Complement Genetics for the Practicing Allergist Immunologist: Focus on Complement Deficiencies

Ágnes Szilágyi, PhD[a,*], Dorottya Csuka, PhD[a,*], Christoph B. Geier, MD, MSc[b,c], and Zoltán Prohászka, MD, DSc[a,d]

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Learning objectives:

1. To choose the most suitable diagnostic approaches towards complement deficiencies.

2. To link the clinical presentation with the appropriate test for diagnostic evaluation.

3. To diagnose patients suffering from hereditary complement deficiencies.

4. To provide improved patient management and choosing appropriate treatment modalities.

Recognition of Commercial Support: This CME has not received external commercial support.

Disclosure of Relevant Financial Relationships with Commercial Interests: All authors and reviewers reported no relevant financial relationships.

Complement deficiencies have been considered to be rare for many decades, but this assumption is changing year by year. Recognition of these conditions significantly increases thanks to the availability of different testing approaches and due to clinical awareness. Furthermore, sequencing technologies (including Sanger sequencing, targeted gene panels, and whole exome/genome sequencing) may facilitate the identification of the underlying disease-causing genetic background. On the other hand, functional characterization of the identified possibly pathogenic variations and performing family studies, as illustrated by some of our cases, remain similarly important to establish a precise clinical diagnosis facilitating the most appropriate management. Here, we present 4 illustrative cases with complement deficiencies of diverse etiologies and also provide an educative, step-by-step description on how to identify the underlying cause of complement deficiency based on the

[a]Department of Internal Medicine and Haematology, Semmelweis University, Budapest, Hungary
[b]Department of Rheumatology and Clinical Immunology, University Medical Center Freiburg, Freiburg, Germany
[c]Center for Chronic Immunodeficiency (CCI), University Medical Center Freiburg, Freiburg, Germany
[d]Research Group for Immunology and Haematology, Semmelweis University—Eötvös Loránd Research Network (Office for Supported Research Groups), Budapest, Hungary
* These authors have contributed equally to this work.

Research in the laboratories of the authors was supported by the following grants: Higher Education Institutional Excellence Programme of the Ministry of Human Capacities in Hungary, within the framework of the molecular biology thematic program of the Semmelweis University; by the National Office for Innovation and Research (2020-1.1.6-JOVO-2021-00013 "JOVO" to Z. Prohászka); Premium Postdoctoral Fellowship Program of the Hungarian Academy of Sciences (PPD2018-016/2018 to D. Csuka); and EU MSCA-ITN 860044project "CORVOS" (to Z. Prohászka). Project no. TKP2021-EGA-24 has been implemented with the support provided by the Ministry of Innovation and Technology of Hungary from the National Research, Development and Innovation Fund, financed under the TKP2021-EGA funding scheme.

Conflicts of Interest: The authors declare that they have no relevant conflicts of interest.

Received for publication December 13, 2021; revised manuscript received and accepted for publication February 21, 2022.

Available online March 8, 2022.

Corresponding author: Zoltán Prohászka, MD, DSc, Department of Internal Medicine and Haematology, Semmelweis University, Szentkirályi St 46, Budapest H-1085, Hungary. E-mail: prohaszka.zoltan@med.semmelweis-univ.hu.

2213-2198

https://doi.org/10.1016/j.jaip.2022.02.036

Abbreviations used
AP- Alternative pathway
C2D- Deficiency of complement C2
CBC- Complete blood count
CD- Complement deficiency
CP- Classical pathway
CRP- C-reactive protein
CSF- Cerebrospinal fluid
ED- Emergency department
FH- Factor H
ICU- Intensive care unit
LP- Lectin pathway
MBL- Mannose-binding lectin
SLE- Systemic lupus erythematosus
WBC- White blood cell

results of complement laboratory testing. **(J Allergy Clin Immunol Pract 2022;10:1703-11)**

Key words: *Complement deficiency; C2; Factor I; C8; Bacterial infections; Systemic autoimmunity; Anti-C1q autoantibody; Mannose-binding lectin*

The complement system is an evolutionarily ancient component of the innate immune system that contributes to the elimination of pathogens, clearance of apoptotic cells, and debris; induces inflammation; and also promotes humoral immunity by enhancing antibody generation, immunological memory, and T-cell responses.[1,2] The sensitive enzymatic cascade of complement components, leading to the generation of powerful biological mediators including anaphylatoxins, opsonins, and lytic complexes, can be activated by carbohydrate patterns of pathogens through its lectin pathway (LP) and by antibodies through its classical pathway (CP), whereas the alternative pathway (AP) is characterized by a constant low level of spontaneous activation and an amplification loop on the level of the central component C3.[3] Insufficient complement activation resulting from the dysfunction of the participating components or impairment in its finely balanced regulation may result in the decline of defense against certain microorganisms, reduction of self-tolerance, excessive inflammation, and tissue injury.[4]

In this study, we focus on the 2 most important clinical phenotypes associated with primary complement deficiencies (CDs) that are severe and/or recurring infections and development of systemic autoimmunity. Primary (inherited) CDs associated with clinical disease collectively account for approximately 5% of the primary immunodeficiencies.[5] CDs are inherited as autosomal recessive traits with some exceptions (X-linked recessive for properdin deficiency, autosomal dominant for C1-inhibitor deficiency). In contrast, secondary CDs are most often linked to systemic autoimmunity, anticomplement autoantibodies (anti-C1q or C3-nephritic factors), or protein loss states. Hereditary angioedema, linked to the deficiency of C1-inhibitor (the most important regulator of the classical and lectin pathways of complement), along with further complement mediated kidney- and inflammatory eye diseases were recently reviewed[6-8] and are not discussed in this work.

The immunological, genetic, and clinical features of the largest worldwide primary complement deficiency cohort and the European Society of Immunodeficiencies registry data have recently been described.[9,10] It is widely recognized and accepted that the incidence of CDs largely depends on the access to expert complement diagnostics, including functional testing of complement, and also genetics. Because of efforts to provide the external quality assurance program for complement laboratories[11] and to standardize various complement measurements,[12] the number of well-prepared and trained diagnostic complement laboratories constantly increases.[13] Furthermore, thanks to the widespread use of next-generation sequencing technologies, the number of cases diagnosed with CDs with an identified complement gene mutation is rising rapidly.[14-16] It is expected, therefore, that practicing allergists and immunologists will have easier access to expert diagnostic services to evaluate patients with suspected CDs, and accordingly, the number of complex complement reports to be interpreted will also increase. The aim of this work is therefore to show 4 selected, illustrative, and educative case descriptions for CDs, and to guide the readers through the process of interpreting complement results of increasing complexity. Because structured information on acquired CDs is largely missing in the literature, we included a case with anti-C1q and deficient complement activity to facilitate the understanding and interpretation of similar results in the daily practice.

CASE PRESENTATIONS

Case 1: an early presentation of complement C2 deficiency

The patient was delivered at 39 weeks of gestation with cesarean section and received ampicillin because of premature rupture of membranes and the elevated C-reactive protein (CRP) level. He had no icterus or soor oris. Until the age of 15 months, he had only mild colds and received the mandatory childhood vaccines (Pentaxim, Prevenar, MMR, Varilrix, and Meningitec). At the age of 15 months, he was admitted to hospital after 4 days of fever with a suspicion of meningitis. A rapid diagnostic test performed on a cerebrospinal fluid (CSF) sample was positive for *Streptococcus pneumoniae* and *Neisseria meningitidis* Y. Brain magnetic resonance imaging revealed disseminated alterations indicating a vasculitic origin. Following discharge after a 1-month-long hospitalization, the patient had hydrocephalus internus and experienced high degree of permanent hearing loss in both ears, strabismus, and imbalance problems. In the next year, he repeatedly had infections with mild fever lasting for a few days, raising the possibility of immunodeficiency; therefore, a complex immunological evaluation was performed at the age of 3 years.

Immunoglobulin levels (IgG, IgA, and IgM) and IgG subclass concentrations were in the normal range. Flow cytometry analysis revealed that the absolute count of white blood cells (WBCs) was high; however, the ratio of WBC subtypes was normal or showed only borderline differences from age-matched reference ranges. Detailed complement analysis was also performed (Table I), showing deficient classical (CH50) and LP activities (LH50) with normal AP activity (AP50), along with normal C3 and C4 levels. As these findings point to a likely deficiency of 1 or more of the early components or regulators of classical and LPs, detailed analysis was carried out for these molecules. The levels of C1q, mannose-binding lectin (MBL), and C1-inhibitor were within reference ranges and antibodies to C1q or C1-inhibitor were not detected, whereas C2 was found to be subnormal. Hence, the whole coding region of the gene (*C2*) encoding C2 protein was analyzed by Sanger sequencing after polymerase chain reaction amplification.

TABLE I. Complement laboratory investigations performed in the 4 illustrative cases with complement deficiencies

Cases	Clinical signs, phenotype	Levels of C3 and C4	Activity of different complement pathways	Further specific complement measurements	Determination of complement autoantibodies	Analysis of complement genes	Final evaluation
Case 1 (C2)	Single episode of severe meningitis Recurring infections with mild fever	Normal C4 Normal C3	Deficient CP Deficient LP Normal AP Normal TCC	Normal C1q Normal MBL Normal C1-inhibitor Decreased C2 (All persist in remission)	Antibodies to C1q or C1-inhibitor not detected	Homozygous *C2* c.839_849+17del28	Inherited *C2* deficiency
Case 2 (C8 and MBL)	Recurrent bacterial upper and lower respiratory tract infections Two episodes of Neisseria infections	Normal C4 Normal C3	Deficient CP Deficient LP Deficient AP	Decreased C8 and MBL Normal C5, C6, C7, and C9	No complement autoantibodies detected	*C8B* 1282C>T, p.Arg428Ter *MBL2* LXPA/LYPB	Inherited C8B and MBL deficiency
Case 3 (CFI)	Viral upper-airway infection Otitis media Bacterial meningitis *S. pneumoniae*	Normal C4 Decreased C3	Moderately decreased CP and LP Deficient AP High TCC	Decreased factor B Decreased factor H Decreased factor I Normal C1q (All persist in remission)	Anti-FH IgG autoantibody not detected	Heterozygous *CFI* c.267_267delG Heterozygous *CFI* c.772G>A Transposition of mutations is verified by family screening No mutations detected in *CFB* and *CFH*	Inherited *CFI* deficiency due to compound heterozygosity for 2 *CFI* mutations
Case 4 (anti-C1q)	SLE-like symptoms Fatigue Fever	Decreased C4 Decreased C3	Deficient CP Deficient LP Deficient AP High TCC	Decreased factor B Decreased factor H (Resolved in remission) Decreased C2 Decreased C1q (Persist in remission)	Extremely elevated IgG-type anti-C1q autoantibody C3-nephritic factor not detected Anti-FH IgG autoantibody not detected	C2 deficiency excluded Complement C4 gene copy number determination: 2 copies of *C4A* and 1 copy of *C4B*	Anti-C1q autoantibody Positive Low number of *C4* alleles (<4), risk factor for the development of SLE
Interpretation, comments	Suspicion for complement deficiency, or complement-mediated disease	Normal levels do not exclude the possibility of complement deficiency Decreased levels may help to orient	Results of functional tests may prove or disprove complement deficiency Patterns of decreased activities narrow down the list of involved components/regulators	A full investigation in serum and plasma samples is necessary to identify the missing component with lack of complement activity, or the presence of regulator deficiency or anticomplement antibody, leading to dysregulation, overactivation, and secondary complement deficiency		Presence or absence of variations verifies or excludes hereditary deficiency of complement components or regulators	
Action	Ordering of complement testing in local laboratories	Functional testing of complement is mandatory, when complement-related conditions are suspected	Detailed investigations in expert complement laboratories help to distinguish between inherited and acquired causes of complement deficiency	If hereditary origin is suspected, genetic analysis of the genes encoding the candidate components/regulators		With the knowledge of missing components, regulators, or anticomplement antibodies, the treatment, additional evaluation, and follow-up of the patient can be planned	

AP, Alternative pathway; *CP*, classical pathway; *FH*, factor H; *LP*, lectin pathway; *MBL*, mannose-binding lectin; *SLE*, systemic lupus erythematosus; *TCC*, terminal complement complex.

The patient was found to be homozygous for a 28-bp-long deletion including the last 9 bases of exon 6 and 18 nucleotides at the 5′-end of the following intron. As this mutation involves the donor splice site of intron 6, it causes aberrant RNA spicing resulting in the deletion of the whole preceding exon (exon 6) from the mature *C2* mRNA and a shift of the reading frame leading to the generation of a premature termination codon and the absence of detectable C2 synthesis.[17] This variation is the most common (>90%) of the mutations causing lack of C2 protein that is the most frequent form (with an estimated prevalence of 1/10,000-30,000 in White populations) of the hereditary deficiencies of CP proteins. Because the C2 protein provides the catalytic subunit of the C3/C5 convertases of the classical and LPs, it is a key component of both pathways; thereby its deficiency (C2D) contributes to impairment in antibody and complement-mediated defense against microbial infections. Accordingly, C2D was found to be associated with frequent occurrence of bacterial infections, particularly with encapsulated bacteria; besides, an increased risk of severe disease course was also reported. The predominant clinical manifestation in patients diagnosed with C2 deficiency is the past history of a single or repeated invasive infections mainly pneumonia, meningitis, or septicemia caused by *S. pneumoniae*, less frequently *N. meningitidis*, *Streptococcus agalactiae*, or *Haemophilus influenzae*. However, many individuals with C2D experience only minor infections such as recurrent otitis media, sinusitis, and upper-airway infections.[18,19] Moreover, based on the known prevalence of mutations causing C2D, deficient individuals are barely recognized in the health care system, implying that a portion of them have minor or no increase in infection susceptibility.

Screening of the patient's family members revealed that both parents and the older brother carried the mutation in the heterozygous form. In contrast to homozygous C2 deficiency, no increased risk for autoimmune or infectious diseases was reported in the heterozygous state, which occurs at a 1% to 2% frequency in White populations.

Case 2: MBL deficiency as a modifier of C8—late complement component deficiency

A 29-year-old woman presented to the emergency department (ED) with a sudden onset of high fever (>40°C), hypotension (109/50), elevated heart rate (115), severe dizziness, and vomiting. Physical examination at admission revealed a severely ill, febrile patient with maculopapular rash and purpura on lower extremities. Initial laboratory examination showed pronounced signs of inflammation (CRP: 16.56 mg/dL; leukocyte count: 38,000/μL). CSF was turbid with predominating polymorphs, raised CSF protein 5.65 g/L, and decreased CSF glucose <0.1 nmol/L. Gram staining of CSF was initially negative, but *N. meningitides* serogroup Z was demonstrated in blood culture and led to the diagnosis of meningococcal sepsis. The patient was subsequently transferred to the intensive care unit (ICU) and treated with intravenous antibiotics including ceftriaxone, vancomycin, and dexamethasone. Parenteral antibiotic therapy led to a complete recovery without any neurological sequela.

Six years earlier the patient was admitted to the ED because of severe right wrist pain on supination and dorsiflexion, as well as inflamed carpometacarpal joints of her left hand. Complete blood count (CBC) showed leukocytosis (21,000/μL) and elevated CRP (9.79 mg/dL). A subsequent arthrocentesis showed purulent synovial fluid with *Neisseria gonorrhoeae* and numerous polymorphonuclear WBCs. The patient was diagnosed with disseminated gonococcal infection and treated with intravenous ceftriaxone.

Additional past medical history was significant for recurrent bacterial infections, including several episodes of bronchitis, sinusitis, and otitis media. Further, she reported 2 additional episodes of hospitalization due to a retropharyngeal abscess (at the age of 17 years) and lobar pneumonia with *H. influenzae* (at the age of 19 years).

Given the history of recurrent severe infections, the patient was evaluated for immunodeficiency at age 29. Her immunological evaluation revealed a normal CBC, normal T- and B-cell subsets, and normal T-cell proliferation to mitogens, as well as normal serum IgG, IgA, IgM levels, and protective post-vaccination antibody titers to tetanus, diphtheria, hemophilus influenza, and streptococcus pneumonia. A dihydrorhodamine test for phagocytic function was normal.

Detailed complement analysis was also performed, showing deficiency in all 3 pathways with absent activity in CH50, AP50, and LH50, with normal C3 and C4 levels (Table I). As multiple pathways were low, a deficiency in the shared terminal pathway (C5-9) was suspected. Examining serum concentrations of individual complement components, C3, C5, C6, C7, and C9 levels were found to be within the normal range, whereas C8 was undetectable. In addition, the MBL serum level with 19 ng/mL was severely reduced. Based on these results, C8 and MBL deficiency were suspected; therefore, targeted sequencing was performed, including *C8A*, *C8B*, and *C8G*, as well as *MBL2* including its 5′ untranslated region. C8 deficiency may result from lack of the α-γ chain or the β chain, the latter by far the most frequent in White populations. The mutation, c.1282C>T, that introduces a premature stop codon (p.R428X) in exon 9 is with approximately 85% of null alleles, the most common cause of C8β deficiency.[10,20-24] Indeed, genetic testing confirmed the homozygous 1282C>T, p.Arg428Ter.

Serum concentration of MBL is dependent on the presence of different mutations (O alleles) in exon 1 of the *MBL2* gene, GGC to GAC in codon 54 (allele B), GGA to GAA in codon 57 (allele C), and CGT to TGT in codon 52 (allele D).[25] These 0 variants result in incorrect assembly of the MBL triple helix structure, thus impairing the ability to form high-order oligomers.[26] Homozygous or compound heterozygous combinations of those O alleles are associated with low to undetectable MBL serum levels, whereas heterozygous variants result in moderately reduced MBL levels in an autosomal dominant effect (B > C > D).[27] In addition, there are 2 polymorphisms at the *MBL2* gene's regulatory promoter region at positions −550 (H/L variant) and −221 (X/Y variant), which modulate MBL serum levels independently of the structural variants.[25] Genotyping of *MBL2* revealed an LXPA/LYPB genotype; LXP is a low expression promoter variant of a functional (variant A = wild-type) MBL protein, whereas LYP is an intermediate expression promoter variant of a defective (variant B) MBL protein, thus explaining the severely reduced serum MBL level.

Deficiency of the terminal components (C5-9) that form the membrane attack complex leads to an increased risk to suffer from infections by Neisseria species (especially meningococcus), whereas otherwise without a susceptibility to bacterial or viral infections.[9] This contrasts with the clinical presentation of our patient; although she had 2 episodes of Neisseria infections, her dominating clinical presentation was an increased susceptibility of bacterial infections especially of the upper and lower respiratory tract. The atypical disease course might be attributed to her

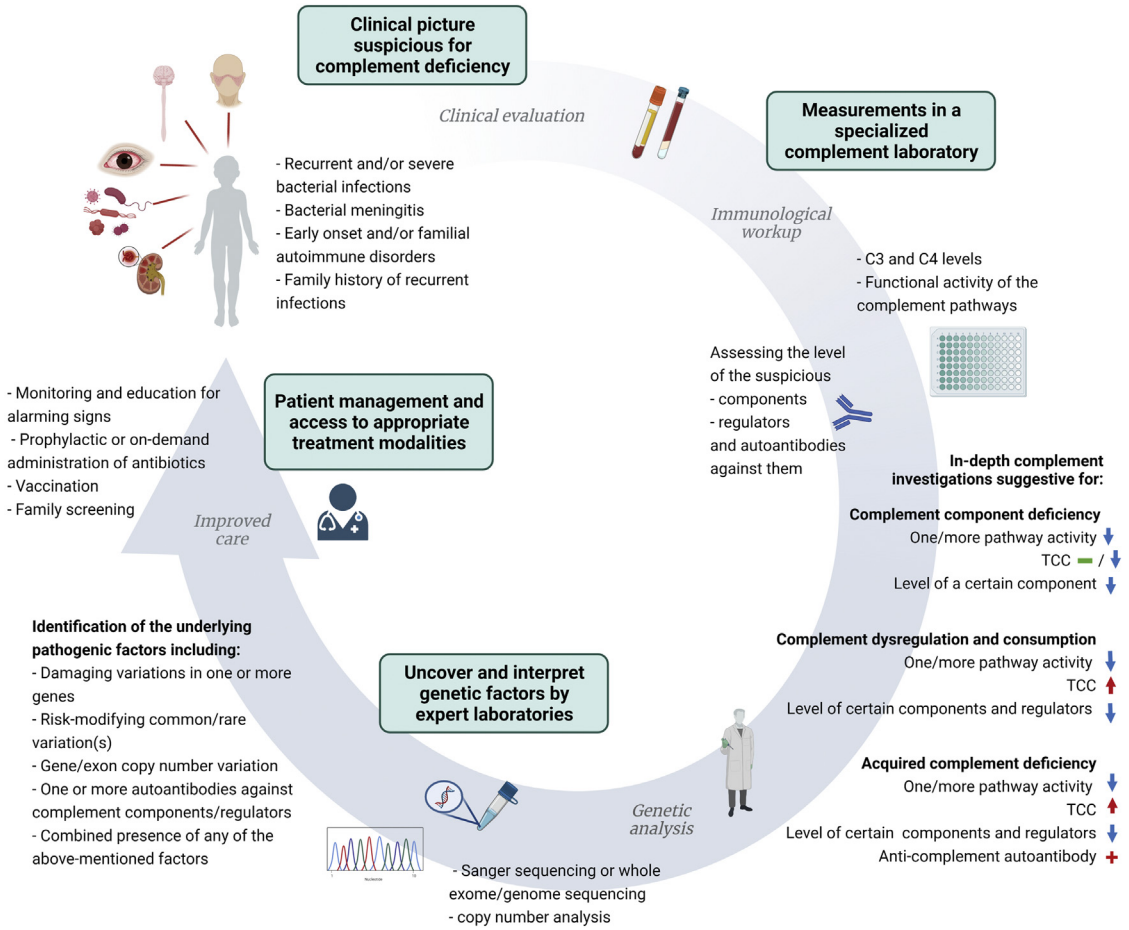

FIGURE 1. Recommended diagnostic approach on observing symptoms suggestive for complement deficiency. Possibility of complement deficiency should be suspected on the presentation of typical signs including meningococcal meningitis, recurrent bacterial infections, and early onset and/or familial autoimmune syndromes. As a first step of the multistage diagnostic protocol, investigating the total activity of the classical, alternative, and lectin pathways is recommended, in order to have an insight about the (dis)integrity of each complement pathway. These initial functional tests should be followed by the in-depth investigation of unique components/regulators/activation products/autoantibodies of the presumably affected pathway (as indicated by the initial functional screening). It should be noted that interpretation of the complement parameters requires complex knowledge about the (patho)physiology of the complement system, because, for instance, an undetectable or significantly reduced activity may indicate both a hereditary and an acquired complement deficiency, or a combination of both. Identification of a missing component or regulator should be followed by its molecular genetic analysis and, if feasible, the functional characterization and/or *in silico* prediction in order to evaluate its molecular pathogenicity and role in disease. Patients with complement deficiency should be provided with an emergency treatment plan and frequently controlled in immunodeficiency centers. *TCC*, Terminal complement complex. This figure was created with BioRender.com.

inherited MBL deficiency. The clinical impact of low levels of serum MBL levels in infectious disease remains unclear. Around 5% to 7% of White populations have inherited MBL deficiency (<100 ng/mL), whereas this does not seem to affect overall mortality or increase susceptibility to community-acquired pneumonia. However, MBL deficiency is described as a modifier in other immunodeficiencies or immunosuppressed patients to predispose for increased susceptibility to infections.[28-31]

Case 3: a rare case of compound heterozygous complement factor I deficiency

A 2-month-old boy presented with subfebrility, signs of viral upper-airway infection, and received symptomatic treatment. After 2 weeks, he presented again with fever, weakness, somnolence, and abdominal pain. An initial physical, imaging, and laboratory evaluation revealed signs of bilateral otitis media, pleuropneumonia with pleural effusion, and bacterial meningitis with elevated inflammatory markers. The microbiology workup identified *S. pneumoniae* (serotype 6C) in both hemoculture and liquor. The clinical state worsened with the development of tachypnea and dyspnea necessitating transfer to the ICU on the second hospital day, necessitating intubation and mechanical ventilation. The empiric antibiotic treatment was changed based on the resistance profile and clinical diagnosis on day 2, and in the next 5 days, gradual improvement started, and the patient was extubated on day 9. Kidney function was normal all the time, and the patient was released in good general state without important sequel on hospital day 42.

Because of the young age, and the quite rapid and severe disease course, complex immunological evaluation was performed. Decreased IgG (2.4 g/L) was observed with increased IgA (0.4 g/L) and IgM (0.9 g/L); therefore, intravenous immunoglobulin therapy was applied in the acute stage (on day 3, 3 g in total). Later, in remission, immunoglobulin levels were in the normal range, and fluorescence-activated cell sorting analysis of peripheral WBCs showed the presence of all lymphocyte and granulocyte subsets with appropriate activity.

Detailed complement analysis was performed (Table I), where the deficiency of AP activity with moderately decreased but not deficient classical and LP activities was remarkable, along with the low C3 level accompanied by the normal C4 level. These signs were indicative for AP dysregulation; therefore, further extended complement analysis was carried out. AP component factor B was decreased, along with the regulators factor H (FH) and factor I. On the other hand, the CP components C1q and C4 were in the reference range. These results raised the possibility of complement AP regulator deficiency; therefore, targeted genetic analysis was performed, including the direct sequencing of the *CFH*, *CFI*, and *CD46* genes (encoding FH, factor I, and membrane cofactor protein, respectively), as well as copy number analysis (by MLPA: multiplex ligation-dependent probe amplification) of exons in the *CFH* and the highly homologues related (*CFHR1, CFHR2, CFHR3, CFHR4*, and *CFHR5)* genes in order to detect possible gene rearrangements, a characteristic feature of this region.

The molecular genetic workup identified 2 relevant rare variations in the gene encoding complement factor I (*CFI*), a plasma serine protease possessing important roles to limit and regulate complement C3 convertase activity. The patient was found to be heterozygous for the deletion of 1 guanine base (c.267_267delG) in exon 2 of *CFI*, which generates a reading frameshift at amino acid 90 and a premature stop codon at amino acid position 102 (p.Ser90Valfs*12). Consequently, no functional factor I can be secreted from the mutated allele. This rare variation was not described previously in patients suffering from complement deficiency or in healthy subjects, and no functional studies were performed on its possible molecular effects. Based on the generated premature stop codon, the c.267_267delG mutation is expected to have a pathogenic role in complement factor I deficiency.

In addition, the patient was found to be heterozygous for a substitution that affects the last nucleotide of exon 5 (c.772G>A) of the *CFI* gene, which is a highly conserved position in the splice site consensus sequence. Previous functional studies confirmed that the identified mutation leads to incorrect splicing, and the consequence is an mRNA lacking exon 5 along with the decreased factor I level.[32,33] The identified mutation was detected previously in patients with complement factor I deficiency,[32,34] in patients with atypical hemolytic uremic syndrome,[35,36] in patients with age-related macular degeneration,[37] and also in healthy subjects with a low frequency (0.05%). Based on the above data, a pathogenic role was assigned to this mutation in factor I deficiency. Taken together, the index patient was found to be compound heterozygous for the *CFI* variations leading to functional factor I deficiency.

Bidirectional sequencing of the *CFH* and *CD46* genes as well as exon copy number determination of *CFH* and related genes did not identify any (likely) pathogenic variants explaining AP dysregulation.

The healthy parents, as well as the healthy brother and sister, were screened for the rare *CFI* variations identified in the index patient. The mutation causing a premature stop codon is carried by the healthy mother and brother, whereas the splice-site mutation showed paternal inheritance. The sister inherited none of the identified *CFI* variations.

At the end of the hospital stay, in infection-free time point, the index patient had a decreased factor I serum level, near the detection limit. In case of missing factor I, an important regulator of complement AP C3-convertase, uncontrolled activation and consumption of the whole AP occurs. This is indicated by the similarly low C3 and factor B levels (FB is the enzymatic component of AP C3-convertase) and missing functional activity of the AP. Remarkably, the components (C1q and C4) of the CP seem to be unaffected, which is a clear distinctive feature between AP dysregulation and global overactivation of the entire complement system. However, as dysregulation occurs at the level of the central component C3, a consequential, moderate decrease in the activity of the classical and LPs could be detected in the patient. An extreme elevation of the terminal pathway activation marker sC5b9 was a remarkable parameter, indicating that the AP C3-convertase and the terminal pathway is constantly "turned on" in this patient, leading to overactivation and consumption of the entire AP, and to constant functional insufficiency of this important arm of innate immunity.

Case 4: systemic lupus erythematosus, accompanied by the presence of anti-C1q autoantibody

A previously healthy 17-year-old female patient presented with repeated episodes of fatigue and fever, 6 months after mononucleosis. Family history was not remarkable for immune-mediated or autoimmune diseases.

Initial workup excluded hematological malignancy, but due to increased erythrocyte sedimentation rate and pancytopenia, complete immunological workup was also performed. The patient had positive antinuclear factor, anti-dsDNA antibodies near the cutoff level with persisting proteinuria and was diagnosed with systemic lupus erythematosus (SLE). A complete complement workup was also performed because of kidney involvement, and also to assess disease activity. The first complement profile, determined in a sample taken before the initiation of any kind of active therapy, showed deficiency for all 3 pathways (classical, lectin, and alternative), with decreased levels of factors C1q, C2, C4, C3, and factor B, low level of FH, and increased terminal pathway activation marker sC5b-9. An extremely elevated IgG anti-C1q autoantibody level (20-fold increase above cutoff) was also observed, whereas the C3-nephritic factor and anti-FH IgG autoantibody were negative. A kidney biopsy was performed and showed a typical picture of full-house proliferative glomerulonephritis, interpreted as focal proliferative sclerosing lupus nephritis (World Health Organization grade III C/A).[38] In the next 3 years, the patient was treated with mycophenolate mofetil and low-dose corticosteroids, anti-C1q declined but remained positive, and hypocomplementemia improved but low levels persisted, with continued proteinuria. Renal function was persevered all the time, and there was no hypertension. Because of the persisting alterations, mycophenolate mofetil was stopped and cyclosporin A treatment was started (taking into account the young age of the patient and the plans for having children in the near future), but low-level anti-C1q and moderate

hypocomplementemia persisted. After 3 more years, proteinuria increased again and the patient was treated with cyclophosphamide based on the Euro-Lupus protocol,[39] and finally 4 times with rituximab. This aggressive treatment finally led to stable remission, declining but still persisting proteinuria (without hematuria, with preserved kidney function), anti-C1q turned to be negative, and complement parameters, including functional activity of the 3 pathways, reached normal levels. Since this time the patient is in stable remission, while on low-dose corticosteroid treatment.

Because persisting complement deficiency was initially observed, genetic analysis was also performed. Complement C4 gene copy number determination identified 2 copies of *C4A*, 1 copy of *C4B*, considered as a risk factor for the development of SLE,[40] because the total number of C4 alleles is less than 4. C2 deficiency, the most common form of inherited complement deficiency in Central Europe,[5] was excluded.

DISCUSSION

The aim of the present work is to provide an educative thread on how to interpret the complex results of complement analysis and how to identify the underlying cause of complement deficiency (Figure 1), as shown by the step-by-step diagnostic algorithm of 4 illustrative real-life cases.

In case of recurring infections, or early onset/familial systemic autoimmunity, or insufficient response to vaccines, one should consider a hereditary or acquired complement deficiency. Detailed investigations should be performed by an expert complement laboratory by a multistep process: as a first step, the affected complement activation pathways (classical, alternative, lectin, or terminal) should be identified, based on functional complement activity assays including either functional ELISA tests or hemolytic/liposome-based assays.[41] CP deficiency is suggested by the severely decreased CH50 activity along with the normal AP activity, whereas normal C3 and CP along with decreased AP suggest a deficiency of the AP components/regulators. Because of the quite high occurrence of MBL deficiency, decreased LP activity is frequently seen. On the other hand, severe terminal pathway deficiency is suggested by the parallel presence of undetectable CP, AP, and LP activity. Absent or severely reduced activity may suggest a primary complement deficiency, but secondary deficiency due to increased/constant complement overactivation and consumption should also be investigated. The first, immediate, and easily available way to rule in or out overactivation is the measurement of C4 and C3 levels. However, detecting normal C3 and C4 levels *per se*, without functional analysis of the complement pathways, is insufficient to rule out complement deficiency, as illustrated by cases 1 and 2 (Table I). In addition, analysis of the terminal complement complex (or sC5b-9), a multiprotein complex indicating the activation of the whole terminal pathway, is an important and reliable tool to differentiate between overactivation and consumption (cases 3 and 4, with a high level), or component deficiency (case 1, with low levels), as the cause behind complement deficiency. It is important to note that results of complement measurements may be significantly influenced by an ongoing infection and related inflammatory processes; therefore, at least 2 concordant functional test results from samples collected at different time points are needed to confirm a suspicious complement deficiency. Finally, one-by-one analysis of the potentially affected components/regulators should be performed on the protein level inside the classical, lectin-induced, alternative and terminal pathway as well as the central component C3.

When suspecting functional or quantitative deficiency of a given complement factor or regulator, molecular genetic determinations should be performed by the screening of the coding regions and the exon-intron boundaries of the gene encoding the candidate protein in order to explore the hereditary background. However, conventional sequencing technologies may fail to identify the underlying genetic variations in certain cases, such as high homology of related complement genes or copy number variation of the affected genes. Family screening including complement and genetic measurements can be of great importance not only in identifying other affected members but also in assessing heritability of a given quantitative or functional deviation.

Patients with inherited or acquired insufficiency of the early components of the CP (C1, C2, C4) are prone to recurrent infections with encapsulated bacteria, and nearly half of them experience 1 or more severe disease manifestation such as meningitis, pneumonia, osteomyelitis, or septicemia. Besides infections, autoimmunity is also a characteristic phenotype in these patients with an elevated risk of SLE, SLE-like syndrome, dermatomyositis, rheumatoid arthritis, and other autoimmune diseases.[9] Diverse genetic factors may stand behind these deficiencies: in case of the first component, the multiprotein complex C1, mainly single nucleotide variations were described in the genes encoding its subcomponents (C1q, C1r, and C1s), responsible for the approximately 100 identified deficient cases. As noted in the description of the first illustrative case, C2 deficiency is mainly caused by a 28-bp-long deletion resulting in aberrant splicing; besides, some small variations causing missense, nonsense, or splice site changes were published.[10] The complement component C4 exists as 2 isoforms encoded by 2 genes, *C4A* and *C4B*, the number of which may vary between 0 and 3 (rarely 4) on a chromosome. The most common allele comprises 1 *C4A* and 1 *C4B* gene; however, both deletion and duplication of either of the genes may occur resulting in a total number of 0 to 5 of *C4A* and 0 to 4 of *C4B* genes in an individual.[42] Deficiency of C4A or C4B is quite common (1:250), but as at least 1 C4 gene is always present on a chromosome, only <50 patients were published with total C4 deficiency resulting from the combined presence of low gene copy number and damaging mutations.[9]

Insufficient activation of the LP due to the functional impairment of its pattern recognition molecule, MBL, is of uncertain clinical significance with no clear clinical picture; however, many studies suggest its association with a somewhat increased risk of infections, especially in individuals with accompanying immunocompromised states.[27] The frequency of functional MBL insufficiency, a result of the combination of promoter and exonic variations (as explained in the description of our second case), is 5% to 15% in White populations. Clinical phenotype along with the frequency of the deficiency of other pattern recognition molecules (ficolin-1/-2/-3, collectin-10/-11) and early serine proteases (MASP-1, -2, and -3) of the LP is quite diverse. These involve an increased susceptibility to infections (in case of ficolin-3−deficient patients, with <10 published cases[43]), no clear connection with infection susceptibility (MASP-2 deficiency due to the p.D120G variant, occurring in

7-15/10,000 individuals with a European origin, which was described as a risk factor for respiratory infections, but later studies revealed mainly asymptomatic homozygous carriers[44]), the distinct clinical phenotype of the rare 3MC syndrome with no elevated risk of infections (in patients with MASP1 or collectin-10/-11 deficiency), and no published case of complete deficiency (ficolin-1 and -2).[45]

Deficiency of complement C3, the central component where all complement pathways converge, is associated with susceptibility to a wide range of severe infectious complications such as meningitis, osteomyelitis, pneumonia, and bacteremia due to *N. meningitidis* or *Haemophilis influenzae*, appearing usually in early ages and recurring later, too. Approximately 40 patients with C3 deficiency have been described so far, who rarely showed SLE-like symptoms in a few cases.[46,47] Considering a further component of the AP, factor B, its deficiency was published only in 1 case who suffered from neisserial and pneumococcal infections.[48] Deficiency of factor D occurs similarly seldom, as it was identified only in 2 families suffering from bacterial infections.[49,50]

Among dysfunctions of the negative regulators of the AP, complete deficiency of FH is the most common with <50 published patients who usually suffer from pyogenic and neisserial infections, which are also characteristic for the similarly rare factor I—deficient patient group.[9] On the other hand, haploinsufficiency of both factors as well as of membrane cofactor protein are predisposing factors for kidney diseases such as atypical hemolytic uremic syndrome and certain types of glomerulopathies. The complete absence or dysfunction of properdin, a positive regulator of the AP, is slightly more common with a few hundred published cases worldwide, who are prone for Neisseria infections.[51,52]

Similarly, a higher incidence of neisserial infections is the main phenotype in patients with terminal pathway insufficiency resulting from various loss-of-function variations in either of the components from C5 to C9. Deficiency of each component is generally rare (each with approximately 100 published cases); however, the occurrence of certain mutations is higher in different ethnic groups (such as the frequency of C6 deficiency is 1:2000 in Afro-Americans, C7 deficiency occurs 1 in 10,000 Moroccan Jews, whereas C9 deficiency has an incidence of 1:1000 in Japan),[9] and altogether nearly quarter of the published complement component—deficient cases (excluding MBL) suffer from terminal complex deficiencies.[5]

Complex investigations for identifying all these diverse genetic variations and the eventually required functional characterization of the identified (likely) pathogenic variants and complement autoantibodies are available only in a few specialized complement laboratories where comprehensive complement diagnostics is provided.[53,54]

Management and treatment options of primary CDs include close monitoring of affected patients with family assessment and screening, education of caregivers for alarming signs of infections and autoimmunity, and guidance for patients regarding surgery, travel, and pregnancy. The use of antibiotic prophylaxis should be based on individual evaluation and decision, but it is advisable for all affected patients to ensure access to emergency antibiotics and prompt medical advice as part of the emergency plan. For patients with CD, the same vaccines are recommended as in healthy individuals; however, vaccinations against encapsulated bacteria such as Pneumococci, Neisseria, and *Haemophilus influenza* should be advised to all patients with CDs. Vaccine responses should be monitored where possible, with necessary booster vaccinations to achieve a sufficient high titer and durable response. Because C3, the central component of complement, is one of the most important opsonins for antigen presentation, patients with the constant low level of C3 are at highest risk of failing vaccine response.[55] Finally, innovative therapeutic trials to replace missing C2 by recombinant protein,[56] or factor I by adeno-associated gene transfer,[57] are underway, and several other additional drugs are in development phase.

CONCLUSION

Early recognition of CDs based on the suggestive signs and symptoms, and applying the required complex diagnostic process, is of utmost importance in order to provide the most suitable and individualized treatment strategy for the patients with CDs. Thanks to the increasing number of well-trained diagnostic complement laboratories and the diagnostic use of next-generation sequencing technologies, the identification of more and more phenotypically diverse CDs is to be expected in the near future.

Acknowledgments

We acknowledge the technical assistance of Márta Kókai, Éva Zsuzsanna Szendrei, Lászlóné Kertész, János Miklós, Kata Marossy, Edina Szabó and Beáta Takács, with many thanks.

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