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Review

Complement deficiencies and dysregulation: Pathophysiological consequences, modern analysis, and clinical management

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ABSTRACT

Complement defects are associated with an enhanced risk of a broad spectrum of infectious as well as systemic or local inflammatory and thrombotic disorders. Inherited complement deficiencies have been described for virtually all complement components but can be mimicked by autoantibodies, interfering with the activity of specific complement components, convertases or regulators. While being rare, diseases related to complement deficiencies are often severe with a frequent but not exclusive manifestation during childhood. Whereas defects of early components of the classical pathway significantly increase the risk of autoimmune disorders, lack of components of the terminal pathway as well as of properdin are associated with an enhanced susceptibility to meningococcal infections. The impaired synthesis or function of C1 inhibitor results in the development of hereditary angioedema (HAE). Furthermore, complement dysregulation causes renal disorders such as atypical hemolytic uremic syndrome (aHUS) or C3 glomerulopathy (C3G) but also age-related macular degeneration (AMD). While paroxysmal nocturnal hemoglobinuria (PNH) results from the combined deficiency of the regulatory complement proteins CD55 and CD59, which is caused by somatic mutation of a common membrane anchor, isolated CD55 or CD59 deficiency is associated with the CHAPLE syndrome and polyneuropathy, respectively. Here, we provide an overview on clinical disorders related to complement deficiencies or dysregulation and describe diagnostic strategies required for their comprehensive molecular characterization – a prerequisite for informed decisions on the therapeutic management of these disorders.

1. Introduction

The complement system is a highly conserved part of the innate immune system (Merle et al., 2015). More than 50 soluble and membrane-bound proteins are involved in a complex mode of activation, serve as regulators or receptors (Ricklin et al., 2010). Upon activation complement significantly contributes to immune surveillance and homeostasis. Complement-mediated opsonisation, as well as the recruitment and activation of inflammatory cells leads to the cytotoxic destruction of microbial pathogens. Complement bridges the innate and adaptive immunity by augmenting the antibody response and supporting the immunological memory. Disposal of waste is mediated through effective clearance of apoptotic cells, cell debris, and immune complexes (Flierman and Daha, 2007). Furthermore, complement has been associated with early embryonic development and tissue repair (Mastellos et al., 2013; Stephan et al., 2012). Multiple interactions exist between the coagulation, fibrinolytic and complement systems where enzymes can cleave and activate one another (Foley, 2016; Oikonomopoulou et al., 2012). This provides a good explanation why many complement-driven diseases (e.g. PNH, aHUS, CHAPLE syndrome) express thrombosis as a hallmark of clinical manifestation (Baines and Brodsky, 2017).

Complement is activated via three distinct enzymatic pathways, the classical, alternative and lectin pathways (Merle et al., 2015; Ricklin et al., 2010). Each of these converge towards the cleavage of the central component C3, followed by the formation of a C5 convertase, which initiates the formation of the lytic membrane attack complex (MAC; terminal complement complex (TCC; C5b-9n) that destroys or damages targeted cells.

The proinflammatory anaphylatoxins C3a and C5a, released upon the activation of C3 and C5, act as potent chemotactic fragments, recruiting immune cells to the site of activation and prime them. Neutrophils and macrophages recognize C3-derived opsonins (C3b, iC3b) on the tagged particles by complement receptors (CR) 1 (CD35) and 3 (CD11b/CD18) and mediate their effective phagocytic removal.

Multiple soluble and membrane-bound regulatory proteins are required that act to prevent complement-mediated damage to the host (Zipfel and Skerka, 2009).

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A broad spectrum of clinical disorders is associated either with complement deficiencies or – even more prevalent – with an over-activated and / or dysregulated complement system (Hajishengallis et al., 2017; Ricklin et al., 2017; Thurman and Holers, 2006).

In this review, we wished to address clinical disorders associated with the various forms of complement abnormalities going beyond classical complement protein deficiencies, by including also mutations leading to loss- or gain-of-function of complement proteins but also clinical relevant autoantibodies mimicking primary defects by their stabilizing or blocking properties.

2. Complement deficiencies

Complement deficiencies can be either primary (hereditary) or acquired (Figueroa and Densen, 1991; Pettigrew et al., 2009; Grumach and Kirschfink, 2014). The mode of inheritance is usually autosomal recessive (exception: properdin deficiency: X-linked) where heterozygous carriers usually remain clinically silent. They need to be identified through accurate medical history and extended laboratory analysis of the entire family (Botto et al., 2009).

Complete defects are described for virtually all complement proteins with the exception of serum carboxypeptidase N (Table 1). Secondary complement deficiencies are most often the consequence of inflammation-induced consumption, functionally active autoantibodies (e.g. against C1q, C1-INH or factor H (FH)), decreased synthesis and/or increased catabolism or protein loss syndromes.

Complement deficiencies represent approximately 5% of all primary immunodeficiencies (PID) – as revealed in the latest evaluation of the European Society of Immunodeficiencies (ESID) registry - but may go up to significantly higher numbers, as demonstrated in recent studies (Blazina et al., 2018; Grumach and Kirschfink, 2014). This clearly indicates that with an increased awareness of clinicians and a more precise testing more complement deficiencies can be identified (certainly applicable to all PIDs).

The prevalence of a congenital complement deficiency has been calculated to be about 0.03%, excluding mannose-binding lectin (MBL) deficiency, which is estimated to occur in about 5% of the Caucasian population. The most frequent complement deficiencies affect C2 and MBL, which often remain clinically silent. The incidence of the hereditary angioedema (HAE, Quincke edema), associated with C1-INH deficiency (HAE-C1-INH), is estimated to be 1:10,000 to 1:50,000.

However, deficiencies of complement proteins are significantly more frequent in individuals with specific diseases. In systemic lupus erythematosus (SLE), 30% of the patients have a preexisting complement deficiency and up to 20% of individuals suffering from disseminated *Neisseria* infections lack any of the late complement components or properdin. Phenotypes of complement deficiencies range from absence of clinical symptoms to severe, partly life-thr9eatening infectious and autoimmune disorders (de Cordoba, 2016). Table 1 outlines known complement deficiencies and associated clinical symptoms/disorders.

Except for HAE, replacement therapy in complement deficiencies, as described for MBL, C2 or FH (Skattum et al., 2011), has never reached clinical routine, in part due to rapid metabolism of complement proteins.

2.1. Complement deficiencies associated with increased susceptibility to infections

Complement is critically involved in host defense against all types of pathogens (bacteria, viruses, parasites, and fungi) and even non-classical host defense mechanisms have been identified. For example, C4 was recently shown to inhibit adenoviral infections by inactivating viral capsids independent of any downstream complement components (Bottermann et al., 2019). Importantly, essential intracellular immune modulatory functions of the complement system have recently been discovered promoting the survival and activation of T lymphocytes (Kolev and Kemper, 2017; West et al., 2018). The critical role of the complement system for host defense is further demonstrated by the multiple complement evasion strategies adopted by pathogens (Laabei and Ermert, 2019; Lambris et al., 2008; Okroj and Potempa, 2018). Accordingly, deficiencies of almost every component of the complement system are frequently associated with an enhanced susceptibility to infections. As estimated in a recent analysis based on data of the ESID registry, about 65% of complement deficient patients suffered from -often recurrent- severe invasive infections predominantly caused by encapsulated bacteria (Turley et al., 2015). In contrast, an increased frequency of viral, fungal or parasitic infections has rarely been reported, which is likely due to a compensation of the complement defect by other effective immune defense mechanisms.

A large part of infections in complement deficient patients is caused by *Neisseria meningitidis* and *Streptococcus pneumoniae*.

2.1.1. Meningococcal disease

N. meningitidis colonizes the nasopharyngeal mucosal surfaces of 5–15 % of healthy young adults and adolescents with higher numbers of carriers being detected during epidemic outbreaks (Lewis and Ram, 2014). The risk of developing a manifest meningococcal disease such as septicemia and meningitis depends on the virulence of the strain as well as the immune competence of the individual. The highest incidence of meningococcal disease is observed below the age of two years (mostly associated with antibody deficiencies), with a second smaller peak emerging at the age of 15–25 years (mostly associated with complement deficiencies) (Lewis and Ram, 2014).

A strong association with meningococcal infections - in particular those caused by rare serotypes such as X, Y, Z, W135, E29 (Fijen et al., 1989) - has been observed for deficiencies of components of the terminal complement complex (TCC), i.e. C5-C9, of C3 as well as of components of the alternative way (properdin, factor D (FD), factor B (FB), FH, factor I (FI)) (Ram et al., 2010). In contrast to deficiencies of the alternative pathway and C3, which are also linked to other invasive bacterial diseases, deficiencies of the TCC are almost exclusively associated with neisserial infections (Ram et al., 2010). In comparison to the general population, patients lacking any one of the TCC components bear a 1,000–10,000 fold higher risk of acquiring neisserial infections (Ross and Densen, 1984) with recurrent infections occurring in about 40% of the patients (Lewis and Ram, 2014). Notably, a secondary C5 deficiency due to treatment with the anti-C5 antibody eculizumab also enhances the risk of severe meningococcal infections, which is significantly reduced by vaccination against these pathogens (see below). The latter findings underline the importance of TCC-mediated bacteriolysis for successful elimination of neisserial pathogens.

Meningococcal infections also represent a prominent clinical feature of properdin deficiency. This deficiency has been categorized into three phenotypes: type I is characterized by complete absence of circulating properdin due to lack of synthesis or defective secretion while type II is defined by low circulating properdin levels (< 10% of the normal range) caused by increased extracellular degradation of abnormally oligomerized properdin molecules; type III deficiency includes patients presenting with normal plasma levels but a reduced function of properdin (Chen et al., 2018a,b). While meningococcal infections in TCC-deficient patients often show a mild disease course – potentially due to reduced release of LPS as a consequence of diminished bacterial lysis (Figueroa and Densen, 1991; Lewis and Ram, 2014) – properdin-deficient patients frequently suffer from fulminant disseminated infections with a mortality rate of up to 65%. However, here recurrent infections are rare (Hellenbrand et al., 2015).

FD or FB deficiencies are extremely rare and are in general associated with neisserial infections. Hiemstra et al. (1989) described a FD-deficient adult patient with a history of *N. meningitidis* infections following one episode of disseminated gonococcal infection. A familial case was recently reported, where members of the family had normal

Table 1
Complement deficiencies.

Deficiency	Gene localisation	Results of complement analysis[2]		Associated Symptoms/Disorders[3]	OMIM database
Components					
C1q	1p36	CH50 C1 C1q	↓	SLE-like (> 90%, mostly clinically severe), infections	613652
C1r/s (mostly combined)	12p13	CH50 C1 C1r	↓	SLE-like, RA, infections	216950
C4 (C4A, C4B)	6p21	CH50 C4	↓	SLE-like, RA, infections, homozygous: severe symptoms; heterozygous: often clinically inapparent	614380 614379
C2	6p21	CH50 C2	↓	SLE-like, RA, infections (pneumonia), vasculitis, often clinically inapparent	217000
C3	19p13	CH50, AH50 C3	↓	Pyogenic infections	613779
C5	9q33-34	CH50, AH50 C5	↓	Meningitis (Neisseriae), SLE	609536
C6	5p13	CH50, AH50 C6	↓	Meningitis (Neisseriae), SLE	612446
C7	5p13	CH50, AH50 C7	↓	Meningitis (Neisseriae), SLE	610102
C8α–γ*/C8β	C8α/β: 1p32 C8γ: 9q34	CH50, AH50 C8	↓	Meningitis (Neisseriae), SLE	613790 613789
C9	5p14-p12	CH50, AH50 C9	↓	Neisserial infections (mostly asymptomatic)	613825
Factor B	6p21	AH50 FB	↓	Neisserial infections	615561
Factor D	19p13	AH50 FD	↓	Neisserial infections	134350
MBL	10q11	LP function MBL	↓	Bacterial infections (mostly asymptomatic)	614372
Ficolin 3 (H-Ficolin)	1p36	Ficolin	↓	Respiratory infections, necrotizing enterocolitis	613860
MASP-2	1p36	LP function MASP-2	↓	Respiratory infections	613791
MASP-3, CL-K1, CL-L1	1p36, 2q25, 3q27	MASP-3 CL-K1, CL-L1	↓	3MC syndrome	257920, 265050, 248340
Regulators					
C1-Inhibitor	11q11–q13	C4 C1-INH	↓	Hereditary angioedema	106100
C4-binding Protein	1q32	C4BP	↓		120830 120831
Properdin	Xp11	(AH50) Properdin	↓	Meningitis (Neisseria),	312060
Factor H	1q32	Homozygous: CH50, AH50 C3 FH	↓	Infections, aHUS /C3G	609814
		Heterozygous: CH50, AH50 n/C3 n/	↓	aHUS, C3G/MPGN	
FHR1 (FHR3)	1q32 (1q32)	CH50, AH50 n C3 n FH n		aHUS, RA, SLE often associated with factor H autoantibodies = > DEAP(deficiency of CFHR proteins and CFH autoantibody positive)-HUS	134371
Factor I	4q25	CH50, AH50 C3	↓	Infections (sepsis, meningitis, pneumonia) aHUS	610984
CD46/MCP	1q32	CH50, AH50 n CD46	↓	aHUS	120920
CD55/DAF	1q32	CH50, AH50 n CD55	↓	CHAPLE syndrome: Severe enteropathy Angiopathic thrombosis Complement hyperactivation	226300 125240
	PIG-A: X			Paroxysmale nocturnal hemoglobinuria (somatic mutation of PIG-A Gene**)	300818
CD59	11p13 PIG-A: X	CH50, AH50 n CD59	↓	GBS-like symptoms, hemolysis Paroxysmale nocturnal hemoglobinuria (somatic mutation of *PIGA* Gene**)	612300 107271 300818
Receptors					
CR3 (CD18/CD11b)	CD18: 21q22 CD11b: 16p11	CD18/CD11b	↓	Leukocyte adhesion deficiency I (LAD I)	116920
CR4 (CD18/CD11c, LFA-1)	CD11c: 16p11	CD18/CD11c			600065

[1]modified from Skattum et al., 2011; Degn et al. 2011, Grumach and Kirschfink, 2014.
[2] n: normal.
[3] SLE: systemic lupus erythematosus; RA: rheumatoid arthritis; 3MC: Malpuech–Michels–Mingarelli–Carnevale syndrome; aHUS: atypical hemolytic uremic syndrome; C3G: C3 glomerulopathy; MPGN: membranoproliferative glomerulonephritis; AMD: age-related macular degeneration; GBS: Guillian-Barré syndrome.

levels of FD with decreased functionality due to a missense mutation (Sng et al., 2018). Surprisingly, only in one case of a 32-year-old woman with recurrent meningococcal and pneumococcal infections a FB deficiency could be identified (Slade et al., 2013).

Complete deficiencies of FH and FI, which are crucial regulators of the alternative pathway, induce a secondary C3 deficiency due to increased complement consumption, which can also be associated with meningococcal infections (Reis et al., 2006; Figueroa and Densen, 1991; Lewis and Ram, 2014).

Finally, low MBL levels have been linked to meningococcal disease, particularly in children during the first 2 years of life (Faber et al., 2007; Hibberd et al., 1999).

Immunization with a tetravalent conjugate vaccine covering serogroups A, C, W-135, Y and a monovalent protein-based vaccine covering the most prevalent serogroup B is highly recommended in patients at risk of invasive meningococcal disease due to complement deficiency (Brady et al., 2016; Hellenbrand et al., 2015); https://www.cdc.gov/vaccines/vpd/mening/hcp/who-vaccinate-hcp.html). The immunization with a tetravalent vaccine has been shown to significantly reduce the risk of recurrent *N. meningitidis*-induced infections in TCC-deficient patients (Lewis and Ram, 2014; Platonov et al., 2003). However, re-infections with meningococci of serogroups targeted by the vaccine still occur in some immunized patients (Fijen et al., 1998; Platonov et al., 2003) suggesting an incomplete protection in complement-deficient patients. In line with this finding, a reduced immunogenicity of the meningococcal B vaccine was noted in complement-deficient children when compared to healthy controls (Martinon-Torres et al., 2018). Furthermore, there are reports on meningococcal

infections in Eculizumab-treated patients despite prior vaccination against the respective serotypes (Lebel et al., 2018; Reher et al., 2018; Struijk et al., 2013). It has been suggested that vaccination failures may be related to a more rapid decline of immunization-induced meningococcal antibody levels in complement deficient vs. normal individuals (Andreoni et al., 1993). Moreover, opsonophagocytic activity - which is regarded as the main immunization-mediated protection mechanism against meningococci in susceptible complement-deficient patients - is inhibited by Eculizumab in blood from vaccinated individuals (Konar and Granoff, 2017). Given these findings, complement deficient patients at risk of meningococcal infections should be closely monitored and instructed to immediately seek medical help when noticing signs of infection.

A pentavalent vaccine aiming to provide protection also against serogroup X is currently being evaluated in clinical trials (Chen et al., 2018a,b).

2.1.2. Recurrent pneumococcal and other infections

While deficiencies of components of the TCC as well as the alternative pathway such as properdin and FD are predominantly associated with an enhanced risk of invasive meningococcal infections (Bousfiha et al., 2018), deficiencies of C3 as well as the alternative pathway regulators FH and FI also predispose to infections with other encapsulated bacteria such as *Streptococcus pneumoniae*, *Hemophilus influenza* or *Staphylococcus aureus* (Reis et al., 2006; Ram et al., 2010). These infections develop early in life and have a tendency to recur (Figueroa and Densen, 1991). For unknown reasons, FI-deficient patients suffer from more severe and more frequent infections than FH-deficient patients (Ram et al., 2010).

Invasive pneumococcal and other infections by encapsulated bacteria are also frequently observed in patients suffering from homozygous defects of components of the classical pathway, i.e. C1, C2, and C4 (Liesmaa et al., 2018; Lipsker and Hauptmann, 2010; Truedsson, 2015). Whereas complete deficiencies of C1 and C4 are rare, C2 deficiency occurs with a prevalence of 1:20,000 in Caucasians and hence represents the most common deficiency of the classical pathway as well the second most common of all complement deficiencies (Kirschfink and Mollnes, 2003; Truedsson, 2015). Patients suffering from partial C4 defects due to homozygous C4B isotype deficiency have also been suggested to be prone to bacterial infections (Bishof et al., 1990); however, this finding remains controversial (Cates et al., 1992; Liesmaa et al., 2018). According to a recent study, 75% of all infections associated with deficiencies of the classical pathway are caused by *S. pneumoniae* indicating the importance of opsonization as a host defense mechanism against these microorganisms (Turley et al., 2015). In contrast, meningococcal infections are rarely observed in patients suffering from deficiencies of the classical pathway (Turley et al., 2015).

Immunization against *S. pneumoniae* is highly recommended in complement deficient patients (Rubin et al., 2014).

Defects of the lectin pathway have also been linked to recurrent infections not only by encapsulated bacteria but also by viral, fungal and protozoan microorganisms (Beltrame et al., 2015; Heitzeneder et al., 2012; Ram et al., 2010). Deficiency of ficolin-3, the most abundant pattern recognition receptor of the lectin pathway with a high lectin pathway-inducing activity, was associated with recurrent infections predominantly by encapsulated bacteria in some patients (Hummelshoj et al., 2008; Michalski et al., 2015; Munthe-Fog et al., 2009). Furthermore, recurrent pneumococcal infections were observed in a patient with complete MBL-associated serine protease 2 (MASP-2) deficiency (Stengaard-Pedersen et al., 2003); however, the clinical penetrance of the latter defect seems to be low as other MASP-2 deficient patients with no signs of enhanced susceptibility to infections were identified (Garcia-Laorden et al., 2006, 2008; Skattum et al., 2011).

Genetic studies further revealed an association of polymorphisms of the genes encoding ficolin 2 (*FCN2*), ficolin 3 (*FCN3*) as well as MASP-2 (*MASP2)* with enhanced susceptibility to leprosy, Chagas disease (*FCN2*) as well as viral infections such as hepatitis C (*MASP2*) (Andrade et al., 2017; Beltrame et al., 2015; Boldt et al., 2013a, b; Luz et al., 2013; Tulio et al., 2011; Zhang et al., 2013).

Homozygous MBL deficiency is observed in about 5% of the Caucasian population (Dahl et al., 2004). In contrast to ficolin-3 and MASP-2 deficiency, MBL deficiency has not been classified as a primary immune defect by the International Union of Immunological Societies (IUIS) (Bousfiha et al., 2018; Picard et al., 2018) as its clinical significance remains controversial. A large population-based study by Dahl et al. (2004) failed to observe an increased susceptibility to infections in homozygous carriers of an MBL-deficient genotype vs. non-carriers. However, an association between *MBL2* gene polymorphisms resulting in MBL deficiency and an enhanced risk for disease progression of specific bacterial, viral or parasitic diseases was reported in other studies (Ram et al., 2010). One factor predisposing to infections in individuals with an *MBL2*-deficient genotype seems to be a very low MBL concentration (< 75 ng/ml) (Heitzeneder et al., 2012; Holdaway et al., 2016). Furthermore, it has been proposed that MBL defects become clinically relevant particularly when present in combination with other immunocompromising factors. MBL deficiency, as defined by low MBL levels or activity, in neonates and infants, whose adaptive immune system has not been fully developed yet, has been linked to an enhanced risk of developing severe bacterial respiratory tract and meningococcal infections as well as an increased risk of death upon pneumococcal sepsis (Eisen et al., 2008; Faber et al., 2007; Frakking et al., 2007; Heitzeneder et al., 2012). Moreover, in a recent study investigating low birthweight infants, the genotype (0/0) - mostly associated with very low MBL levels - was linked to an increased frequency of gram-negative sepsis in children born during the 32th–36th week of gestation as well as to urinary tract infections and vesicular stomatitis during the first 24 months of life (Hartz et al., 2017). Whether MBL deficiency contributes to the risk of infections in patients under chemotherapy-induced immunosuppression remains controversial (Heitzeneder et al., 2012). There is also recent evidence that MBL deficiency increases the risk of infections in patients suffering from rheumatoid arthritis and receiving disease-modifying antirheumatic drugs (Carroll et al., 2017) further suggesting a contribution of MBL deficiency to infection under conditions of an altered immune system.

In contrast, MBL deficiency may also exert protective effects in some infectious conditions, such as tuberculosis (reviewed in Ram et al., 2010). A high frequency of MBL variants associated with low serum MBL concentrations has been observed in populations with a high prevalence of tuberculosis suggesting that MBL deficiency may be protective. In line with this assumption, a MBL-deficient genotype was found to be associated with protection against *Mycobacterium africanum*-induced tuberculosis, potentially by reducing the MBL-mediated uptake of these intracellular pathogens by macrophages (Thye et al., 2011). Furthermore, in a cohort of patients suffering from chronic obstructive pulmonary disease (COPD), MBL-deficient individuals showed a higher microbial diversity and reduced colonization with *H. influenzae*, an important COPD pathogen, in the airways. This was associated with reduced inflammation of the airways and less severe exacerbations of the disease (Dicker et al., 2018).

2.2. Complement deficiencies associated with autoimmune disorders

Autoimmune manifestations are frequently observed in patients deficient in early components of the classical pathway often presenting with SLE, dermatomyositis, Henoch-Schönlein purpura, juvenile rheumatoid arthritis and glomerulonephritis (Ballanti et al., 2013; Conigliaro et al., 2019; Vignesh et al., 2017). SLE is the prototype of immune complex (IC) diseases with disease activity varying over time. Autoantibodies, esp. so-called antinuclear antibodies, are frequently observed (Macedo and Isaac, 2016) and those directed against C1q are considered of clinical relevance (Bock et al., 2015).

It is most remarkable that about 90% of C1q-deficient, about 75% of

C4-deficient, and about 60% of C1r/C1s-deficient patients suffer from partly severe SLE or SLE-like symptoms (Jonsson et al., 2005; Macedo and Isaac, 2016; Stegert et al., 2015). Also the lack of either C4A or (less often) C4B, the two alloptypic variants of C4, significantly increases the risk to develop SLE (Yang et al., 2004). In contrast, only about 10% of C2-deficient, about 15% of C3-deficient patients and occasionally TCC-deficient patients present with autoimmune disorders (Ricklin et al., 2016). The fact that also MBL deficiencies are found in patients with SLE and rheumatoid arthritis (Seelen et al., 2005b) raises the question if the lectin pathway like the classical pathway may contribute to disposal of autoantigenic waste.

Impaired clearance of immune complexes and apoptotic cells as well as loss of complement-dependent B cell tolerance are thought to be the major underlying mechanisms (Carroll, 2004; Pickering et al., 2000; Truedsson et al., 2007). Defective clearance of apoptotic cells due to genetic or acquired deficiency of complement results in continuous presentation of autoantigens to dendritic cells. Failure to clear complexes from the circulation could cause tissue injury with subsequent release of autoantigenic materials promoting the development of an autoimmune response. Abnormal lymphocyte activation may result in failure of B cell negative selection, with autoreactive clones escaping anergy and resulting in the production of autoantibodies (Lewis and Botto, 2006).

Individuals with heterozygous C2 or C4 deficiency often remain asymptomatic (Jonsson et al., 2005). However, many of these patients also develop severe bacterial infections including meningitis, pneumonia, arthritis, or septicaemia, caused by encapsulated bacteria, most commonly *S. pneumoniae*.

The deficiency of C1 inhibitor, resulting in classical pathway activation with subsequent C4 and C2 consumption, appears to increase the risk for SLE, discoid lupus erythematosus (DLE) or lupus-like disorders as shown in 23 cases of hereditary angioedema (Koide et al., 2002). Anti-C1-INH levels, indicative for acquired angioedema, were higher in SLE patients than in healthy controls and correlated with the duration and activity of the disease (Meszaros et al., 2010).

2.3. Complement deficiencies associated with malformation

The Malpuech–Michels–Mingarelli–Carnevale (3MC) syndrome comprises a group of autosomal recessive disorders with a wide range of clinical features such as dysmorphic facial features (e.g. cleft lip and palate), hypertelorism, postnatal growth retardation, intelectual disability and hearing loss (Rooryck et al., 2011; Sirmaci et al., 2010; Titomanlio et al., 2005). Type 1 (MIM 257920) it is caused by mutations in the MASP1 gene which encodes MASP-1, MASP-3, and MBL-associated protein 44 (MAp44). Type 2 3MC syndrome (MIM 265050) is caused by mutations in *COLEC11*, which encodes collectin kidney 1 (CL-K1) (Rooryck et al., 2011), whereas 3MC syndrome type 3 (MIM 248340) is caused by mutations in the collectin liver 1 (CL-L1)-encoding *COLEC10* gene (Munye et al., 2017). Only recently it was found that MASP-3 serves as an important enzyme, converting pro-FD to the active enzyme (Dobo et al., 2016). It is therefore not surprising that plasma of 3MC patients predominantly contains pro-FD (Pihl et al., 2017). However, since also detectable levels of active FD could be found, alternative pathway function appears not to be significantly impaired.

Results from *colec11* and *masp1* zebrafish experiments suggest that CL-K1 and MASP-1 are critically involved in the migration of neural crest cells during embryonic development (Rooryck et al., 2011), providing for the first time evidence that complement components are implicated in developmental disorders.

2.4. Diseases associated with complement dysregulation

Dysregulation of the alternative pathway with subsequent mostly local overactivation of the complement cascade is the driving force for renal (aHUS, C3G) and occular (AMD) disorders (Wong and Kavanagh, 2018). Next generation sequencing has become a valuable tool in the further identification of this new class of complement defects - particularly in the clinical setting. However, a meaningful association of the discovered variants with pathogenesis and clinic is still highly needed (Liszewski et al., 2017).

2.4.1. Atypical hemolytic uremic syndrome (aHUS)

aHUS is characterized by microangiopathic hemolytic anemia, thrombocytopenia and acute renal failure. In about 50% of cases, loss (e.g. *CFH*, *CFI*, *MCP/CD46*)- or gain-of-function (*C3*, *CFB*) mutations of complement factor genes, deletions, gene rearrangements (e.g. between *CFH* and *CFHR1/CFHR3*) or autoantibodies (e.g. against FH) provide the molecular background for an unrestricted local complement activation at the level of the vascular endothelium (Feitz et al., 2018; Michels et al., 2018; Noris and Remuzzi, 2010). As reported recently (Rodriguez et al., 2014), most genetic aHUS cases are heterozygous and are attributed to variants in the genes CFH (25–30%), followed by CD46 (8–10%), C3 and CFI (4–8% each), and CFB (1–4%) (Noris and Remuzzi, 2015).

However, also diarrhea-positive HUS, caused by Shiga toxin-producing *Escherichia coli* (STEC HUS) (Morigi et al., 2011; Orth and Wurzner, 2010), as well as thrombotic thrombocytopenic purpura (TTP) (Turner and Moake, 2013) have been associated with activation of the complement alternative pathway via mechanisms involving P-selectin and platelet thrombi, respectively.

While in aHUS the regulatory function of FH in the fluid phase is usually not impaired by the mutations identified, the majority of aHUS-related mutations leads to a lack of FH binding to the endothelial surface. This impaired recognition of “self” structures (glycosaminoglycans, anionic phospholipids or sialic acid) by FH and subsequent targeted attack of the complement alternative pathway against endothelial cells is the underlying cause of aHUS related to *CFH* mutations. Similarly, defects in the membrane-anchored complement regulatory proteins (e.g., MCP/CD46) also allow for unrestricted progression of the complement cascade on the vascular endothelium. Finally, gain-of-function mutations in *C3* result in a decreased decay of the alternative pathway C3 convertase, due to impaired regulator binding (Schramm et al., 2015) whereas that of *FB* accelerate formation and generate a more stable C3-convertase (Goicoechea de Jorge et al., 2007).

As recently described, the clinical picture of a thrombotic microangiopathy (TMA) can also be elicited by mutations in the gene encoding diacyl protein kinase ε (*DGKE*), affecing the phosphatidyl-inositol pathway with increased levels of protein kinase C (PKC) (Lemaire et al., 2013; Ozaltin et al., 2013). *DGKE* mutations were so far only identified in infant aHUS patients, typically refractory or poorly responsive to eculizumab treatment. The underlying molecular mechanisms, how these mutations cause disease, are still unclear. It is, however, conceivable, that an increased prothrombotic activity of the target cells may subsequently lead to complement activation.Thus, *DGKE* mutations should be considered in the panel of genes tested at least in young children (Azukaitis et al., 2017).

2.4.2. C3 glomerulopathy (C3G)

C3G is a recent disease classification comprising several rare types of glomerulonephritis, including dense deposit disease (DDD) and C3 glomerulonephritis (C3GN) (Cook, 2017; Medjeral-Thomas et al., 2014). Disease pathogenesis is heterogeneous and is induced by both autoimmune and genetic factors (Ravindran et al., 2018). Potential causative genetic variants in the *C3*, *CFB*, *CFH*, *CFI* and *CFHR1–CFHR5* genes have been found. Both rare and common variants can coexist and are associated with increased susceptibility to disease (Smith et al., 2019). The most common histological feature in these diseases is the glomerular deposition of C3 within the mesangium and along the glomerular basement membrane (GBM) in the subendothelial area or within the GBM. Low serum C3, but normal C4 levels are a common

finding. C3NeF activity is found in approximately 80% of patients with DDD and in 45% of patients with C3GN (Servais et al., 2013).

Dense deposit disease (DDD) – previously called membranoproliferative glomerulonephritis type II (MPGN II) – was the first nephropathy, for which excessive activation of the complement alternative pathway was identified (Appel et al., 2005; Barbour et al., 2013). In DDD - and less frequently also in other forms of MPGN - C3NeF is found (Schwertz et al., 2001). A severely reduced total complement activity with low C3 plasma levels is typical and warrants testing for C3NeF. Besides C3NeF, additional autoantibodies against C3b, FH and FB have been detected in DDD. Similar to aHUS, DDD and MPGN I patients can also carry mutations or polymorphisms in the *CFH* (Servais et al., 2012), *CFHR3 - CFHR1* (Malik et al., 2012), *CFHR5 (*Gale et al., 2010*)*, *CFI (*Servais et al., 2012*), MCP/CD46* (Radhakrishnan et al., 2012; Servais et al., 2007), and *C3* genes (Martinez-Barricarte et al., 2010). Different from aHUS, mutations in DDD typically do not impair complement control on endothelial cells but result in complement dysregulation in plasma reflected by significantly decreased C3 levels. This pathogenetic difference is exemplified by the finding of a disease-causing *CFH* mutation (ΔK224 in SCR4) in two siblings with DDD, which abolished FH cofactor activity while leaving the regulator's surface binding capacity intact (Licht et al., 2006).

The prognosis of C3-positive and immunoglobulin-negative *C3 glomerulonephritis (C3GN)* is unfavorable, and end-stage kidney disease (ESKD) develops in up to 50% of the patients during late childhood (Zand et al., 2014). C3GN results from dysregulation of the alternative complement pathway in the fluid-phase or on specific glomerular surfaces (i.e. GBM, mesangium). This is due to either immunological factors, mainly antibodies that activate the alternative pathway via stabilizing the C3 convertase or by mutations in complement-associated genes, mainly *CFH*, *CFI*, *CFB*, and *C3* (Servais et al., 2012). Additionally, risk polymorphisms in the membrane-cofactor protein (*MCP/CD46*) gene have been described.

Complement-factor H-related protein 5 (CFHR5) nephropathy, a subtype of C3GN with autosomal dominant inheritance, has been discovered in Cypriotic families (Gale and Maxwell, 2013). An abnormality in chromosome 1q31-32 which includes *CFH* and *CFHR* genes was associated with subendothelial or mesangial deposits and presents with microscopic and synpharyngitic macrosopic hematuria, finally leading to renal failure. It is caused by an internal duplication of exons 2–3 within the *CFHR5* gene resulting in dysregulation of the alternative complement pathway.

Treatment with eculizumab has become the therapy of choice for aHUS and has been occasionally also applied to other complement-mediated nephropathies (Wehling et al., 2017). This antibody prevents the cytolytic effects of the TCC, whereas its impact on the release of the proinflammatory C5a is still under debate. Since it acts downstream of C3, eculizumab preserves the autoimmune-protective and immune-enhancing functions of the early components of the complement pathways.

2.4.3. Age-related macular degeneration (AMD)

AMD represents the most common cause of blindness in the Western world affecting an estimated number of 150 million people (Geerlings et al., 2017; Warwick and Lotery, 2018; Wong et al., 2014). The disease develops in the elderly (> 60 years old) and is characterized by a progressive loss of central vision. The latter is caused by degeneration of photoreceptor cells in the macula following formation of so-called drusen, which represent the accumulation of retinal waste products in and around the Bruch's membrane (Geerlings et al., 2017; McHarg et al., 2015). Two forms of AMD can be distinguished: "dry" AMD is characterized by geographic retinal atrophy associated with gradual degeneration of photoreceptors and retinal pigmental epithelial cells while neovascularization of the retina with aberrant blood vessels represents a hallmark of "wet" AMD (Geerlings et al., 2017). Rupture of those vessels and subsequent leakage of blood products into the retina may cause a sudden loss of vision.

Apart from aging processes and environmental influences such as smoking, genetic factors have been shown to significantly contribute to the development of AMD. Based on twin studies, the heritability for AMD is estimated to be 46–71% (Geerlings et al., 2017). A recent genome-wide association study (GWAS) has identified 52 common and rare gene variants independently associated with AMD (Fritsche et al., 2016). Among them, more than one third (19/52) are located in or near a complement-related gene (Fritsche et al., 2016; Geerlings et al., 2017; Park et al., 2019; Warwick and Lotery, 2018). Specifically, gene variants in the complement genes *CFH, CFI, C2/CFB, C3, C9*, and *vitronectin (VTN)* have been associated with an enhanced risk of developing AMD. These genes are involved in the alternative pathway of complement activation suggesting that a dysregulation of this pathway may significantly contribute to disease pathogenesis. Results of functional investigations propose an impairment of control of this pathway resulting in increased complement activation in AMD, e.g. due to a reduced function of the complement regulators factor H and factor I (Park et al., 2019). In line with this notion, several studies described increased levels of complement activation products in the plasma from AMD patients vs. controls – though with a high overlap of activation product levels between cases and controls (Ristau et al., 2014; Scholl et al., 2008). In addition, significantly enhanced concentrations of the complement activation products C3a and Ba have been observed in the vitreous fluid of AMD patients when compared to controls (Schick et al., 2017). Notably, in the latter study, no differences in plasma levels of these split products could be detected between the two cohorts suggesting that local rather than systemic measurements of complement parameters may be a reliable way to determine complement activation in AMD patients, e.g. for therapeutic stratification purposes.

Compounds targeting different components of the alternative pathway of complement activation have been or are currently being tested in clinical studies for AMD. An overview on ongoing and completed clinical trials and their outcome is given in Park et al. (2019).

2.5. Diseases associated with complement membrane regulator and receptor deficiencies

2.5.1. Paroxysmal nocturnal hemoglobinuria (PNH)

PNH is a rare hemolytic disorder, clinically associated with anemia, thrombosis, dyspnoea, chest and abdominal pain, chronic kidney disease, and bone marrow failure (Brodsky, 2014; Hill et al., 2017). With an incidence of about 1–1.5 cases per million individuals worldwide it mainly affects individuals at ages between 30–59 years (Socie et al., 2016).

Complement-mediated hemolysis is the central mechanism underlying the morbidity and mortality associated with PNH (Brodsky, 2014). Thrombosis, occuring in up to 40% of patients, is the leading cause of death in PNH (Pu and Brodsky, 2011) and frequently involves multiple sites such as hepatic veins (Budd-Chiari syndrome), cavernous sinus, central nervous system veins, mesenteric veins or dermal veins (Weitz, 2011; Ziakas et al., 2007).

PNH is the consequence of somatic mutations in the phosphatidylinositol glycan anchor biosynthesis class A *(PIGA)* gene in one or more hematopoietic stem cell (HSC) clones. *PIGA* is involved in the biosynthesis of glycosylphosphatidylinositol (GPI), the anchoring structure of several molecules including the membrane complement inhibitors CD55 and CD59 (Kinoshita, 2018).

The severity of clinical symptoms determines treatment, which includes allogeneic hematopoietic stem cell transplantation and complement inhibition with eculizumab or the recently FDA-approved ravulizumab (McKeage, 2019). However, due to C3-dependent extravascular hemolysis, which cannot be prevented by anti-C5 inhibitors, about 25% of all patients remain to depend on transfusion (Notaro and Sica, 2018).

2.5.2. CHAPLE syndrome

CD55 (decay accelerating factor, DAF), which is broadly expressed on hematopoietic, stromal, endothelial, and epithelial cells (Koretz et al., 1992; Mikesch et al., 2006), confers protection to host cells against complement activation by accelerating the decay of the C3 and C5 convertases of the classical and alternative pathway (Merle et al., 2015). Recently, CD55 deficiency due to homozygous loss-of-function mutations of the *CD55* gene was identified as the molecular cause of a rare autosomal recessive syndrome characterized by complement hyperactivation, angiopathic thrombosis, and early-onset protein-losing enteropathy (CHAPLE syndrome) (Kurolap et al., 2017; Ozen et al., 2017). Protein-losing enteropathy likely results from primary lymphangiectasia and is – in some patients - linked to mucosal inflammation. Clinical symptoms of protein-losing enteropathy include gastrointestinal disease (diarrhea, abdominal pain, vomiting), edema due to hypoalbuminemia as well as consequences of malabsorption such as growth retardation and anemia. Moreover, patients may be affected by multiple severe thrombotic events. Abrogation of CD55 protein expression is associated with complement activation as indicated by enhanced deposition of C3d and TCC on peripheral blood leukocytes and submucosal arterioles, respectively (Kurolap et al., 2017; Ozen et al., 2017). Complement activation seems to significantly contribute to the pathogenesis of the disease as off-label treatment of CD55-deficient patients with eculizumab leads to an improvement of clinical symptoms and laboratory parameters (Kurolap et al., 2017).

2.5.3. Congenital CD59 deficiency

Congenital isolated deficiency of CD59 is a rare disorder. From early infancy on, the patients suffer from chronic hemolysis, recurrent strokes and episodes of Guillain-Barré syndrome (GBS)-like disease pointing to chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) (Karbian et al., 2018; Tabib et al., 2017). Several mutations in the CD59-coding sequence have been identified. Complications include acute renal failure and thromboembolic events, which are probably mediated by intravascular hemolysis. Therapy with eculizumab proved to be succesful in improving not only the hemolytic anemia but also the neurologic symptoms (Hochsmann et al., 2014; Mevorach et al., 2016).

2.5.4. Leukocyte adhesion deficiency I

Leukocyte adhesion deficiency I (LAD I) represents a rare autosomal recessive disorder characterized by recurrent life-threatening bacterial infections predominantly affecting the skin and mucosal surfaces. Furthermore, wound healing processes are impaired in patients suffering form this disease (Almarza Novoa et al., 2018; Hanna and Etzioni, 2012). It is caused by mutations of the *ITGB2* gene encoding CD18, the common ß2 subunit of a subgroup of integrins, which includes LFA-1 (CD11a/CD18), complement receptor 3 (CR3; CD11b/CD18) and complement receptor 4 (CR4; CD11c/18). More than 200 different mutations of CD18 have been described which result in decreased expression of the respective ß2-integrin receptors (Fagerholm et al., 2019). As these receptors are essential mediators of leukocyte adhesion and migration as well as C3-dependent opsonophagocytosis and are also involved in the regulation of adaptive immune responses (Fagerholm et al., 2019), their lack or low expression is associated with a severely immunocompromised state.

LAD I develops during the first months of life with disease severity and survival rates correlating with the degree of CD18 expression levels on leukocytes (Hanna and Etzioni, 2012). Hematopoietic stem cell transplantation (HSCT) represents the only treatment for severely affected patients; in the absence of HSCT the survival rate to the age of 2 is 39% for these patients (Almarza Novoa et al., 2018).

2.6. Hereditary angioedema

Despite the fact that hereditary (C1-INH-HAE) or acquired (C1-INH-AAE) angioedema is NOT a complement, but a bradykinin-triggered disease, for historical reasons this disorder needs to be included in an overview on complement deficiencies.

Primary angioedema, a recently suggested new classification of recurrent angioedema without urticaria, includes hereditary and non-hereditary forms with C1 inhibitor deficiency (Caccia et al., 2014) and with normal C1-INH (Bork, 2013).

In addition to its role as an inhibitor of C1r and C1s of the classical pathway and MASP1 and MASP2 of the lectin pathway, C1-INH also regulates factor XIa, XIIa, kallikrein and tissue plasminogen activator. Impaired inhibition of these enzymes leads to excessive bradykinin generation, which in turn increases vascular permeability leading to angioedema (Cugno et al., 2009).

Clinical symptoms include recurrent and episodic swelling affecting the facial area, upper airways, upper or lower limbs, genital area, or intestinal tract. Attacks usually have a relatively slow onset (hours) and may be preceded by prodromal symptoms including erythema marginatum, muscle pain or itching. Swelling located at the pharyngeal and laryngeal area is the most serious clinical manifestation as this may lead to airway obstruction and even asphyxia. Laryngeal complications have been reported in more than 20% of patients, sometimes leading to the requirement of tracheal intubation or even emergency tracheotomy (Levi et al., 2019).

It is well known that a large number of patients suffering from primary angioedema present with normal C1-INH levels. Mutations especially in the factor XII gene (*F12*-HAE), but also in the genes encoding plasminogen (*PLG*-HAE) and occasionally angiopoietin-1 (*ANGPT1-HAE*) have been linked to this newly defined group of primary angioedema patients with normal C1-INH (nlC1-INH- HAE) (Zuraw, 2018). Even where the underlying genetic defect has been defined, the pathogenetic mechanism of angioedema formation remains elusive. Furthermore, for a large number of these patients the molecular background has not been defined yet.

Management of hereditary angioedema aims at reducing the severity and duration of an angioedema attack but should also prevent angioedema attacks in high risk situations for angioedema formation (e.g. surgical procedures).

The current most rational approach for prevention and treatment is the administration of C1 inhibitor concentrate derived from plasma (Bork et al., 2013; Longhurst et al., 2017). Alternatively, a recombinant concentrate (Ruconest), produced in transgenic rabbits expressing the human C1 inhibitor gene, can be administered (Choi et al., 2007). More recently, effective treatment for hereditary angioedema has been achieved by icatibant, a selective bradykinin-2 receptor antagonist (Cicardi et al., 2010). Like the plasma kallikrein inhibitor, ecallantide, icatibant is effective for treatment of acute attacks, but a short half-life limits its use for prophylaxis (Sabharwal and Craig, 2015). If C1 inhibitor or icatibant is not available, attenuated androgens, such as danazol and stanozol, can be used for treatment. Since hereditary angioedema is a heterozygous disease, upregulation of the healthy gene by these drugs may result in higher levels of C1 inhibitor (Gelfand et al., 1976). In addition, tranexamic acid has been shown to be effective in the prevention and treatment of angioedema attacks, and is often used for long-term prophylaxis in children, in whom androgens are contraindicated (Farkas et al., 2017).

3. Clinical and laboratory assessment of complement abnormalities

Recognition of the following warning signs may help clinicians in the diagnosis of complement deficiencies (Grumach and Kirschfink, 2014):

- Meningococcal meningitis at > 5 years of age
- Recurrent systemic bacterial infections with encapsulated organisms (particularly *S. pneumoniae* and more rarely gonococcal disease)
- Autoimmune diseases (particularly SLE)

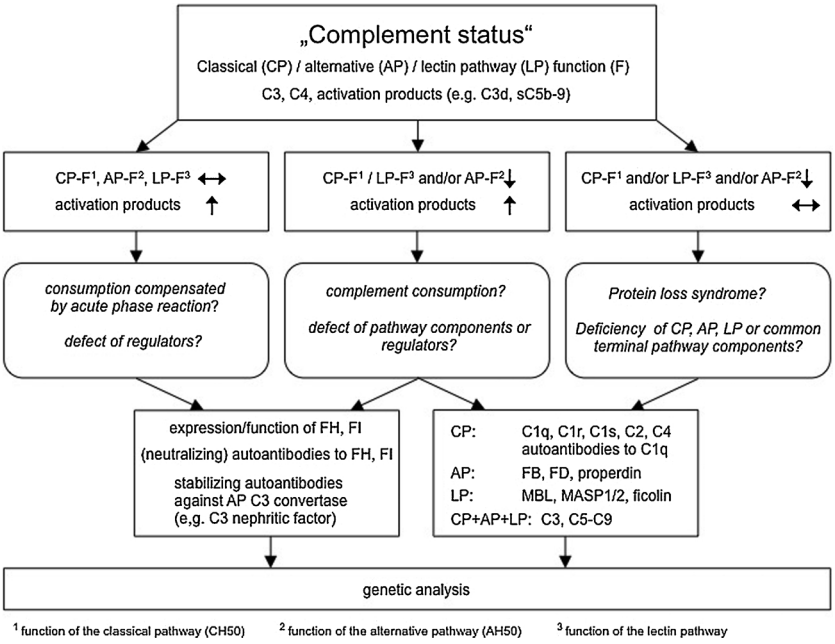

Fig. 1. Laboratory analysis of complement abnormalities. When complement abnormalities are suspected, the determination of the „complement status“, i.e. the analysis of the functions of the classical (CP-F), alternative (AP-F), and lectin (LP-F) pathway as well as the measurement of C3, C4, and activation products, is recommended as a first step (*level 1*). The results of this analysis (*level 2*) and their interpretation (*level 3*) determine further diagnostic steps (*level 4/5*). *Left*: A normal function of CP-F, AP-F, and LP-F associated with enhanced levels of activation products indicates complement activation even if compensated by acute phase reaction. Depending on the clinical context, a defect of complement regulation has to be considered and should be further characterized by analysis of complement regulators as well as autoantibodies against these regulators or against the AP convertase. *Middle:* Decreased CP-F, LP-F and / or AP-F in the presence of enhanced levels of activation products indicates complement activation associated with complement consumption, which may also be a sign of defective regulation. Alternatively, this result constellation may also be compatible with a complement deficiency associated with complement activation- in particular if clinical signs of autoimmune disease or infections are present. Accordingly, expression and function of complement regulators or pathway components as well as relevant autoantibodies against these proteins should be analysed. *Right*: Decreased CP-F and/or LP-F and/or AP-F in the absence of complement activation suggests a deficiency of components of the respective pathway or the common terminal pathway. Accordingly, measurement of the expression and function of pathway components will contribute to the identification of the defective protein. Protein loss syndromes should be excluded. Finally, genetic analysis should be performed in order to characterize genetic variants underlying suspected complement deficiencies or dysregulation. The latter is of particular importance when complement protein levels are within the normal range and functional assays are not available.

- Angioedema without urticaria
- Inflammatory disorders involving the kidney or eyes

3.1. Laboratory analysis

The work-up of any complement abnormality requires a comprehensive laboratory analysis of the complement system. It involves the determination of the functionality and the activation state of the different pathways, the concentration and function of single components and regulators, the search for autoantibodies as well as the molecular analysis of complement genes (Fig.1).

When complement deficiency is considered as a differential diagnosis, in a first step, a set of tests assessing the functions of the classical and the alternative pathway (e.g. CH50 and AH50) as well as the activation state of the complement system should be performed (“complement status”). If indicated clinically, the integrity of the lectin pathway should also be investigated. The combined results of these assays allow to determine whether a loss of pathway function is due to a primary (inherited) defect of pathway-associated complement factors or most likely caused by consumption of complement factors following strong complement activation, e.g. due to complement dysregulation. Furthermore, this allows to distinguish between deficiencies of early pathway-specific components and those of C3 and of the common terminal sequence (C5-C9).

In case the results of these assays suggest a primary complement deficiency or complement dysregulation, the analysis of individual complement components is indicated. The latter includes the determination of the concentration of single factors as well as functional tests including reconstitution assays (see below). Flow cytometric analysis is employed to examine expression levels of complement regulators or receptors on the cell surface. Finally, molecular analysis will provide insight into gene variants underlying suspected inherited complement defects.

Importantly, laboratory analysis of complement abnormalities also involves the measurement of clinically relevant inhibitory or activating autoantibodies targeting individual complement components, regulators or convertases such as C1 inhibitor, C1q, factor H, and C3 nephritic factor. These autoantibodies have been demonstrated to be useful as diagnostic or prognostic markers as well as for monitoring therapeutic responses (Dragon-Durey et al., 2013).

3.1.1. Preanalytics

Conclusive complement analysis critically depends on correct sampling and subsequent preanalytical handling of the samples. Whereas serum is best suited for functional analysis of the complement pathways and for measuring the concentration of complement components as well as autoantibodies, the quantification of activation products needs to be performed using EDTA-plasma. By chelating divalent cations such as Ca^{2+} and Mg^{2+}, EDTA at concentrations of 10 mM or higher inhibits complement activation as occurring rapidly *ex vivo* (Prohaszka et al., 2018). Importantly, as another measure to prevent *ex vivo* complement activation, serum and EDTA-plasma have to be separated from whole blood as rapidly as possible. Subsequently, they need to be subject to immediate analysis or to be frozen at −80 °C until being assayed or shipped to specialized laboratories (http://www.ecomplement.org/european-complement-labs.html) on dry ice.

3.1.2. Assessment of the functional integrity of complement pathways

Several methods have been developed to monitor the integrity of the different complement pathways. Traditionally, hemolytic assays are used for assessing the function of the classical (CH50) or alternative pathway (AH50). In these assays, activation of the classical pathway is initiated by antibody-coated sheep erythrocytes, while rabbit, chicken or guinea pig erythrocytes are employed to induce activation of the alternative pathway (Mayer, 1961; Rapp and Borsos, 1970; Joiner et al., 1983). Notably, assays monitoring the function of the alternative pathway are performed in the presence of the calcium chelator EGTA, which blocks activation of the classical and lectin pathway. If all pathway components are present in sufficient concentrations and functional, complement activation will proceed to the formation of the TCC in the cell membrane of the erythrocytes and ultimately induce their lysis. The degree of lysis is determined by measuring the amount of hemoglobin released into the serum and serves as a measure of complement pathway activity in the sample. Results of these assays are

conventionally reported as the reciprocal serum dilution required to lyse 50% of a defined amount of erythrocytes under standardized conditions. Alternatively, they can be expressed as percent activity of a standard given as 100%. As standard CH50 and AH50 assays require laborious preparation of serial dilutions of serum samples, a faster hemolytic assay relying on the preparation of a single dilution (one tube assay) has been established for routine analysis of complement pathway activity (Nilsson and Nilsson, 1984).

Given the shortage of high quality erythrocytes, alternative assay systems are of increasing importance. A cytolytic assay using sensitized liposomes for measuring classical pathway activity (Masaki et al., 1989) is commercially available. The performance of this assay exceeds that of conventional erythrocyte-based methods with regard to reproducibility and suitability for automation. Furthermore, in a functional enzyme-linked immunosorbent assay (ELISA) for the classical, the alternative as well as the lectin pathway, complement activation is induced by target molecules specific for each of these pathways (IgG-CP, mannan-LP, LPS-AP) coated to the surface of wells of microtitre plates. For quantification of complement activity the amount of the terminal complement complex, C5b-9, generated is determined using a neoepitope-specific monoclonal antibody (Seelen et al., 2005a).

However, cryoglobulinemia but also protein loss syndromes have to be ruled out in order to confirm that the functional defect is due to a specific complement deficiency.

3.1.3. Assessment of complement activation products

Information on the state of complement activation is provided by quantification of split products of single complement components, e.g. C4a, C4d, C3a, C3c, C3d, Ba, Bb, and C5a, which are generated by enzymatic cleavage during complement activation. Further biomarkers for complement activation are soluble protein complexes such as the properdin-containing alternative pathway convertase (C3bBbP) as well as the soluble terminal complex (sC5b-9).

For detection of most of these complement activation products, ELISAs are commercially available. They rely on monoclonal antibodies specifically recognizing neoepitopes solely exposed following activation-induced conformational changes of the respective complement components (Mollnes et al., 1993). Furthermore, a bead-based multiplex assay using Luminex XMap technology was recently described for the simultaneous detection of C3a, C5a, and sC5b-9 (Gallenkamp et al., 2018).

If only polyclonal antibodies are available for the analysis of activation-induced split products, the latter have to be separated from their zymogens prior to immunodetection, e.g. by using rocket immune electrophoresis (RIE) technology. This procedure is currently employed for measuring C3d (Brandslund et al., 1986). However, a monoclonal antibody specifically recognizing C3d has recently been characterized which may prove useful for the development of immunoassays allowing detection of C3d without the need for prior laborious separation steps (Rasmussen et al., 2017).

For routine diagnostic purposes, complement activation is assessed by quantification of those activation products which show a high stability *in vivo* such as C3d and sC5b-9. The half-lives of the latter activation products in the circulation *in vivo* are 4 h (Teisner et al., 1983) and 50–60 min (Mollnes, 1985), respectively, while e.g. C5a possesses a half-life of 1 min *in vivo* due to rapid receptor binding (Oppermann and Gotze, 1994). The determination of complement activation products (in combination with functional assays) allows to distinguish between a primary deficiency and complement consumption as a cause of low functionality of one or more pathways. This distinction is not possible if only the total concentrations of complement components (e.g. C3, C4) are analysed. Since most complement factors are acute phase reactants, their concentration may be in the normal range even in the presence of strong inflammation-induced complement activation due to masking of complement consumption by concomitant upregulation of protein expression. This further underlines the diagnostic significance of determining the level of complement activation products for assessing complement activation. In line with this notion, a recent study demonstrated the superiority of the complement activation product C3dg vs. total C3 as a diagnostic biomarker for SLE (Troldborg et al., 2018).

Complement activation can also be monitored locally in tissues by determining the deposition of C3b, C4b, C4d, and C5b-9 *in situ* employing antibodies suitable for immunohistochemical detection of these antigens in frozen or formalin-fixed paraffin embedded (FFPE) sections.

3.1.4. Analysis of individual complement components

If the results of the screening assays for complement pathway integrity and activation ("complement status") provide evidence for a primary complement deficiency, analysis of individual complement factors is performed in order to identify the defective component. Various test systems, such as nephelometry, radial immunodiffusion (RID), and ELISAs have been established for measuring the concentration of individual complement proteins in serum. Furthermore, the activity of single components can be tested by hemolytic titration assays. In these assays, the ability of a sample to restore the hemolytic activity of a human serum deficient for or depleted of the respective complement component is determined. In addition, in order to further verify the deficiency of a given component, the purified functionally active component can be added to the patient's serum, and the effect on the hemolytic activity of the complement pathway affected can be measured (reconstitution assays).

Finally, genetic analysis allows to characterize gene variants underlying the identified or suspected complement defect. It is of particular diagnostic importance in cases of suspected complement deficiencies, where concentrations of the complement factors are within the normal range and functional assays are not available. Sanger sequencing can be applied to detect single nucleotide variants in specific DNA regions using oligonucleotide primers (Jamuar et al., 2016). Next-generation sequencing (NGS) is now routinely applied to detect known and novel mutations in complement genes that are associated with diseases, and may have implications for both diagnosis and treatment (Turley et al., 2015). For example, genetic sequencing and multiple ligation-dependent probe assessment screening panels for aHUS and C3G typically include up to 10 complement genes (CFH, CFHR1, CFHR3, CFHR4, CFHR5, CFI, C3, CD46, CFB, and CFP), two coagulation genes (THBD (thrombomodulin) and *PLG* (plasminogen)), and one non-complement gene (DGKE) (Osborne et al., 2018). Finally, with the rapid improvement in whole genome and whole exome sequencing its application for diagnosing complement deficiencies will certainly gain increasing attention (Liszewski et al., 2017).

3.1.5. Analysis of autoantibodies targeting complement factors

Autoantibodies to complement proteins have been implicated in the pathogenesis of a number of complement-related diseases. In particular, autoantibodies against C1q, C1 inhibitor, factor H as well as the C3 convertase of the alternative pathway (e.g. C3NeF) represent important diagnostic parameters impacting therapeutic decisions (Dragon-Durey et al., 2013; Ekdahl et al., 2018). The detection of these antibodies is mostly performed using commercially available or in-house ELISAs with the corresponding antigen being immobilized on microtiter plates. For detection of C3NeF, a semiquantitative hemolytic assay, demonstrating the presence of C3 convertase stabilizing activity in patient samples, is frequently conducted (Rother, 1982). Furthermore, functional assays, testing the neutralizing effect of C1 inhibitor and factor H autoantibodies, have been described (Jozsi et al., 2007; Ziccardi and Cooper, 1980). A comprehensive overview on clinical and diagnostic aspects of autoantibodies against complement proteins is given by Dragon-Durey et al. (2013) and Ekdahl et al. (2018).

4. Conclusions

In recent years, the spectrum of clinical disorders associated with

complement deficiencies and dysregulation has been expanding. An in-depth knowledge of their pathophysiology, clinical phenotypes and their diagnostic evaluation will be helpful for clinicians in order to timely identify complement deficient patients. Recently discovered complement deficiencies such as the CHAPEL syndrome and the 3MC syndrome shed new light on the importance of complement in organ-specific severe inflammatory disorders and even developmental processes.

With various compounds being currently tested in clinical trials for the treatment of complement deficiencies and dysregulation, the therapeutic options for these rare disorders may improve in the near future.

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