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Review

Are complement deficiencies really rare? Overview on prevalence, clinical importance and modern diagnostic approach☆

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ABSTRACT

Complement deficiencies comprise between 1 and 10% of all primary immunodeficiencies (PIDs) according to national and supranational registries. They are still considered rare and even of less clinical importance. This not only reflects (as in all PIDs) a great lack of awareness among clinicians and general practitioners but is also due to the fact that only few centers worldwide provide a comprehensive laboratory complement analysis. To enable early identification, our aim is to present warning signs for complement deficiencies and recommendations for diagnostic approach. The genetic deficiency of any early component of the classical pathway (C1q, C1r/s, C2, C4) is often associated with autoimmune diseases whereas individuals, deficient of properdin or of the terminal pathway components (C5 to C9), are highly susceptible to meningococcal disease. Deficiency of C1 Inhibitor (hereditary angioedema, HAE) results in episodic angioedema, which in a considerable number of patients with identical symptoms also occurs in factor XII mutations. New clinical entities are now reported indicating disease association with partial complement defects or even certain polymorphisms (factor H, MBL, MASPs). Mutations affecting the regulators factor H, factor I, or CD46 and of C3 and factor B leading to severe dysregulation of the alternative pathway have been associated with renal disorders, such as atypical hemolytic uremic syndrome (aHUS) and – less frequent – with membranoproliferative glomerulonephritis (MPGN). We suggest a multi-stage diagnostic protocol starting based on the recognition of so called warning signs which should aid pediatricians and adult physicians in a timely identification followed by a step-wise complement analysis to characterize the defect at functional, protein and molecular level.

1. Introduction

Several hallmarks related with a dramatic decrease of the mortality in children had great influence on the recognition of PIDs: (1) the effective control of infections through universal childhood immunization, (2) the discovery of penicillin by Alexander Fleming in 1928 opening the era of antibiotics and (3) the introduction of oral hydration (Shulman, 2004). Equally important was that the concept of adaptive and innate immunity, established during the late 19th and in 20th centuries, became part of medical reasoning and ensured progress in diagnosis, treatment, and the genetics of PID (Ochs and Hitzig, 2012). The classical definition of PIDs refers to inherited disorders of the immune system that predispose affected individuals to increased rate and severity of infection, immune dysregulation with autoimmune disease, and malignancy. However, many other PID-related clinical phenotypes have been identified, including angioedema, granulomas, autoinflammation, hemophagocytosis, thrombotic microangiopathy and predisposition to allergy (Casanova and Abel, 2007).

Multiple infections have been the main feature associated with PIDs. It has been well accepted that infections in children with PIDs commonly persist or recur. However, in certain disorders, the patients suffer only from one or two episodes of infection with no relapses (Casanova et al., 2008). In these PIDs, the adaptive immunity may compensate for an impaired innate response as observed in patients with terminal complement components deficiency. Although C5–C9 deficient patients are susceptible to meningococcal meningitis, specific immunization against *Neisseriae* decreases the recurrence of this infection, probably due to the induction of an adaptive immune response (Fijen et al., 1989; Keiser and Broderick, 2012). Furthermore, some PIDs remain clinically silent for a long period and first manifest in adulthood like common variable immunodeficiency (CVID). Here, additional MBL deficiency

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appears to influence disease severity (Litzman et al., 2008). Many clinically significant complement deficiencies, such as those of the late C components, C5–C9, of properdin and of C1 inhibitor, are often recognized in the second or third decade of life.

PID definition was established for defects leading to total absence of protein level or function. However, the occurrence of renal complications in heterozygous individuals with intermediate factor I concentrations indicate that even partial defects present with a clinical phenotype (Grumach et al., 2006). Interestingly, MBL deficiency may even be protective against mycobacterial infections and other intracellular microorganisms (Søborg et al., 2003).

Primary immunodeficiency disorders are relatively rare with a prevalence of approximately 1:2000 in the general population, with variable degrees of ascertainment in different countries (Bonilla et al., 2005; Boyle and Buckley, 2007). The rapid advances in analytical technology, including the widespread use of whole exome and whole genome sequencing, allows to identify gene defects in affected families and even in single individuals with inherited diseases faster and with more precision (Locke et al., 2014). Today, more than 200 PIDs have been characterized down to the molecular level (Chapel, 2012; Al-Herz et al., 2014). Only between the two recent IUIS reports on Al-Herz et al. (2011, 2014), over 30 new PIDs have been identified (Al-Herz et al., 2011; Al-Herz et al., 2014). However, many patients suffering from primary immunodeficiency disease still remain without correct diagnosis for many years either due to a general lack of medical aid or lack of experience of medical professionals. According to a large population-based study in the US a delay in diagnosis is common and is associated with increased morbidity and an older age at diagnosis was significantly associated with mortality (Joshi et al., 2009).

2. Complement deficiencies

In 1919 a complement defect recognized in guinea pigs was the first immunodeficiency identified (Moore, 1919). This was long before a C2 deficiency was reported as the first PID affecting the complement system in man. Since the 1960s deficiencies of literally all complement components, regulators and receptors could be characterized (Klemperer et al., 1966; Lee and Lau, 2009). As 'experiments of nature' complement deficiencies in men and animals have significantly furthered our knowledge on the role of complement in host defense and in maintaining homeostasis (Botto et al., 2009; Ricklin et al., 2010). A broad spectrum of clinical disorders is associated with complement deficiencies dependent on which complement protein and activation pathway is affected (Figueroa and Densen, 1991; Pettigrew et al., 2009).

Complement deficiencies can be primary (hereditary) or secondary (acquired). The inheritance is usually autosomal recessive (exception: properdin deficiency: X-linked; factor B, C1-INH and MCP/CD46 deficiency: autosomal dominant). Heterozygous carriers usually remain clinically silent. Hereditary defects can be identified through accurate medical history and family analysis. Complete defects are described for virtually all complement proteins with the exception of serum carboxypeptidase N (Table 1). Secondary deficiencies are caused by inflammation-induced complement consumption, auto-antibodies (e.g. against C1q or C1 inhibitor), decreased synthesis and/or increased catabolism (Agnello, 1986; Botto et al., 2009).

Complement deficiencies represent approximately 1–6% of all primary immunodeficiencies but may go up to 10% in certain national registries (Modell et al., 2011; Naidoo et al., 2011). In 2005 the newly established registry of the European Society of Primary Immunodeficiencies (ESID) comprised 0.5% complement deficiencies (of 1173 patients). Today, from 19,091 PID cases 4.9% are complement deficiencies (www.esid.org). In 2013, the Latin American Society of Primary Immunodeficiencies (LASID) reported approximately 5000 PID cases registered from 12 countries with 2% of them to be complement deficient (personal communication). Despite a higher rate of consanguinity, in recent reports from Oman, Turkey and Iran the prevalence of complement deficiencies did not exceed 6% (Al-Tamemi et al., 2012; Aghamohammadi et al., 2014; Kilic et al., 2013). With the improvement of PID analysis in general and complement diagnostics in particular higher prevalences are expected.

The most convincing demonstration of the rarity of complete complement deficiencies in the general population comes from a Japanese study in 145,640 blood donors (Fukumoru et al., 1989; Inai et al., 1989). With the exception of 139 individuals with isolated deficiency of C9, nobody else showed alterations in the hemolytic activity of the classical and alternative pathways. From this and other studies the prevalence of a congenital complement deficiency has been calculated to be about 0.03% in the general population, excluding MBL deficiency, estimated to occur in its homozygous form in about 5% of the population (Dahl et al., 2004).

The most frequent complement deficiency affects C2 which occurs in about 1:20,000 individuals. The incidence of the hereditary angioedema with C1-INH deficiency is estimated in 1:10,000 to 1:50,000. Partial C4 deficiency was reported in 1:250 individuals with C4A deficiency more frequently observed in Caucasians. However, deficiencies of complement proteins are significantly more frequent in people with specific diseases. In Caucasians with rheumatic diseases such as systemic lupus erythematosus, a C2 deficiency is detected in about 1% of the patients (Atkinson, 1989; Johnson et al., 1992). Complement deficiencies are estimated to occur in up to 20% of patients with disseminated Neisseria infections (Figueroa and Densen, 1991; Platonov et al., 1993; Rasmussen et al., 1988).

3. Warning signs for primary immunodeficiencies

Ten warning signs for primary immunodeficiencies have been first promoted by Jeffrey Modell Foundation (http://www.jmfworld.com) and American Red Cross as a screening tool for use by both the general public and physicians (http://www.info4pi.org/aboutPI). When it was designed, our knowledge about PIDs was mostly associated with the occurrence of infections. According to a recent study, except for family history, the need for intravenous antibiotics and failure to thrive, the other warning signs are not considered a useful screen of PIDs (Arkwright and Gennery, 2011; O'Sullivan and Cant, 2012; http://www.info4pi.org/aboutPI). Nowadays, it is recognized that PIDs may present with sporadic rather than recurrent infections, but also with autoimmunity, autoinflammation or malignancy (Bousfiha et al., 2010).

Late manifestations and a better survival in developed countries led to an increased number of adults affected by PIDs. Therefore, the European Society of Primary Immunodeficiencies recommended warning signs for adults: Four or more infections requiring antibiotics within one year (otitis, bronchitis, sinusites, pneumonia); recurring infections or infections requiring prolonged antibiotic therapy; two or more severe bacterial infections (osteomyelitis, meningitis, septicemia, cellulitis); two or more radiologically proven pneumonia within 3 years; infections with unusual localization or unusual pathogen and PID in the family (de Vries, 2012). Again, infections represented the main indication for immunologic investigations in this group.

The increasing list of PIDs, the diversity of clinical presentations and the need to reach non immunologists led to a recent publication re-organizing the warning signs according to the specialty. The

Table 1
Primary deficiencies of components of complement system, gene defects and symptomatology.

Deficiency	Gene localization	Cases (*n*)/frequency	Associated symptoms/disorders
C1q	1p36	>40	SLE-like, infections
C1r/s (mostly combined)	12p13	<20	
C2	6p21	1:10,000 to 1:20,000	
C4 (C4A, C4B)	6p21	<30 (homozygous)	SLE-like, RA, infections, heterozygous: often clinically inapparent
C3	19p13	<30	Pyogenic infections
		>10 (gain-of-function mutation)	aHUS
C5	9q33-34	40	Meningitis (*Neisseriae*), SLE
C6	5p13	>50	
C7	5p13	>50	
C8α–γ*/C8β	C8α/β: 1p32 C8γ: 9q34	>50 (mostly C8β)	
C9	5p14-p12	1:1000 (Japan), rare	Neisserial infections (mostly asymptomatic)
Factor B	6p21	2	Neisserial infections
		<10 (gain-of-function mutation)	aHUS,
Factor D	19p13	<10	Neisserial infections
MBL	10q11	5% (Caucasians)	Bacterial infections (mostly asymptomatic)
Ficolin 3 (H-ficolin)	1p36	<10	Respiratory infections, necrotizing enterocolitis
MASP-2	1p36	<10	Respiratory infections
C1-Inhibitor	11q11–q13	1:50,000	Hereditary angioedema
C4-binding Protein	1q32	<10	Atypical Morbus Behçet, Angioedema
Properdin	Xp11	>100	Meningitis (*Neisseriae*),
Factor H	1q32	<30	Infections, aHUS
		>100	aHUS, C3G, DDD, AMD
FHR1 (FHR3)	1q32	>100 (5% Caucasians)	aHUS, C3G; AMD, RA, SLE **
Factor I	4q25	>30	Severe infections
		>50	aHUS, C3G
Thrombomodulin (CD141)	20p11	>10 (5% of aHUS?)	Ahus
CD46/MCP	1q32	>50	aHUS, C3G
CD55/DAF***	1q32	Rare	*** Paroxysmal nocturnal hemoglobinuria hemolysis, chronic hemolysis and relapsing peripheral demyelinating disease cerebral infarction
	11p13	<10	*** Paroxysmal nocturnal hemoglobinuria
CD59***			
CR2 (CD21)	1q32	Rare	Infections, associated with CVID
CR3(CD18/CD11b)	CD18: 21q22	1:1 million	Leukocyte adhesion deficiency(LAD)
CR4(CD18/CD11c, LFA-1)	CD11b: 16p11 CD11c: 16p11		

Modified from Skattum et al. (2011), Degn et al. (2011), Pettigrew et al. (2009), Wahn and Späth (2008), Al-Herz et al. (2014), Nakashima et al. (2014). RA: rheumatoid arthritis; SLE: systemic lupus eritematosus; aHUS: atypical hemolytic uremic syndrome; C3G: C3 glomerulopathy; DDD: dense deposit disease; AMD: age related macular degeneration; CVID: common variable immunodeficiency.

* Always as C8α–γ-deficiency, as the γ-chain is covalently bound to the α-chain. The C8γ-gene is normal.

** Often associated with anti-factor H antibody (Ab) = DEAP (deficiency of CFHR proteins and CFH autoAb positive)-HUS.

*** PNH occurs due to somatic mutation of PIG-A gene coding for the enzyme *N*-acetylglucosaminyltransferase, which is needed for the formation of the glucosylphosphatidylinositol (GPI) anchor of various membrane molecules, such as CD55 and CD59.

intension was to direct specific educational programs with the clear aim of improving awareness of PIDs (Costa-Carvalho et al., 2014).

With respect to support general practitioners and clinicians in early recognition of complement deficiencies, we refrained from presenting a systemic overview. Here we refer to previous comprehensive reviews (Figueroa and Densen, 1991; Botto et al., 2009; Pettigrew et al., 2009; Degn et al., 2011; Skattum et al., 2011; Tichaczek-Goska et al., 2012; Mayilyan, 2012; Al-Herz et al., 2014). We rather suggest to follow a multi-stage diagnostic protocol starting from clinical presentation of so called warning signs which should aid pediatricians and adult physicians in a timely identification of potential complement deficiencies. Simple screening tests in the initial phase should be followed by in-depth analysis in specialized laboratories.

4. Warning signs for complement deficiencies

Based on the several reviews previously mentioned and case reports of complement deficiencies, certain clinical warning signs become obvious.

- Meningococcal meningitis >5 years of age;
- other recurrent bacterial infections, esp. *Pneumococcus*;
- autoimmune manifestations;
- angioedema without urticaria; and
- renal and ophthalmic inflammatory disorders.

4.1. Meningococcal meningitis >5 years of age

Community-acquired infections are more evident in the period after 5–6 months of age and before 2 years of age. In this period infants have decreased levels of transplacental maternal antibodies and their adaptive immunity is not well developed (Carvalho et al., 1999). The lack of an adequate specific protective response to *Pneumococcus* has been demonstrated in children (Sorensen et al., 1998). Although meningococcal infection may occur at all ages, it also has a peak incidence in children aged <2 years, and is usually due to an absence of specific protective antibodies.

Meningococci colonize the nasopharynx of 5–15% of individuals in non-edemic areas, reaching up to 50% during epidemy (Lewis and Ram, 2014). Individuals, deficient of properdin and of the terminal pathway components (C5 to C9) are highly susceptible to meningococcal disease. In patients lacking late C components C5–C8 the risk of meningococcal disease compared with the general population is 1000 to 10,000-fold increased. This reflects the importance of the cytolytic complement activity in the host defense against *Neisseriae*.

Meningitis occurs in approximately 40% of individuals with late component complement deficiencies (Ross and Densen, 1984) and

in 6% of those with properdin deficiencies (Sjöholm et al., 1988). MBL deficiency has also been associated with a higher risk for invasive meningococcal disease (Hibberd et al., 1999). Also the rare factor D deficiency had been described as predisposing to invasive meningococcal disease (Sprong et al., 2006). Factor H and Factor I deficiencies result in uncontrolled activation of the alternative complement pathway with subsequent consumption of complement. This creates a functionally complement deficient state that predisposes the affected individuals to meningococcal disease (Lewis and Ram, 2014).

Meningococcal disease due to "uncommon" serotypes (X, Y, Z, W135 or 29E) is described more frequently in complement deficient patients (Fijen et al., 1998).

Immunization against *Neisseria meningitidis* with a tetravalent conjugate vaccine is strongly recommended, although not all disease-related serotypes are recognized (Overturf, 2003) Successful immunization diminished the occurrence of meningitis due to *N. meningitidis* in complement deficient patients (Drogari-Apiranhitou et al., 2000).

Although the introduction of routine vacination against *N. meningitidis* type C increased the protection against this serotype, quadrivalent meningococcal polysaccharide–protein conjugate vaccines against meningococcal serogroups A, C, W, and Y appear superior for long term individual protection (Cohen and Levy, 2012; Cohn et al., 2013).

However, more recently the effect of meningococcal polysaccharide vaccine for long term protection was questioned, suggesting a more aggressive vaccination strategy in this patient population (Keiser and Broderick, 2012).

4.2. Other recurrent bacterial infections

Recurrent bacterial infections in infancy may be the consequence of a deficiency of literally all complement components, regulators and receptors (Table 1). Increased susceptibility to infections is the predominant clinical symptom in patients with deficiency of C3 and of control proteins factor H and factor I which lead to consumption of C3. The predominant organisms are encapsulated pyogenic bacteria, pneumococci, *H. influenzae* and streptococci reflecting the relevance of C3 as an opsonin (Lokki and Colten, 1995; Pettigrew et al., 2009).

Defects of CR3 and CR4 (CD11b,c/CD18; β2-integrins) result in severe bacterial infection. This Leukocyte Adhesion Deficiency 1 (LAD I) is a life-threatening impairment of leukocyte function and often presents first with a delayed rejection and inflammation of the umbilical cord stump of the newborn. Due to a defect of adhesion leukocytes cannot bind to the vessel wall to migrate into the tissue. The cause is the absence of the common subunit of the CD18 of CR3 (CD11b/CD18) and CR4 (CD11c/CD18). The diagnosis is based on the cytofluorometric analysis of the CD18 molecule. A stem cell therapy is required, especially in severe cases (Hanna and Etzioni, 2012).

As mentioned before, low MBL levels either augment the susceptibility to bacterial or fungal infections or may be associated with protection to intracellular microorganisms (Hibberd et al., 1999; Dahl et al., 2004). MBL deficiency should principally be considered in diseased newborns where the adaptive immunity has not yet been developed and defense depends on maternal antibodies and innate immunity (Frakking et al., 2006). Since premature neonates can have low MBL concentrations despite wild-type haplotypes, MBL deficiency at birth should be defined by decreased MBL concentrations and not by *MBL2* genotype (Thiel et al., 1995).

However, there are numerous reports indicating that an impairment of the lectin pathway may be associated with increased risk, severity, and frequency of infections and autoimmunity (Larsen et al., 2004). Vallès et al. (2010) suggest that the susceptibility to pneumococcal disease among MBL-deficient patients may be influenced by serotype invasiveness. The type-specific capsular serotypes of *Streptococcus pneumoniae* need to be taken into account in further genetic association studies of invasive pneumococcal disease.

There is no consensus on the definition of MBL deficiency, as due to a high rate of haplotype variations between different ethnic groups and within these groups MBL concentrations vary considerably (Ivanova et al., 2008). Therefore, studies in adults usually define MBL deficiency according to the *MBL2* genotype (Thiel et al., 1995).

MASP2 and ficolin deficiencies have also been related to susceptibility to infections but the impact of these defects has to be defined (Stengaard-Pedersen et al., 2003; Thiel et al., 2007).

4.3. Autoimmune disorders

Rheumatic disorders are another manifestation of complement deficiencies. Besides diseases resembling systemic lupus erythematosus (SLE), cutaneous lupus, dermatomyositis, Henoch–Schonlein purpura, membranoproliferative glomerulonephritis and vasculitis have been reported (Pettigrew et al., 2009). The association of genetic deficiency of any early component of the classical pathway (C1q, C1r/s, C2, C4) oro f MBL with SLE-like disorders has been explained by the failure of clearance of immune complexes and apoptotic materials and impairment of normal humoral response (Agnello, 1986; Elkon et al., 2012).

The occurrence of autoimmunity associated with a deficiency of classical components of complement system is variable. C1q deficiency is associated with severe autoimmune disease in 95% of the cases whereas less than 40% complete C2 deficient patients develop autoimmunity (Pickering and Walport, 2000). SLE-like diseases associated with complement deficiency differ clinically from those in complement competent patients. In complement deficient SLE patients, the onset is at a younger age with less renal, pulmonary or pericardial involvement, but more prominent annular photosensitive skin rashes (Pettigrew et al., 2009). Remarkable in this connection is the observation that even a deficiency of C1 inhibitor, resulting in an activation of the classical activation pathway, increases the probability to develop SLE, probably due to a subsequent acquired C4 and C2 deficiency (Sturfelt, 2002).

Decreased CR1 expression (complement receptor 1, CD35) in erythrocytes and other cell types have been described in patients with SLE, and stimulated an extensive investigation of the role of CR1 regarding the pathogenic events in immunecomplex disease. The reduction of CR1 was also found in other diseases, including reactive arthritis due to *Yersinia enterocolitica* (Lahesmaa et al., 1992). SLE patients also have decreased expression of CR2 on B lymphocytes but deficiencies may be acquired through disease-related mechanisms (Kavai, 2008), whereas the only described CD21 deficiency was associated with hypogammaglobulinemia and CVID (Thiel et al., 2012).

4.4. Angioedema without urticaria

The occurrence of angioedema without urticaria is estimated as 2% of all the clinical cases of angioedema and/or urticaria. It can be classified as histaminergic or non-histaminergic, the latter being related to bradykinin accumulation (Walford and Zuraw, 2014). Hereditary angioedema (HAE) due to C1 inhibitor deficiency has an estimated prevalence of 1 to 50,000 individuals. HAE, also known as Quincke's edema, is caused by acute, paroxysmal swelling of the lips and eyelids and of the gastrointestinal tract (colic). If localized in the larynx and pharynx the edema is particularly dangerous. Other organs can be affected causing intestinal obstruction, urinary retention and headache. Identified triggering factors are stress, trauma,

infections and hormonal variations (menses, pregnancy, contraceptives, hormonal reposition). Prodromal symptoms can precede the attacks: erythema serpiginosus, irritability and anxiety among others. The attacks last 3–7 days and if not treated can lead to asphyxia in 25–40% of the cases (Cicardi et al., 2014).

Acquired angioedema develops upon hypercatabolism of C1-INH or as a consequence of blocking autoantibodies (Cicardi and Zanichelli, 2010).

HAE with a quantitative defect of C1-INH (previously type I—85% of cases) can be identified by decreased plasma levels of C1-INH as well as of C4. The synthesis of a dysfunctional inhibitor (previously called type II) leads to HAE (about 15%), in which the C1-INH plasma levels are often normal, but the function is significantly reduced. This laboratory diagnostic constellation is also found in the much rarer acquired form of autoimmune angioedema, where also C1q is reduced (Cicardi et al., 2014).

The therapy of this angioedema is fundamentally different from that of allergic edema or even urticaria through the lack or an only poor efficacy of corticosteroids and antihistamines. A prophylactic treatment with androgens (danazol, stanazolol) leading to increased C1-INH synthesis or with plasmin inhibitors such as tranexamic acid has been successfully applied, especially where C1-INH was not available. Nowadays, for acute attacks, C1-INH substitution (plasma derived C1-INH as Berinert® and Cinryze® and recombinant C1-INH as Rhucin®) is available and new drugs were introduced with specific activity in the kinin–kalikrein system as outlined in detail in comprehensive reviews (Lang et al., 2012; Zuraw et al., 2012; Craig et al., 2012; Cicardi et al., 2014).

In 2000, Bork et al. described a large group of patients with similar clinical manifestations to HAE but without C1-INH defect. Here, sporadically so-called gain-of-function mutations in the FXII gene of the clotting system were found, which also ultimately lead to the increase of angioedema-inducing bradykinin. In these cases of HAE without C1-INH deficiency, clinical manifestations, normal C4 and C1-INH and familial history could be criteria for the diagnosis (Zuraw et al., 2012).

4.5. Renal and ophthalmic disorders due to complement dysregulation—A new class of complement deficiencies?

Although the inheritance of the following deficiencies has not been proven in general yet, mutations of genes associated with the regulation of the alternative pathway (de Cordoba et al., 2012) appear to be noteworthy to be included in a review on complement deficiency disorders (Maródi and Casanova, 2009).

4.6. Hemolytic uremic syndrome

The hemolytic uremic syndrome (HUS) is characterized by diarrhea-associated kidney disease associated with the toxin from bacteria, usually *Escherichia coli* O157:H7. In children, about 90% of HUS is mediated by Shiga toxin and is traditionally referred to as typical diarrhea positive (D+) HUS. The remaining ~10% of cases are classified as atypical HUS (aHUS), which has a poorer prognosis compared with shiga toxin HUS (Joseph and Gattineni, 2013). Within the group of thrombotic microangiopathies the atypical hemolytic uremic syndrome (aHUS, D(−) HUS) is characterized by the triad of microangiopathic hemolytic anemia, thrombocytopenia and acute renal failure in the absence of an *ADAMTS13* defect (Noris and Remuzzi, 2009).

Variable age of presentation and the presence of triggering factors (~80%) in patients with aHUS indicate that genetic mutations provide a predisposition to develop aHUS (Joseph and Gattineni, 2013). Atypical HUS may develop in patients of any age, though 70% of pediatric diseases have an onset before two years and 25% have an onset before the age of 6 months (Loirat and Fremeaux-Bacchi, 2011), Several genetic mutations in complement regulatory proteins have been identified in patients with both familial and sporadic aHUS. Mutations in complement factor H (CFH) (20–30% of the cases), complement factor I (CFI) (5–10% of the cases), complement factor B (CFB) (1–4%), MCP/CD46 (10–15%), thrombomodulin (3–5%) and C3 (2–10%) comprise about 50% of known mutations in patients with aHUS (Loirat and Fremeaux-Bacchi, 2011). These gene defects result in defective alternative pathway regulation, favoring complement mediated cell injury and kidney damage. In vivo, the alternative complement pathway is continuously activated by the spontaneous hydrolysis of C3 to C3 (H_2O). Under physiologic conditions, with factor H and fH related proteins as cofactors C3b is cleaved by factor I (C3b inactivator) to iC3b. In case of mutation in CFH family proteins, excessive accumulation of C3b and inflammatory cells on the glomerular endothelium, lead to detriment of cells, loss of endothelial integrity, activation of complement pathways, and development of thrombotic microangiopathy (Maródi and Casanova, 2009; Skerka et al., 2013).

The majority of mutations in aHUS are heterozygous and familial occurrence is reported in up to 20% of the patients. aHUS can be inherited in an autosomal dominant or recessive fashion. Despite the advances in the field, about 30–40% of patients with aHUS do not have an identifiable abnormality in the complement system (Joseph and Gattineni, 2013). In aHUS, occasionally also in the more common infectious form of this disease (D (+) HUS, EHEC-HUS), total complement function (e.g. CH50, AH50), and C3 levels are reduced, going along with increased plasma concentrations of the complement activation products C3a/C3d, Bb, and SC5b-9. Besides analysis of autoantibodies against factor H, the simultaneous molecular genetic analysis of the complement regulators fH, fI, CD46/MCP, of the components C3 and fB and of thrombomodulin is essential for differentiation from other forms of microangiopathic thrombocytopenia (e.g. TTP). Eculizumab has been proven to be first-line therapy for the treatment of aHUS (Christmann et al., 2014).

4.7. C3 glomerulopathy and dense deposit disease

Occasionally, mutations of alternative pathway regulators are also found in patients suffering from C3 glomerulopathies, esp. in MPGN (de Cordoba et al., 2012). Particularly in the histologically defined MPGN type 2 (DDD, dense deposit disease) a dysregulation of the complement C3 convertase through the autoantibody C3 nephritic factor needs to be assessed. First reports indicate an improvement of the disease upon eculizumab treatment. Despite the finding that with an improvement of clinical symptoms the terminal sequence of the cascade is completely blocked (CH50 undetectable, SC5b-9 levels low), C3 activation is often seen to continue (C3 reduction, elevation of C3a/C3d), since a possibly ongoing activation of the initiation phase of all pathways, esp. in the presence of C3 nephritic factor, is not affected by the drug (Skerka et al., 2013; Appel, 2013).

4.8. Age-related macular degeneration (AMD)

Age-related macular degeneration, a genetically complex, multifactorial disease of the retina and adjacent structures is considered the leading cause of blindness in old age in many countries. The formation of so-called drusen leads to a pronounced central visual loss. Replacement of thymine (T) by a cytosine (C) in the FH gene (Y402H polymorphism) poses a significantly increased risk of disease to the affected individual (Fritsche et al., 2014; Calippe et al., 2014). Only occasionally AMD is associated with alterations in routine complement analysis (Scholl et al., 2008) but increased levels of complement activation products in vitreous fluid speak

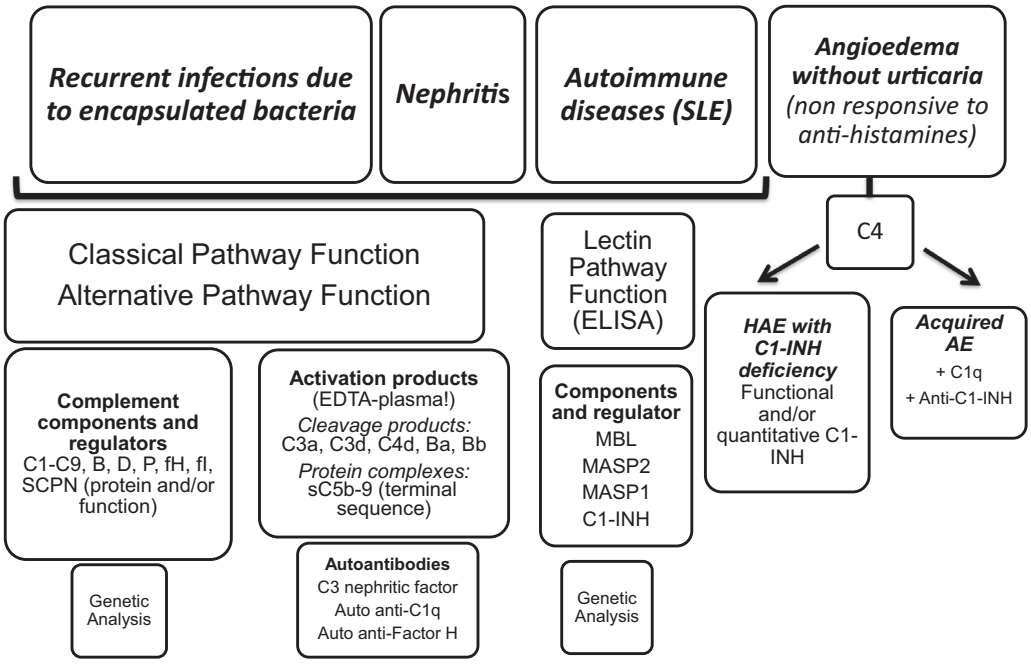

Fig. 1. Laboratorial complement evaluation according to warning signs for complement deficiencies.

in favor for a local inflammatory process (Fauser, Kirschfink et al., manuscript in preparation).

5. Diagnostics of complement deficiencies

The diagnostic approach leading to the identification of a complement deficiency involves a multistep process that starts with functional screening of each activation pathway and proceeds in specialized laboratories with the characterization of the defect at functional, protein and molecular level (Fig. 1) (Mollnes et al., 2007). A modern complement analysis goes far beyond the traditional parameters C3 and C4, which allow only in a few cases a statement about a deficiency, a functional disorder or the activation state of this complex system. Only in a few specialized laboratories a comprehensive diagnostics is provided (www.IUIS.org).

It is absolutely essential that blood samples are processed as quickly as possible to serum and EDTA plasma. If the analysis is not immediately performed, samples need to be frozen until assayed or shipped on dry ice to a specialized laboratory by courier. Repeated freezing and thawing should be avoided because of the risk of in vitro activation. While serum is sufficient for the analysis of the total function, of complement proteins and regulators as well as of autoantibodies or even mandatory (e.g. functional test for the C3 nephritic factor), a quantitation of activation products requires the use of EDTA plasma. EDTA at $\geq$10 mM final concentration is used as standard anticoagulant since it blocks the in-vitro activation of the complement system by way of its Mg^{2+} and Ca^{2+} complexing properties. Heparin and citrate are less useful.

In recent years, great progress has been made in complement analysis to better define disease severity, evolution and response to therapy (Tudoran and Kirschfink, 2012). Every complement diagnosis should first determine the total activity of the classical and alternative pathway either by functional ELISA or by hemolytic or liposome-based assays. These global tests provide information about the integrity of the entire complement cascade. A missing or greatly reduced activity indicates a primary complement deficiency, but may also be due to a secondary deficiency caused by increased consumption. The analysis of individual components (or regulators) indicating, in which portion of the complement cascade (over-) activation occurs, must follow in such cases. For rapid deficiency analysis, an ELISA has been developed that examines all three activation pathways in parallel (Seelen et al., 2005).

Modern diagnostic technologies focus on the quantification of complement-derived split products or protein–protein complexes thereby providing a comprehensive insight into the activation state of the system. By choosing the appropriate parameter, it is possible to determine exactly, which pathway is activated.

Recently, the soluble activation product of the terminal complement cascade, SC5b-9, has received more attention as unrestricted progression to its final steps was linked to specific pathology as in atypical hemolytic uremic syndrome (aHUS), (Noris and Remuzzi, 2009) and the efficacy of the recently introduced C5 antibody eculizumab in treating aHUS patients is reflected by SC5b-9 suppression (Legendre et al., 2013; Wehling, Kirschfink et al., manuscript in preparation). Thus, like C3a or C3d, SC5b-9 can also be utilized as global marker of complement activation and appears to be useful in monitoring patients under (eculizumab) therapy. An algorithm of complement analysis is shown in Fig. 1.

6. Conclusion

The idea to establish warning signs for early recognition of complement deficiencies is part of a program to attract attention to these rare diseases and guide the clinician to their diagnosis. Increasing awareness of the clinical relevance of complement deficiency disorders have led to the development of new drugs to restrict severe inflammation such as in HAE, paroxysmal nocturnal hemoglobinuria (PNH) and renal disorders. With the better availability of modern complement analysis the identification of many more complement deficiencies in the near future is anticipated, especially of those with a less overt clinical phenotype. This implies that diagnostic algorithms need to be revised from time to time. It appears that complement deficiency disorders – albeit with varying severity – are more common than we think at present.

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