANA) were detected by indirect immunofluorescence and levels of anti-dsDNA antibodies were tested by ELISA (U/mL) in University Hospital "Tzaritza Ioanna – ISUL"– Sofia. Pathologically elevated ANA titers (over 1:80) were found in 50 (69.4%) of patients and pathologically elevated levels of anti-dsDNA were found in 31 (40.8%) of patients.

The C4 and C3 complement components in plasma were measured by immunodiffusion. Reference ranges for C3 were from 0.75 to 1.65 g/L, and for C4 – 0.20 to 0.65 g/L. C3 hypocompletemia was detected in 14 (19.7%, 14/66). C4 hypocompletemia was detected in 28 (39.4%, 28/71). Both C3 and C4 hypocompletemia were detected in 13 (19.8%).

Seventy two healthy volunteers, age and gender matched to the patients, were included as a control group. All healthy volunteers were without autoimmune and infectious inflammatory diseases, and without renal, hepatic and haematopoietic dysfunctions.

The study had the approval of the Ethics Review Board of Medical University of Varna (protocol 62/04.05.2017) and each patient and healthy volunteer signed a consent form of enrolment.

ELISA for detecting anti-properdin autoantibodies

ELISA plate (Greiner bio-one(r)) were coated with either 20 μg/ml of test antigens – human Properdin (Complement Technology, Ins) in sodium carbonate buffer (35mM $NaHCO_3$, 15mM Na_2CO_3, pH 9.6) for

overnight at 4*C. Blocking of the plates was done by 1% BSA in PBS for 1h at 37*C and washed three times with PBS containing 0.05% Tween-20. Plasmas were diluted 1/100 in PBS-0.05% Tween 20. After washing, HRP-conjugated anti-human IgG (Southern Biotech) was applied in 1/1000 dilution in PBS-0.05% Tween 20. After washing three times, the color was developed with 0.5 mg/ml o-phenylenediamine (OPD) (Thermo, Scientific). The reaction was stopped with 2N H_2SO_4 and absorbance at 490 nm was measured using an ELISA plate Reader – Synergy 2.

Alternatively, plasma samples were serially diluted starting from 1/50 and applied on coated and blocked plates to evaluate the dose-response of the binding of the anti-properdin IgG to their antigen.

A sample was considered positive if its optical density exceeded the average of the optical density of the samples of the healthy volunteers + 3SD.

IgG purification

IgG was purified from plasma of patients with LN or of healthy donors, by using Protein G beads (GE Healthcare), as recommended by the manufacturer. The concentration of the IgG was determined by a Nanodrop and the purity of IgG – by 10% SDS-PAGE electrophoresis (Invitrogen, Life technologies, Novex), followed by a Coomassie Blue staining of the gel.

Characterization of the interaction of the anti-properdin IgG with their antigen by Surface plasmon resonance (SPR)

The interaction of the IgG with properdin was analyzed in real time using a ProteOn XPR36 SPR equipment (BioRad, Marne-la-coquette, France) and BiaCore2000 (GE Healthcare, France). Properdin was covalently immobilized to a GLC sensor chip (BioRad) following the manufacturer's procedure. Alternatively, CM5 chips for BiaCore were used. Protein G purified IgG from LN patients or healthy donors were injected for 300s at 6 different concentrations (300, 150, 75, 35, 17.5 and 0 μg/ml) diluted in PBS 0.005% Tween or 10mM Hepes, 145mM NaCl, 0,005% Tween 20 running buffers. The dissociation was followed for 300s. Bound protein was regenerated with 1M NaCl, 50mM NaOH regeneration buffer.

Effect of anti-properdin IgG on formation of C3 convertase

The effect of total IgG, positive for anti-properdin autoantibodies on the interaction of properdin with C3b, C3bB and C3bBb was analyzed in real time using a ProteOn XPR36 SPR equipment (BioRad, Marne-la-coquette, France). Properdin was covalently immobilized to a GCL sensor chip (BioRad), following the manufacturer's procedure. Protein G purified IgG from LN patients, positive for anti-properdin autoantibodies (but negative for anti-C3b and anti-FB), and healthy donors were injected for 300s at dilution 1:15 in 10 mM Hepes, 50 mM NaCl, 10 mM MgCl, 0.005% Tween 20, pH 7.4 buffer. The same buffer was separately injected to serve as a control. Following 300s of dissociation, C3b (13μg/ml), C3b (13μg/ml) + Factor B (10μg/ml) and C3b (13μg/ml) + Factor B (10μg/ml) + Factor D (0,5μg/ml) (Complement Technology) were injected for 300s of association, followed by 300s of dissociation.

Effect of anti-properdin IgG on C3 activation fragments and properdin deposition on apoptotic cells

Human endothelial cells from an umbilical vein (HUVEC) were used to study the possibility of anti-properdin to modulate the opsonization of apoptotic cells by the complement. The cell were characterized as late apoptotic cells after detection of phosphatidylserine on the cell membrane (binding to anexin V), impaired membrane permeability (permeability for propidium iodide) and DNA fragmentation (DAPI). The apoptotic cells were incubated with 1:10 diluted human sera mixed with IgG from LN patients with high levels of anti-properdin or anti-C3 autoantibodies. The dilution buffer contained 10 mM EGTA and 7 mM $MgCl_2$ to allow activation only of the alternative pathway. Also, IgG from patients with anti-properdin positivity, but negative for anti-C1q, anti-C3b, anti-FB, anti-FH were selected for this experiment to minimize the confounding effects of other autoantibodies. EGTA-Mg allows activation of alternative complement pathway by inhibition of classical and lectin pathways. After incubation for 30 min/37°C the cells were labeled with mouse anti-C3c antibody (Quidel) or anti-properdin antibody (Quidel), diluted 1:50, followed by Alexa Fluor

555-conjugated anti-mouse IgG antibody (1:100) (Thermofisher). The cells were analyzed by FACS on an LSRII machine (BD Biosciences) and with FlowJo software.

Effect of anti-properdin on alternative pathway activation in serum

IgG from LN patients, positive for anti-properdin autoantibodies and healthy donors were added to normal human serum and incubated 1h at 37°C to test their capacity to activate complement in fluid phase. The released Ba fragment was measured as an indicator for the formation of a C3 convertase. The test was performed according manufacturer's procedure, MicroVue Ba kit (Quidel).

Prediction of the antigenic determinants

The B cell epitopes on human properdin were predicted using IEBD server B Cell Epitope Prediction Tools (Prediction of linear epitopes from protein sequence) (http://tools.iedb.org/main/bcell/). The crystal structure of the properdin monomer [1] was used as input file. The predicted antigenic determinants were visualized using PyMOL.

Statistical analysis

Statistical analysis was carried out using software GraphPad Prism 6.01. Quantitative data were expressed as mean ± standart deviation (SD). For comparison between groups of patients and of healthy volunteers, the Mann-Whitney U test for continuous variables for 2-group comparisons was used. Fisher's exact test was used for the analysis of data also. Odds ratio (OR) and 95% confidence interval (CI) were calculated. The Spearman correlation was used to analyze the relativeness of the study parameters. Statistical significance was considered as $p<0.05$.

Results

Anti-properdin autoantibodies in patients with LN

The screening by ELISA revealed a presence of autoantibodies against properdin in patients with LN. 22.53% (16/71) of the patients were seropositive for anti-properdin IgG autoantibodies. For positivity cut-off was taken the average value of determined autoantibodies against properdin in healthy volunteers +3SD. (Fig.1A). The binding of IgG from positive patients to properdin was dose-dependent (Fig. 1B). Detection of anti-properdin antibodies by ELISA was validated by SPR. Purified IgG from three LN patients positive for anti-Properdin antibodies (P33, P35 and P38) showed presence and specificity the binding of purified IgG from patients to immobilized properdin (Fig. 1C, D, E). The SPR analyses revealed slow off-rate and lack of complete dissociation of the complexes, suggesting stable interaction.

Positive men for anti-properdin were 4/15 (26.67%) and positive women – were 12/56 (21.43%). The presence of elevated levels of anti-properdin antibodies was not realated to the sex of the patients (p=0.112, data not shown). There was no correlation between the age of patients (r=-0.022, p=0.854) or duration of LN (r=-0.083, p=0.498) and levels of anti-properdin (data not shown).

Association of the anti-properdin IgG with markers of disease activity

The associations between levels of anti-Properdin and proteinuria, urinary sediment and renal function were investigated.

The median level of proteinuria in anti-properdin seropositive patients was 1.56 g/24h (from 0.05 to 15.72) and the median level of proteinuria in negative for anti-Properdin antibodies patients was 0.32 g/24h (from 0.02 to 8.73). There was a trend toward that positive for anti-properdin patients had higher proteinuria than in negative ones (p=0.056, Fig. 2B) but there were no correlation between anti-properdin and proteinuria (r=0.165, p=0.170, data not shown) and between anti-properdin and eGFR (r=-0.225, p=0.059, Fig. 2C). Also, the presence of anti-properdin did not determine the presence of active urinary sediment (more than 8 erythrocytes/μl, or more than 8 leukocytes/μl, or cellular casts in non-centrifuged urine sample) (p=0.931, data not shown).

The median level of anti-Properdin antibodies in patients positive for anti-dsDNA levels – 0.232±0.292 – were higher than median level of anti-properdin in negative for anti-dsDNA patients (0.070±0.134) (p=0.013, Fig. 2E) Positive for anti-properdin and for anti-dsDNA patients were 9/14 (64.3%). Negative for anti-Properdin and negative for anti-dsDNA patients were 31/47 (66.0%). We found a trend toward an association of serological status of anti-Properdin with that of anti-dsDNA (p=0.064).

Patients with low C3 levels had higher median level of anti-properdin – 0.243±0.253 in comparison with patients with reference levels of C3 – 0.108±0.204; (p=0.008, Fig. 2F). The patients with increased anti-properdin levels and low levels of C3 were 6/14 (42.9%) and patients with reference levels of anti-properdin and C3 were 6/53 (11.3%). The presence of pathologically increased anti-properdin statistically significant determines the presence of C3 hypocomplementemia with relative risk 3.79; 95% CI: 1.44 – 9.95, p=0.013.

Moderate correlations between anti-properdin and ANA titers (r=307, p=0.020, Fig. 2I), and between anti-properdin and anti-dsDNA (r=309, p=0.017, Fig. 2J) were established. Weak and negative correlations between anti-properdin and C3 (r=-256, p=0.036, Fig. 2K) and between anti-properdin and C4 levels (r=-270, p=0.034, Fig. 2L) were found.

Positive for anti-properdin patients in category A, according BILAG Renal score were 6/21 (28.6%), in category B – were 6/24 (25.0%), in category C – 2/8 (25.0%) and in category D – 2/18 (11.1%). (Fig. 2A). Statistically significant difference in the levels of anti-properdin in different categories of BILAG Renal score has not been established (Fig. 2A).

Category A BILAG patients had a higher anti-properdin titer in comparison to patients in other BILAG categories (Fig. 2A). The significance of anti-properdin alone to identify patients in category A BILAG or in a group with other markers of LN activity was evaluated (Table 1). Anti-C1q alone and in combination with anti-dsDNA or in combination with anti-dsDNA and levels of complement C3 and C4 showed significant specificity to identify patients with A BILAG category (Table 1). Although anti-properdin alone could not be used for identification A BILAG patients (p=0.275), they in combination with anti-dsDNA could significantly increase sensitivity (70.7%) and NPV (89.8%), but decrease the specificity (57.3%) in the identification of patients in A BILAG category in comparison with anti-C1q and anti-dsDNA together (sensitivity 38.1% and specificity 91.4%, Table 1).

Comparative analysis between levels of anti-properdin in the groups of patients with and without histological signs of LN activity and chronicity were made (Table 2). High levels of anti-properdin significantly associated with renal histologic lesions, like sudendothelial immune deposits type "Wire loop" (p=0.009, Table 2), cellular (p=0.009, Table 2) and fibrous crescents (p=0.008, Table 2). A statistically significant correlation between levels of anti-properdin and histological activity and chronicity indexes did not found (r=0.175, p=0.190 and r=0.094, p=0.482, data not shown).

Functional consequences of anti-properdin IgG

Positive for anti-properdin autoantibodies LN patients, who showed dose response reactivity, were used for functional analysis. The presence of IgGs from positive for anti-properdin patients showed very weak effects on the capability of properdin to bind C3b, C3bB and C3bBb. There was a weak increase in Properdin binding to C3b (Fig. 3A, D and G) and to pro-converatase (C3b+Factor B) (Fig. 3B and H) in patients 33 and 38 in presence of anti-properdin antibodies. These effects were weak and inconsistent among the tests and patients and hence could not be considered to affect the stabilizing function of properdin.

The functional effect of anti-properdin containing IgG on the activation of the alternative pathway in serum was measured by the release of Ba. No significant difference was detected between levels of Ba fragments in IgGs from LN patients in comparison with the IgGs from healthy volunteers (data not shown).

To explore the capacity of anti-properdin positive IgG to activate complement on dying cells, purified IgGs from positive patients and healthy volunteers were incubated with late apoptotic cells in alternative pathway favoring conditions. In two patients (P9 and P35, Fig. 3J, K) was detected increased deposition of C3b on

late apoptotic cells. Deposition of C3 fragments was not observed in the other two patients, positive for anti-properdin (P33 and P38, data not shown). They were the same patients in whom anti-properdin antibodies weakly increased the binding of Properdin to C3b (Fig. 3A and G) and to pro-convertase (Fig. 3B and H). IgGs isolated from two patients, who were negative for anti-properdin, but positive for anti-C3 (P32 and P17) also increased deposition of C3b on late apoptotic cells (Fig. 3L, M).

Purified IgGs from the same patients (P9, P17, P32, P33, P35, P38) as well IgGs from healthy volunteers (K85, K3, K2 and collective K) were studied for their effect on properdin deposition on late apoptotic cells. The presence of patients or healthy donors IgG did not affect the deposition of properdin on late apoptotic cells (date not shown).

Prediction of epitopes of anti-properdin

Anti-Properdin epitopes were predicted using the IEBD server - http://tools.iedb.org/bcell/ (Fig. 4A). The majority of the binding epitopes were outside of the "vertex", formed at the junction of two monomers and responsible for the convertase binding. They were located in the linker regions between different "vertexes". The predicted peptide antigenic determinants of a molecule of prorperdin with a size greater than 3 amino acid residues are presented in Table 3.

Discussion

Our study showed that anti-properdin IgG were present in 22.5 % of patients with LN and correlated with some clinical parameters. These antibodies were specific and enhanced the deposit of C3 activation fragments on apoptotic cells.

Properdin stabilizes the alternative pathway C3 and C5 convertases and hence it is tempting to speculate that autoantibodies binding to it may enhance the stabilization capacity, increasing the half-life of these otherwise labile enzymes. Such enhanced stabilization could result in complement overactivaition and pathological consequences similar to the C3Nef, found in C3 glomerulopathies [27]. LN is a hallmark of a disease with complement overactivaiton in the kidney and indeed we discovered that more than 20% of the patients in our cohort were positive for anti-properdin IgG. Anti-properdin IgG titres showed a trend to negative correlation with eGFR, suggesting a possible association with renal damage. Moreover, higher levels of anti-properdin IgG were related to high levels of anti-dsDNA and ANA and to low concentrations of C3 and C4. The correlation with C3 complement consumption could be either related to the overall autoimmunity status, where the anti-properdin IgG are just an epiphenomenon or could indicate functional relevance.

To understand whether anti-properdin IgG affect the functions of properdin as complement regulator, their functional consequences were characterized. The anti-properdin IgG formed stable complexes with its target. Except to stabilize the C3bBb, it is reported that properdin binds to C3b, promoting it subsequent association with Factor B [28]. We found that in some patients anti-properdin positive IgG weakly increase the binding of properdin to C3b and to pro-convertase (C3bB) and did not affect the alternative complement pathway C3 convertase unlike C3NeF which react with C3 convertase and stabilized it [19]. In a case report anti-properdin positive IgG activated complement in serum [18]. We did not detect fluid phase complement activation by anti-properdin IgG, contrary to autoantibodies against other alternative pathway components, such as anti-Factor B, anti-C3b or anti-FH [29-32]. A possible explanation for the weak or absent effect of anti-properdin IgG could be the usage of low pH elution buffer for IgG purification, which may have a dramatic effect on the biological activity of IgG and their antigen-binding behavior [33]. Nevertheless, purified IgG showed strong and dose-dependent interaction with properdin by SPR, suggesting preserved binding capacity. Another possibility is that the epitopes of anti-properdin IgG are outside of the C3-convertase binding region of properdin. Indeed, predicted epitopes showed higher density of antigenic determinants outside the C3bBb-binding area, suggesting that the effect of these antibodies may be indirect, and likely affecting other functions.

It has been reported that properdin binds specifically to late apoptotic cell, but not to early ones and this occurs independently of C3b [8]. We performed an analysis with late apoptotic cells in order to understand

weather anti-properdin affect the C3b and properdin deposition. Anti-properdin IgG did not contribute to properdin deposition on late apoptotic cells in all studied patients. Nevertheless, we found that anti-properdin increased the C3b deposition in 2/4 tested patients to similar levels as anti-C3b IgG from LN patients, which have overt functional consequences [30]. These two patients were the same in whom anti-properdin slightly increased binding of properdin to C3b and pro-convertase, but not to the convertase. The C3 levels in both patients were in the reference range. This suggests that in those patients there was not excessive consumption of C3, following by increased C3b deposition. Taken together, these results suggest that anti-properdin IgG could contribute to the complement overactivaiton in a subgroup of patients, but that this is not a general phenomenon and the functional consequences of these autoantibodies are rather weak.

Further we explored whether the anti-properdin positivity could serve as a biomarker in combination with other characteristics of LN patients to predict flares and severity. Anti-C1q are the more often parameters correlated with the renal flares in LN. It is known that they are associated with LN activity and severity with renal histological lesion [34-40]. Anti-C1q are positively associated with BLAG renal score [41] as well as with SLEDAI score [42]. The combination of anti-C1q and anti-dsDNA was reported as a stronger marker for renal involvement and increased specificity for the identification of LN activity. Julkunen et al., 2012 found that anti-C1q and complement C3 and C4 are better markers for lupus nephritis activity than anti-dsDNA, and that anti-dsDNA and complement C3 and C4 were better than anti-C1q to evaluate the overall and nonrenal activity of SLE [43]. In our study anti-C1q alone and in combination with anti-dsDNA and in combination with anti-dsDNA and serum levels of C3 and C4 could significantly increase the specificity but decreased the sensitivity for identification of patients in category A according to BILAG Renal score. These findings confirmed established trends in the study of Chi et al., 2015 who evaluated the role of anti-C1q alone and in combination with other serological markers to identified patients with active LN [35]. Anti-properdin alone could not be determinant for high category of LN according to the BILAG Renal score. But in combination with anti-dsDNA anti-properdin could significantly increase sensitivity and NPV in the identification of patients in A BILAG category. The high NPV of anti-properdin and anti-dsDNA combination suggested that patients will not have severe nephritis in the absence of anti-properdin and anti-dsDNA. Although anti-properdin did not associate with more active and severe LN, they were significantly associated with renal flares. We found that pathological high levels of anti-properdin were associated with some renal histologic lesions, such as "Wire loop" deposits, fibrous and cellular crescents.

In conclusion, we found the presence of anti-properdin autoantibodies in the patients' sera with LN. Their presence correlate with clinical parameters and affect properdin function in a subgroup of patients. Although likely not a driver of the disease, these autoantibodies may be a contributing factor with pathological relevance for LN.

Acknowledgements

GM, DI and MD carried out sample preparation and performed experiments. VV, VL were responsible for the selection and diagnosis of LN patients. MR, LR and GM wrote the manuscript. MR, LR, and VV conceived and designed the study and edited the manuscript. All authors have read and approved the final manuscript.

This work was supported by the Bulgarian National Science Fund (DNTS/France 01/11/09.05.2017).

Conflicting interest

The authors have no conflicts of interest to declare.

References

1. Pedersen DV, Gadeberg TAF, Thomas C et al. Structural basis for properdin oligomerization and convertase stimulation in the human complement system. *Front Immunol* 2019; 10: 2007.
2. van den Bos RM, Pearce NM, Granneman J, Brondijk THC, Gros P. Insights into enhanced complement activation by structures of properdin and its complex with the C-terminal domain of C3b. *Front Immunol* 2019; 10: 2097.

3. Pangburn MK. Analysis of the natural polymeric forms of human properdin and their functions in complement activation. *J Immunol* 1989; 142: 202–7.
4. Smith CA, Pangburn MK, Vogel CW, Muller-Eberhard HJ. Molecular architecture of human properdin, a positive regulator of the alternative pathway of complement. *J Biol Chem* 1984; 259: 4582–8.
5. Zaferani A, Vivès RR, van der Pol P et al. Identification of tubular heparan sulfate as a docking platform for the alternative complement component properdin in proteinuric renal disease. *J Biol Chem*2011; 286: 5359–67.
6. Kemper C, Atkinson JP, Hourcade DE. Properdin: emerging roles of a pattern-recognition molecule. *Annu Rev Immunol* 2010; 28: 131–55.
7. Spitzer D, Mitchell LM, Atkinson JP, Hourcade DE. Properdin can initiate complement activation by binding specific target surfaces and providing a platform for de novo convertase assembly. *J Immunol*2007; 179: 2600–8.
8. Xu W, Berger SP, Trouw LA, de Boer HC, Schlagwein N, Mutsaers C, Daha MR, van Kooten C. Properdin binds to late apoptotic and necrotic cells independently of C3b and regulates alternative pathway complement activation. *J Immunol* 2008; 180: 7613–21.
9. Gaarkeuken H, Siezenga MA, Zuidwijk K, van Kooten C, Rabelink TJ, Daha MR, Berger SP. Complement activation by tubular cells is mediated by properdin binding. *Am J Physiol Renal Physiol* 2008; 295: F1397–403.
10. Harboe M, Johnson C, Nymo S et al. Properdin binding to complement activating surfaces depends on initial C3b deposition. *Proc Natl Acad Sci USA* 2017; 114: E534–9.
11. Al-Rayahi IA, Browning MJ, Stover C. Tumour cell conditioned medium reveals greater M2 skewing of macrophages in the absence of properdin.*Immun Inflamm Dis* 2017; 5: 68–77.
12. Dupont A, Mohamed F, Salehen N et al. Septicaemia models using Streptococcus pneumoniae and Listeria monocytogenes: understanding the role of complement properdin. *Med Microbiol Immunol* 2014; 203: 257–71.
13. Steiner T, Francescut L, Byrne S et al. Protective role for properdin in progression of experimental murine atherosclerosis. *PLoS One*2014; 9(3): e92404.
14. van Essen MF, Ruben JM, de Vries APJ, van Kooten C. Role of properdin in complement-mediated kidney diseases. *Nephrol Dial Transplant*2019; 34(5): 742-50.
15. Ziegler JB, Rosen FS, Alper CA, Grupe W, Lepow IH. Metabolism of properdin in normal subjects and patients with renal disease. *J Clin Invest* 1975; 56: 761–7.
16. Perrin LH, Lambert PH, Miescher PA. Properdin levels in systemic lupus erythematosus and membranoproliferative glomerulonephritis. *Clin Exp Immunol* 1974; 16: 575–81.
17. Józsi M, Reuter S, Nozal P, López-Trascasa M, Sánchez-Corral P, Prohászka Z, Uzonyi B. Autoantibodies to complement components in C3 glomerulopathy and atypical hemolytic uremic syndrome. *Immunol Lett* 2014; 160(2): 163-71.
18. Nozal P, Garrido S, Martínez-Ara J. Case report: lupus nephritis with autoantibodies to complement alternative pathway proteins and C3 gene mutation. *BMC Nephrol* 2015; 16: 40.
19. Tanuma Y, Ohi H, Hatano M. Two types of C3 nephritic factor: properdin-dependent C3NeF and properdin-independent C3NeF. *Clin Immunol Immunopathol* 1990; 56(2): 226-38.
20. Appel GB, D'Agati VD. Lupus nephritis-pathology and pathogenesis. In Wallace DJ, Hahn BH, ds. *Dubois' Lupus Erythematosus*. 7th ed. Philadelphia: Lippincott Williams & Wilkins 2007; 1094-112.
21. Gerald BA, Radhakrishnan J, D'Agati VD, et al. Systemic Lupus Erythematosus. In: Brener & Rector's *The Kidney*. 9th ed. Taal M, Chertow G, Marsden P, Skorecki K, Yu A, Brenner B eds. Philadelphia. Elsevier Saunders 2012; 1193-208.
22. Appel GB, D'Agati VD. Renal involvement in systemic lupus erythematosus. In: Massry S, Glassock R, eds. *Textbook of Kidney Disease*. St. Louis: Williams & Wilkins 2001; 2000: 787-97.
23. Hay EM, Bacon PA, Gordon C et al. The BILAG index: a reliable and valid instrument for measuring clinical disease activity in systemic lupus erythematosus. *Q J Med* 1993; 86(7): 447-58.
24. Isenberg DA, Rahman A, Allen E, et al. BILAG 2004. Development and initial validation of an updated version of the British Isles Lupus Assessment Group's disease activity index for patients with systemic

lupus erythematosus. *Rheumatology (Oxford)* 2005; 44(7): 902-6.
25. Weening JJ, D'Agati VD, Appel GB, et al. The Classification of glomerulonephritis in systemic lupus erythematosus revisited. *J Am Soc Nephrol* 2004; 15: 241-50.
26. Markowitz GS, D'Agati VD. Classification of lupus nephritis. *Curr Opin Nephrol Hypertens* 2009; 18(3): 220-5.
27. Marinozzi MC, Chauvet S, Le Quintrec M et al. C5 nephritic factors drive the biological phenotype of C3 glomerulopathies. *Kidney Int* 2017; 92(5): 1232-41.
28. Hourcade DE. Properdin and complement activation: a fresh perspective. *Curr Drug Targets* 2008; 9(2): 158-64.
29. Marinozzi MC, Roumenina LT, Chauvet S et al. Anti-Factor B and Anti-C3b Autoantibodies in C3 Glomerulopathy and Ig-Associated Membranoproliferative GN. *J Am Soc Nephrol* 2017; 28(5): 1603-13.
30. Vasilev VV, Noe R, Dragon-Durey MA et al. Functional Characterization of Autoantibodies against Complement Component C3 in Patients with Lupus Nephritis. *J Biol Chem* 2015; 290(42): 25343-55.
31. Vasilev VV, Radanova M, Lazarov VJ, Dragon-Durey MA, Fremeaux-Bacchi V, Roumenina LT. Autoantibodies Against C3b-Functional Consequences and Disease Relevance. *Front Immunol* 2019; 10: 64.
32. Blanc C, Togarsimalemath SK, Chauvet S, et al. Anti-factor H autoantibodies in C3 glomerulopathies and in atypical hemolytic uremic syndrome: one target, two diseases. *J Immunol* 2015; 194(11): 5129-38.
33. Djoumerska-Alexieva IK, Dimitrov JD, Voynova EN, Lacroix-Desmazes S, Kaveri SV, Vassilev TL. Exposure of IgG to an acidic environment results in molecular modifications and in enhanced protective activity in sepsis. *FEBS J* 2010; 277(14): 3039-50.
34. Moroni G, Quaglini S, Radice A, Trezzi B, Raffiotta F, Messa P, Sinico RA. The value of a panel of autoantibodies for predicting the activity of lupus nephritis at time of renal biopsy. *J Immunol Res* 2015; 2015: 106904.
35. Chi S, Yu Y, Shi J, Zhang Y, Yang J, Yang L, Liu X. Antibodies against C1q Are a Valuable Serological Marker for Identification of Systemic Lupus Erythematosus Patients with Active Lupus Nephritis. *Dis Markers* 2015; 2015: 450351.
36. Tan Y, Song D, Wu LH, Yu F, Zhao MH. Serum levels and renal deposition of C1q complement component and its antibodies reflect disease activity of lupus nephritis. *BMC Nephrol* 2013; 14: 63.
37. Matrat A, Veysseyre-Balter C, Trolliet P, Villar E, Dijoud F, Bienvenu J et al. Simultaneous detection of anti-C1q and anti-double stranded DNA autoantibodies in lupus nephritis: predictive value for renal flares. *Lupus* 2011; 20: 28-34.
38. Meyer OC, Nicaise-Roland P, Cadoudal N, Grootenboer-Mignot S, Palazzo E, Hayem G, et al. Anti-C1q antibodies antedate patent active glomerulonephritis in patients with systemic lupus erythematosus. *Arthritis Res Ther* 2009; 11(3): R87.
39. Orbai AM, Truedsson L, Sturfelt G et al. Anti-C1q antibodies in systemic lupus erythematosus. *Lupus* 2015; 24(1): 42-9.
40. Akhter E, Burlingame RW, Seaman AL, Magder L, Petri M. Anti-C1q antibodies have higher correlation with flares of lupus nephritis than other serum markers. *Lupus* 2011; 20(12): 1267-74.
41. Marto N, Bertolaccini M, Calabuig E, Hughes G, Khamashta M. Anti-C1q antibodies in nephritis: correlation between titres and renal disease activity and positive predictive value in systemic lupus erythematosus. *Ann Rheum Dis* 2005; 64(3): 444–8.
42. Bock M, Heijnen I, Trendelenburg M. Anti-C1q Antibodies as a Follow-Up Marker in SLE Patients. *PLoS One* 2015; 10(4): e0123572.
43. Julkunen H, Ekblom-Kullberg S, Miettinen A. Nonrenal and renal activity of systemic lupus erythematosus: a comparison of two anti-C1q and five anti-dsDNA assays and complement C3 and C4. *Rheumatol Int* 2012; 32(8): 2445-51.

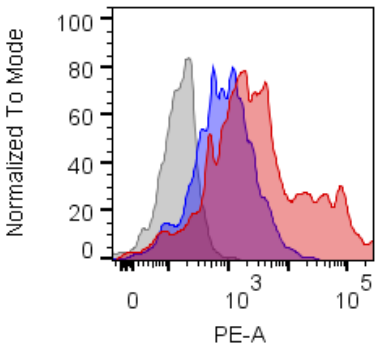
Fig. 1 Detection of anti-properdin autoantibodies: A. Levels of anti-properdin in 74 patients with LN and 72 healthy volunteers. **B.** Dose dependent ELISA with anti-properdin positive patients. **C.** SPR senzograms of binding of purified IgG from seropositive patient 33 (P33), **D.** - from seropositive patient 35 (P35) and **E.** - from seropositive patient 38 (P38) to properdin. **F.** SPR senzograms of binding of purified

IgG from healthy volunteer (K1) and **G.** - from healthy volunteer (K2) to properdin.

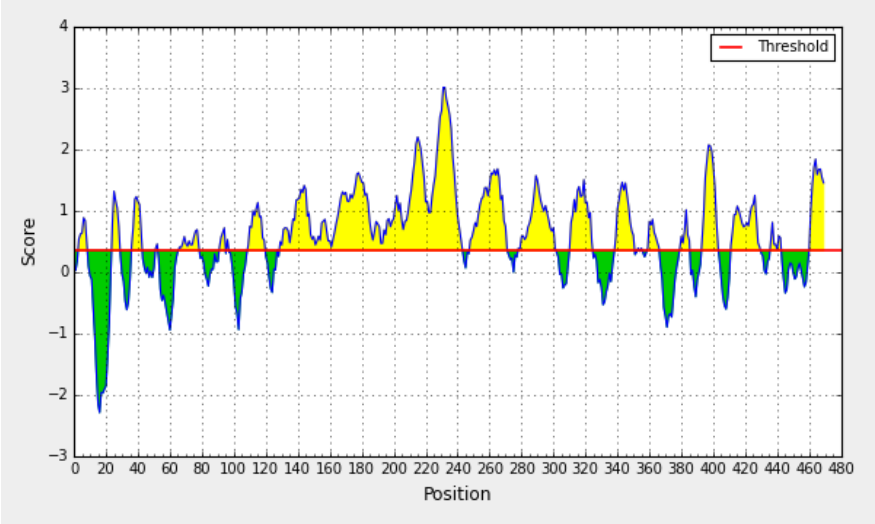
Fig 2. Statistical analysis with anti-properdin autoantibodies: A. Levels of anti-properdin autoantibodies in patients divided on the complex clinical-laboratory estimation for LN activity according BILAG Renal Score. **B.** Comparative analysis of proteinuria in anti-properdin positive and anti-properdin negative patients in cross-section analysis. **C.** Correlation between levels of anti-properdin antibodies and eGFR. **D.** Levels of anti-properdin in patients with LN depending on the presence or absence of pathological ANA, **E.** anti-dsDNA, **F.** C3 hypocomplementemia, **G.** C4 hypocomplementemia and **H.**anti-C1q. With "+" is marked the presence of increased ANA, anti-dsDNA and anti-C1q; with "-" are marked the reference values of ANA, anti-dsDNA and anti-C1q in the samples. The two groups in every graphic were compared via Mann-Whitney, nonparametric t-test. **I.**Correlation between levels of anti-properdin and levels of ANA,**J.** anti-dsDNA, **K.** C3, **L.** C4 and **M.**anti-C1q in patients with LN. In order to estimate the correlations between anti-properdin with every immunological markers a nonparametric Spearman analysis was used.

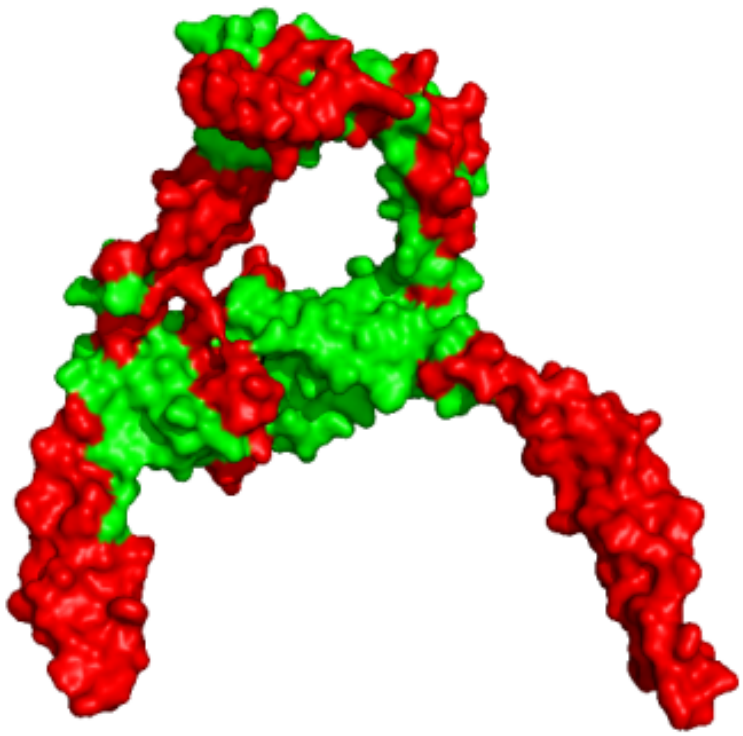
Fig. 3. Functional analysis with anti-properdin autoantibodies: A. SPR sensograms for the effect of purified IgG from patient 33 (P33), positive for anti-properdin antibodies and from healthy volunteer (K1) on properdin binding with C3b, **B.** with C3b+FB (proconvertase),**C.** and with C3b+FB+FD (convertase) in real time. **D.**The effect of purified IgG from patient 35 (P35), positive for anti-properdin and from healthy volunteer (K1) on properdin binding with C3b, **E.** with C3b+FB (proconvertase), **F.** and with C3b+FB+FD (convertase) in real time. **G.** The effect of purified IgG from patient 38 (P38), positive for anti-properdin and from healthy volunteer (K1) on properdin binding with C3b, **H.** with C3b+FB (proconvertase), **I.** and with C3b+FB+FD (convertase) in real time. Properdin is immobilized on SPR chip and then expose to IgG from patients positive for anti-properdin (P33, P35 and P38) and IgG from healthy volunteer (K1), followed by C3b (**A., D., G.**), C3b+FB (**B., E., H.**) and C3b+FB+FD (**C., F., I.**) addition.**J.** Histogram of FACS analysis of C3 deposition in the presence of purified IgG from patients 9 (P9) and **K.** - purified IgG from patients 35 (P35), both positive for anti-properdin. **L.**Histogram of FACS analysis of C3 deposition in the presence of purified IgG from patients 32 (P32) and **M.** - purified IgG from patients 17 (P17), both positive for anti-C3. All patients are compared with a control sample (K85).

Fig. 4 Prediction of the B cell epitopes of properdin: A. Epitopes, predicted by the IEDB server http://tools.iedb.org/bcell/.**B.** Visualizaiton of the predicted peptides (red) on the surface of a properdin monomer (green)



	Sample Name	Subset Name	Count	Geometric Mean : PE-A
	Specimen_001_P9 aC3c.fcs	Single Cells	1251	2863
	Specimen_001_K85 aC3c.fcs	Single Cells	1506	790
	Specimen_001_Iso aC3c.fcs	Single Cells	1302	144





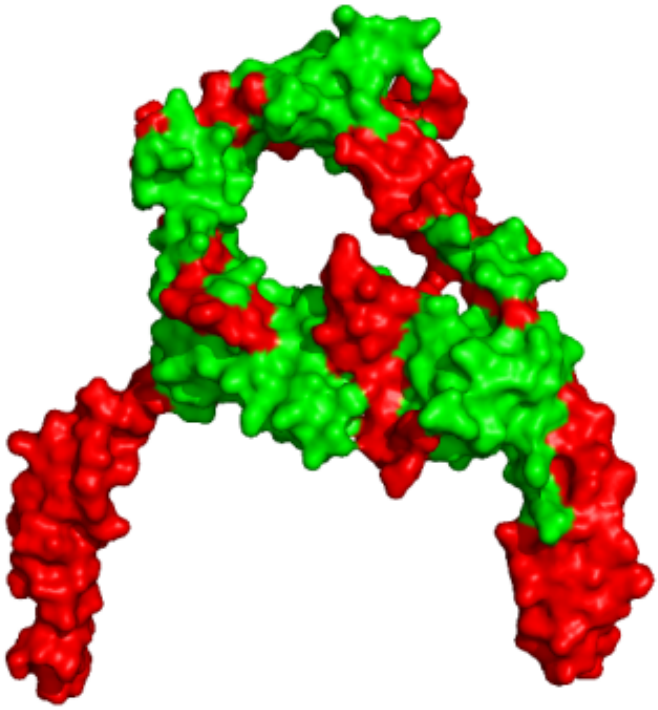


Table 1

Table 1. Significance of anti-Properdin antibodies alone and in combination with conventional markers for LN activity for determination of category A according BILAG Renal score

BILAG A vs BILAG B, C, D	**Specificity, %**	**NPV, %**	**Sensitivity, %**	**PPV, %**	**P**
Anti-C1q	87.9 (188/214)	85.5 (188/219)	40.4 (21/52)	44.7 (21/47)	**0.000**
Anti-Properdin	18.7 (40/214)	75.5 (40/53)	74.5 (38/51)	17.9 (38/212)	0.275
Anti-C1q and anti-Properdin	88.3 (189/214)	85.1 (189/222)	35.3 (18/51)	41.9 (18/43)	**0.000**
Anti-C1q and anti-dsDNA	91.4 (169/185)	86.7 (169/195)	38.1 (16/42)	50.0 (16/32)	**0.000**
Anti-Properdin and anti-dsDNA	57.3 (106/185)	89.8 (106/118)	70.7 (29/41)	26.9 (29/108)	**0.001**
Anti-C1q, anti-dsDNA and C3 and C4	97.8 (176/180)	84.2 (176/209)	19.5 (8/41)	66.7 (8/12)	**0.000**

BILAG A vs BILAG B, C, D	**Specificity, %**	**NPV, %**	**Sensitivity, %**	**PPV, %**	**P**
Anti-Properdin, anti-dsDNA and C3 and C4	94.4 (170/180)	85.9 (170/198)	30.0 (12/40)	54.5 (12/22)	**0.000**

Table 2

Table 2. Comparative analysis between levels of anti-Properdin in groups of patients with and without histological signs of LN activity and chronicity

Histological features	**Anti-Factor P** Median (from-to)	**Anti-Factor P** Median (from-to)	**p-value**
	Presence	**Absence**	
Endocapillary proliferation	0.048 (0.000-0.942)	0.054 (0.000-0.326)	0.797
"Wire loop" deposits	0.238 (0.000-0.942)	0.047 (0.000-0.788)	**0.009**
Fibrinoid necrosis/karyorrhexis	0.076 (0.000-0.666)	0.048 (0.000-0.942)	0.655
Cellular crescents	0.124 (0.073-0.666)	0.043 (0.000-0.942)	**0.009**
Interstitial inflammation	0.061 (0.000-0.942)	0.051 (0.000-0.846)	0.740
Glomerular sclerosis	0.053 (0.000-0.788)	0.061 (0.000-0.942)	0.697
Fibrous crescents	0.124 (0.073-0.788)	0.043 (0.000-0.942)	**0.008**
Tubular atrophy	0.078 (0.000-0.942)	0.045 (0.000-0.846)	0.349
Interstitial fibrosis	0.064 (0.000-0.942)	0.048 (0.000-0.846)	0.829

Table 3

Table 3. Peptide antigenic determinants in the properdin molecule predicted by IEBD.

Start	End	Peptides longer than 3 aminoacids properdin
3	8	TEGAQA
24	28	ATGSD
36	42	YEESSGK
66	78	KRSGGLCQPCRSP
91	94	VTCS
109	119	GQCSGKVAPGT
129	243	DQQCCPEMGGWSGWGPWEPCSVTCSKGTR TQQVCPTHGAWATWGPWTPCSASCHGGPH
248	271	CTGLPPCPVAGGWGPWGPVSPCPV
278	301	TMEQRTCNHPVPQHGGPFCAGDAT
310	324	VPCPVDGEWDSWGEW
339	350	EIPCQQSRGRTC
359	366	RCAGQQQD
379	385	KGSWSEW
393	403	PPCGPNPTRAR
411	429	LPKYPPTVSMVEGQGEKNV

Start	End	Peptides longer than 3 aminoacids properdin
436	442	LPRCEEL