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MINIREVIEW

Human parvovirus B19: general considerations and impact on patients with sickle-cell disease and thalassemia and on blood transfusions

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Abstract

Human parvovirus B19 (B19V) is a small (22–24 nm) nonenveloped DNA virus belonging to the genus *Erythrovirus* (family *Parvoviridae*). Although it generally causes self-limiting conditions in healthy people, B19V infection may have a different outcome in patients with inherited hemolytic anemias. In such high-risk individuals, the high-titer replication may result in bone marrow suppression, triggering a life-threatening drop of hemoglobin values (profound anemia, aplastic crisis). To date there is no consensus concerning a B19V screening program either for the blood donations used in the hemotherapy or for high-risk patients. Moreover, questions such as the molecular mechanisms by which B19V produces latency and persistent replication, the primary site (sites) of B19V infection and B19V immunopathology are far from being known. This review summarizes general aspects of B19V molecular characteristics, pathogenesis and diagnostic approaches with a focus on the role of this pathogen in blood transfusions and in patients with some hemoglobinopathies (sickle-cell disease, thalassemia).

Introduction

Human parvovirus B19 (parvovirus B19, B19V) is a small, nonenveloped DNA virus belonging to the genus *Erythrovirus* (*Parvoviridae* family) (International Committee on Taxonomy of Viruses (ICTV), 2007). Its capsid comprises 60 capsomers (VP1 and VP2) surrounding a single-stranded (ss) DNA genome (Kaufman *et al.*, 2004). VP1 protein is a dominant target for the neutralizing antibodies and is located on the external surface of the virion (Bönsch *et al.*, 2008). The mechanism of B19V replication is exceptional: DNA is replicated through high-molecular-weight intermediates linked through hairpin structures because of the presence of genomic palindromes (Ozawa *et al.*, 1986).

The pattern of clinical disease caused by B19V varies and is influenced by both the hematological and the immunological status of the infected individual. In healthy hosts, B19V generally causes self-limiting subclinical erythroid aplasia followed by a rash or arthralgia. However, in patients with diminished production or increased loss of erythrocytes, it is now clear that B19V infection results in a severe drop of hemoglobin values and anemia which could be life-threatening (Heegaard & Brown, 2002; Zaki *et al.*, 2006; Krishnamurti *et al.*, 2007; Toyokawa *et al.*, 2007).

In 1981, B19V was implicated as the etiologic agent of severe aplastic crises in children with sickle-cell disease (SCD), in whom this infection can evolve into various life-threatening conditions (Serjeant *et al.*, 1981) including *acute encephalopathy* (Bakhshi *et al.*, 2002), *nephrotic syndrome* (Quek *et al.*, 2010), *splenic sequestration* (Yates *et al.*, 2009) and *fatal bone marrow embolism* (Rayburg *et al.*, 2010). This raises important questions related to the safety of transfused blood and B19V transmission by blood transfusion. B19V infection transmitted by transfusions has already been described as a cause of chronic anemia (Cohen *et al.*, 1997; Jordan *et al.*, 1998) as well as transient heart failure (Zanella *et al.*, 1995) in affected individuals. Seronegative children with inherited hemolytic diseases are particularly vulnerable to B19V, in whom the introduction of the virus via transfusion can seriously disturb erythropoiesis (Heegaard & Brown, 2002; Zaki *et al.*, 2006; Yates *et al.*, 2009). However, most hematological units worldwide do not carry out

routine screening of donated blood or high-risk patients for B19V, despite the unpredictable infection outcome and the lack of treatment options (Prowse *et al.*, 1997; Blumel *et al.*, 2002).

This review summarizes general aspects concerning the molecular, genotypic and pathogenetic features of B19V with a focus on its impact on blood transfusion and patients with hemoglobinopathies (SCD and thalassemia).

Discovery, brief history and taxonomy of B19V

Discovery and brief history

B19V was discovered accidentally in 1974 in the serum of an asymptomatic blood donor by Yvonne Cossart's group in London (Cossart *et al.*, 1975). The viral name originated from the identification of the tested sample: number 19 of panel B. The electron microscopy studies which followed revealed 23-nm viral particles similar to those of the animal parvoviruses (Heegaard & Brown, 2002; Brown, 2009). Characterized during 1983–1984, the viral genome of B19V proved to be a single ~5.5 kb DNA molecule (Summers *et al.*, 1983) surrounded by superficial proteins copurifying at a density of 1.43 g cm^{-3} (buoyant density for parvoviruses in CsCl: 1.38–1.45 g cm^{-3}) (Clewley, 1984; ICTV, 2007).

Description of B19V clinical entities coincided with its virological characterization. The first connection between B19V and symptomatic disease was made in 1981, when it was demonstrated that it causes transient aplastic crisis (TAC) (Pattison *et al.*, 1981) in patients with SCD. Erythema infectiosum ('fifth disease') in children was consequently linked to acute B19V infection (Anderson *et al.*, 1983). Following studies described B19V-induced fetal loss (hydrops fetalis) (Brown *et al.*, 1984) during pregnancy and symmetrical arthropathy in adults (Reid *et al.*, 1985; White *et al.*, 1985).

Taxonomy

B19V is a member of the family *Parvoviridae*, subdivided into *Parvovirinae* and *Densovirinae* depending on the type of the infected host (vertebrate or invertebrate). *Parvovirinae* is further divided into five genera (*Amdovirus*, *Bocavirus*, *Dependovirus*, *Erythrovirus* and *Parvovirus*) due to differences in transcription, organization of their terminal repeats and host range. B19V is an autonomously replicating virus, as its life cycle is supported only in rapidly dividing erythroid cells. Therefore, B19V is classified within the genus *Erythrovirus* as a prototype. The genus *Parvovirus* comprises most of the parvoviruses infecting vertebrates with a prototype member minute virus of mice (MMV). *Dependovirus* includes adeno-associated viruses and two avian viruses (duck and goose parvovirus). *Amdovirus* has only one member, the Aleutian mink disease virus, which causes lethal infection among minks. The *Bocavirus* genus has as a prototype the bovine parvovirus (ICTV, 2007).

Viral structure

B19V is a nonenveloped virus whose icosahedral virion is composed of two structural proteins (VP1 and VP2) surrounding a linear, ssDNA molecule with positive or negative polarity.

Viral capsid proteins

The virion consists of 60 structural subunits, ~95% of which belong to VP2 (58 kDa) and 5% to VP1 (83 kDa) (Kaufman *et al.*, 2004; Bönsch *et al.*, 2008). VP1 and VP2 originate from overlapping reading frames and are identical with the exception of 227 additional amino acids at the N-terminus of VP1, called VP1 '*unique region*' (VP1u) (Kaufman *et al.*, 2004). VP1u exhibits relatively high sequence variability in persistently infected individuals ranging from 3.5% (Heegard *et al.*, 2002) to 6.8/8.2% (Hemauer *et al.*, 1996). Such variation could cause nonconservative amino acid alterations (uncharged amino acid residues are replaced with polar amino acids). This region plays an important role in eliciting immune responses, with this type of mutations probably leading to a modified viral surface and inability of the infected organism to clear B19V, and the development of persistent infection (Hemauer *et al.*, 1996).

In contrast to the mammalian parvoviruses and despite its low concentration in the virion, *VP1u* is the major antigenic target for neutralizing antibodies. It also possesses phospholipase A_2 (PLA_2) motif, which takes part in the initiation of the infection. *VP1u* is thought to play an important role in the induction of autoimmune responses and chronic inflammation by mechanisms that are not fully understood (Bönsch *et al.*, 2008).

Nonstructural proteins, NS1

B19V possesses several nonstructural (NS) proteins, the most abundant of which is NS1. It has a molecular mass of ~77 kDa (Sol *et al.*, 1999) and multiple functions, including site-specific DNA-binding, DNA-nicking, helicase function and regulation of gene transcription. NS1 *trans*-regulates the viral P6 promoter as well as some cellular promoters and thus controls B19V replication (Zhi *et al.*, 2006). This protein is highly cytotoxic for erythroid precursors and can induce apoptosis via interaction with caspase 3 (Moffat *et al.*, 1998; Morita *et al.*, 2001). Most of the B19V-associated clinical manifestations are thought to be related to damage of the infected erythroid precursors by NS1 cytolytic or apoptotic activity (Morita *et al.*, 2001).

Viral genome

B19V genome is composed of internal 4830 nt coding sequences flanked by 383 nt terminal repeats (Heegaard & Brown, 2002). The distal 361 nucleotides of each repeat form a palindrome, capable of assembling hairpins usually designated as 'forks' (Mori *et al.*, 1987). The hairpins exist in two distinct configurations, named 'flip-flop', which are found in all parvoviruses (Deiss *et al.*, 1990) and serve as initiation sites for replication (Ozawa *et al.*, 1987).

B19V genome is divided hypothetically into two main ORFs with NS1 protein encoded by the 'left' genome portion and the capsid proteins by the 'right' one (Heegaard & Brown, 2002). The coding potential of such a small genome is increased by the utilization of multiple reading frames that generate large, overlapping transcripts. B19V has approximately nine overlapping poly-(A) transcripts that differ from the other parvoviruses (Ozawa *et al.*, 1987). A short ORF encoding protein X and located within the VP1 gene has been described recently. The functions of this hypothetical protein for the B19V infectious process have not been defined due to limitations of viral cultivation and lack of infectious clones (Zhi *et al.*, 2006). It is has also been proposed that the invasion of the single-stranded B19V genome during infection can induce DNA damage and cell-cycle arrest (Chen & Qiu, 2010).

B19V genetic variability

At present, three B19V genotypes (1, 2 and 3) have been identified with an estimated divergence of more than 10% among them (Servant *et al.*, 2002). Genotype 1 is the most prevalent genotype worldwide with a prototype B19V. Because of genotype 1 intercluster variability of approximately 2% it is additionally subdivided into two subgroups (1A and 1B) (Toan *et al.*, 2006). Genotype 2 (prototypes A6 and LaLi) has been detected in patients from several European countries, the United States and Brazil (Toan *et al.*, 2006; Freitas *et al.*, 2008; Grabarczyk *et al.*, 2011). Genotype 3 (prototypes V9 and D91.1) is divided into two subgenotypes: 3A and 3B. Circulation of genotype 3 has been described mainly in tropical countries such as Brazil (Freitas *et al.*, 2008) and Ghana (Parsyan *et al.*, 2007). Recently, a new parvovirus sequence *PARV4* was discovered in clotting factor concentrates (Jones *et al.*, 2005). *PARV4* differs genetically from the B19V genotypes described to date and is probably a new genetic type of the family *Parvoviridae* (Simmonds *et al.*, 2008). Although there is a significant divergence among the sequences of the viral genotypes, most of the nucleotide changes are at a third position and the amino acid variation is reduced. As a consequence, the structural proteins have approximately 96% similarity and low antigenicity, which is responsible for the high serological degree of cross-reactivity among the different B19V genotypes in clinical samples (Heegard *et al.*, 2002).

B19V life cycle

The life cycle, tropism and transcriptional map of B19V are highly specific for this pathogen, distinguishing it from all other members of the *Parvoviridae* into a separate genus (*Erythrovirus*). B19V is an autonomously replicating parvovirus. Compared with MMV, which produces three transcripts (R1, R2 and R3) terminating at one single polyadenylation site (Wan *et al.*, 2002), B19V utilizes at least 12 RNA transcripts, all processed by alternative splicing (Ozawa *et al.*, 1987). B19V has multiple polyadenylation sites located in the middle of the genome and its right-hand portion (St Amand *et al.*, 1991). B19V replication occurs in the nucleus of the infected cell and includes common stages for the most DNA viruses: (1) attachment of the virus to the host cell receptors; (2) internalization; (3) translocation of the genome to the nucleus; (4) DNA replication; (5) RNA transcription; (6) assembly of the capsids; (7) packaging of the genome; and (8) cell lysis with release of infectious virions (Heegaard & Brown, 2002) (Fig. 1).

Tropism, viral receptors and uncoating

B19V has exceptional tropism to human erythroid progenitors, fetal liver and umbilical blood erythroblasts (Heegaard & Brown, 2002). Factors responsible for this property include the possession by erythroid cells of blood group P antigen (globoside, Gb4), which is the main B19V receptor

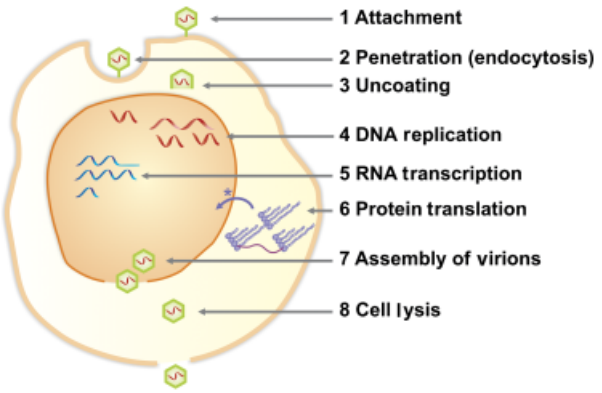

Fig. 1. Life cycle of B19V in an erythroid progenitor cell. The attachment (1) is probably mediated by the P-antigen in combination with (α)-5 (β)-1 integrins, after which B19V is translocated into the cytoplasm by endocytosis (2). The uncoating (3) occurs in the cytoplasm and the viral DNA is transported to the nucleus, where replication (4) and transcription (5) take place. After the translation (6) of the viral proteins in the cytoplasm, they are transported back to the nucleus (arrow with asterisk), where the assembly (7) of the virions into large inclusion bodies occurs. B19V leaves the cell by lysis (8) and causes a pronounced cytopathic effect in the bone marrow.

and the rapid division of erythroid cells, which provides indispensable intracellular factors for optimal B19V replication and transcription (Weigel-Kelley *et al.*, 2006). Some individuals who genetically lack P antigen are naturally resistant to B19V infection (Brown, 2004).

Nevertheless, other cellular types (especially those in kidney, liver tissues, synoviocytes, platelets, endothelium and vascular smooth muscle) that express P antigen do not allow B19V entry (Simeoni *et al.*, 2010). Thereby, P antigen is a necessary but not a sufficient factor for productive infection. That is why it is suggested that the cell entry is additionally mediated by (α)-5 (β)-1 integrins in high-affinity confirmation. Mature red blood cells that express high levels of P antigen, but not (α)-5 (β)-1 integrins, bind B19V but do not allow viral entry. In contrast, erythroid progenitors that possess high levels of P antigen and (α)-5 (β)-1 integrins allow P antigen/integrin-mediated entry. It is believed that in the natural course of the viral pathogenesis, red blood cells serve as efficient disseminators of B19V in the body (Weigel-Kelley *et al.*, 2003). The globoside is also thought to mediate the capsid rearrangements required for the subsequent interactions leading to virus internalization (Bönsch *et al.*, 2010a). These findings may explain why virus tropism is restricted to erythroid progenitor cells that contain high levels of P antigen and coreceptors, and a number of P antigen-positive nonerythroid cells that do not permit efficient replication because of the lack of coreceptors (Simeoni *et al.*, 2010).

After binding to the receptors, B19V probably enters the target cell through clathrin-mediated endocytosis. The uncoating mechanism of B19V is not clear but the rigid organization of the capsid suggests that the genome is released without virion disassembly (Ros *et al.*, 2006).

Replication

Despite the small genome, B19V replication is a complex process because the DNA is both single-stranded and linear. B19V disrupts the functions of the synthetic machinery of the host cell for its own replication purposes using an ancient strand-displacement mechanism called *rolling-circle replication*. As a consequence, B19V replicates through DNA intermediates that are linked through their terminal hairpins (Ozawa *et al.*, 1986). The 3′ terminal end of each palindrome serves as a self-primer for the initiation of the synthesis for a plus sense DNA. During B19V replication, the imperfectly paired termini provide an energetically favorable environment for unfolding and refolding the hairpin. B19V dimeric replicative forms are usually detected in infected bone marrow and therefore it is believed that the growing strand replicates back on itself to produce a tetrameric form, from which are generated two plus and two minus strands (White & Fenner, 1994).

Transcription

During B19V transcription at least 12 transcripts are generated by alternative splicing and polyadenylation from a single pre-mRNA. The polyadenylated transcripts are synthesized as spliced or unspliced mRNAs. The unspliced mRNAs encode the NS1 protein, and the transcripts polyadenylated internally at pA-*p* (proximal) position encode a 7.5-kDa protein with unknown function. All B19V transcripts polyadenylated at the distal polyadenylation site (pA-*d*) excise their first intron (D1 to A1-1 or D1 to A1-2). Those that are not additionally spliced encode the capsid proteins VP1 and VP2. mRNAs further subjected to splicing with excision of the second intron (D2 to A2-1) encode one nonstructural 11-kDa protein thought to play a role in B19V transportation from the nucleus (Guan *et al.*, 2008).

Pathogenetic and clinical aspects of B19V infection

Transmission

The transmission of B19V occurs mainly via respiratory droplets but it can also be spread by contaminated blood, organ transplantation and vertical transmission from mother to fetus (Heegaard & Brown, 2002). After respiratory acquisition of B19V, a short replication occurs in the nasopharyngeal lymphoid tissue, followed by a massive viremia with a viral load that can exceed 10^{13} copies mL^{-1} (Anderson *et al.*, 1985). Subsequently, B19V is disseminated throughout the body and enters the bone marrow microenvironment, producing generalized erythroblast infection (Anderson *et al.*, 1985; Heegaard & Brown, 2002). The lymphopenia, neutropenia and thrombocytopenia that occur during the acute viremic phase are not significant. The etiology of the thrombocytopenia found in the productive B19V infection can be partly explained by the existence of viral replication in the thrombocytes, without synthesis of structural proteins (Pallier *et al.*, 1997).

Transmission via blood products

Transmission of B19V by blood products is favored by two important viral characteristics: (1) persistent infection in the bone marrow of asymptomatic individuals (Cassinotti *et al.*, 1997), and (2) prolonged replication (up to several years) after initial infection/reinfection (Lefrere *et al.*, 2005). The prevalence of B19V DNA in asymptomatic blood donors (~1%) is sufficient to contaminate the plasma pools used for fractionation (Azzi *et al.*, 1999; Ke *et al.*, 2011). During epidemics the asymptomatic donors frequently show high viral titers $> 10^6$ IU mL^{-1} without concurrent antibodies that prompt unrestrained contamination of blood collections (Kooistra *et al.*, 2011). As a consequence, B19V is easily

transmitted by blood transfusion or hemotherapeutic procedures. Acute B19V infection was reported after transfusion of heat-treated factor IX concentrates (Lyon *et al.*, 1989), high-purity heated or solvent–detergent-treated factor VIII concentrates (Azzi *et al.*, 1992) and factor VIII or prothrombin complex concentrates (Williams *et al.*, 1990).

Such types of transmission may have serious consequences in several categories of high-risk patients: (1) individuals with shortened red cell survival (chronic hemolytic conditions); (2) pregnant women; and (3) immunocompromised patients (Lefrere *et al.*, 2005). In immunocompromised patients B19V infection can result not only from blood transfusion or airway transmission but also from reinfection and reactivation. It is difficult to distinguish reinfection and reactivation in such patients but both mechanisms can lead to persistent reticulocytopenia and chronic anemia (Broliden, 2001). The presence of viral DNA in blood donations is usually associated with the presence of anti-B19V immunoglobulin G (IgG) (Daly *et al.*, 2002), but whether these antibodies eliminate or reduce the infectivity in the transfused fraction is controversial. Nevertheless, transfusion transmission of B19V in the presence of neutralizing anti-B19V IgG antibodies is possible and is explained by their qualitative inability to inhibit the virus infectivity (qualitative defects) (Kurtzman *et al.*, 1989).

Transplacental transmission

B19V vertical transmission occurs in approximately 30% of the cases of maternal infection and in 2–5% of these cases, results in *fetal hydrops* or *fetal death*. The exact mechanism of B19V fetal transmission and the consequent development of fetal infection have not been elucidated fully. Recent studies indicate that fetal capillary endothelium in the placental villi can support B19V replication. Infection of the placental endothelium may lead to structural and functional destruction of the blood exchange between the mother and the fetus and facilitate the fetal involvement of B19V (Pasquinelli *et al.*, 2009). The highest risk of transplacental transmission is between the first and second trimesters. Termination of pregnancy is not indicated because B19V has no oncogenic properties. Intravascular and intraperitoneal transfusion of high doses of intravenous globulin may help to clear B19V and normalize the blood circulation and anemia (Hsu *et al.*, 2007).

Immune response to B19V infection and mechanisms of autoimmunity

B19V infection is a common event in children and adults and more than 50% have lifelong immunity (Tolfvenstam & Broliden, 2009). Acute B19V infection is controlled by the neutralizing antibodies of the humoral immune response: the viremia declines with the appearance of IgM, followed by synthesis of lifelong anti-B19V IgG (Lefrere *et al.*, 2005). IgM antibodies are directed mainly against the conformational VP2-specific epitopes. They persist up to several weeks after acute viremia but in some patients can be found for up to several months. In the natural course of the humoral response, IgM antibodies are substituted by IgG directed to VP1 and VP2 conformational and, to some extent, VP1 linear epitopes (Gray *et al.*, 1993).

Cell-mediated immunity is also developed during B19V infection, as demonstrated by Th1 and Th2 clonal proliferation in B19V-seropositive patients, but this is difficult to analyze (Franssila *et al.*, 2005). Following VP1/VP2 antigen stimulation in previously infected individuals, an elevated secretion of interferon-γ and interleukin (IL)-2 is observed, indicating the existence of Th1 cell-mediated response (Corcoran *et al.*, 2000). B-cell memory can also be established and is maintained against the nonlinear conformational VP2 epitopes and the linear VP1 ones (Corcoran *et al.*, 2004). B19V persistence probably contributes to the development of autoimmune responses (Lunardi *et al.*, 2008). PLA_2 activity (von Landenberg *et al.*, 2003) belonging to the VP1 region as well as molecular mimicry events (Reitblat *et al.*, 2000) are probably involved in the development of antiphospholipid antibodies (autoimmunity). Another possible cause may be increased gene expression of tumor necrosis factor-α and IL-6, provoked by the NS1 cytotoxic properties, which transactivate the promoter regions of these two genes (Lunardi *et al.*, 2008).

Viral persistence

Low-level persistent B19V viremia has been described in various studies. Persistent viremia has been found to last from 10 months (Fattet *et al.*, 2004) to 5 years (Kerr *et al.*, 1995) after acute infection. PCR data indicate also that B19V can persist in the circulation of immunocompetent individuals (Cassinotti & Siegl, 2000; Lefrere *et al.*, 2005). The asymptomatic presence of B19V in healthy hosts is not generally recognized because such a phenomenon is mainly observed in cases associated with disturbed immune status or specificities in the immune response. Lefrere *et al.* (2005) examined multiply transfused patients with congenital hemoglobinopathies that could already have a relative degree of immunomodulation due to the repetitive transfusions, probably predisposing them to B19V persistence (Klein, 1999).

Obviously, viral persistence can be caused by mechanisms other than immune status disturbance. Norja *et al.* (2006) proposed that the different human tissues, or 'bioportfolio' of the organism, can have a capacity for lifelong storage (> 70 years) of *Erythrovirus* genomes. Such 'deposits of B19V DNA' could serve as a source of persistent replication. Another possibility that has been discussed is the existence

of specific B19V DNA integration sites into the human genome (Reed *et al.*, 2000), as has demonstrated for the MMV (Corsini *et al.*, 1997) and human dependoviruses (Chiorini *et al.*, 1996). Nevertheless, there are no categorical experimental data demonstrating the integration of B19V into human DNA. The establishment of B19V persistence is of critical importance in high-risk patients and blood transfusion. There is the theoretical risk that approximately 1% of the blood donations can contain B19V DNA which will cause symptomatic disease, but parameters such as the role of anti-B19V IgG in donated blood and the minimum B19V infectious dose should also be carefully examined.

Clinical syndromes commonly associated with B19V

The development of B19V disease is influenced by the host's hematological and immunological status. Healthy children usually develop asymptomatic infection, nonspecific illness or benign *erythema infectiosum*. But in patients suffering from decreased production or increased loss of erythrocytes, B19V can cause a severe drop in hemoglobin values, leading to aplastic crisis and anemia, which can be fatal. Immunocompromised patients can develop a state of chronic anemia due to their inability to clear the persistent B19V replication (Heegaard & Brown, 2002; Broliden *et al.*, 2006).

Erythema infectiosum

Erythema infectiosum ('fifth disease') is the major manifestation of B19V infection in children. It is a self-limiting, contagious exanthema (Anderson *et al.*, 1983) that has been recognized by pediatricians for over a century. Typically, the rash involves the cheeks, hence its other name *slapped cheek syndrome*, as the child has the appearance of having been slapped on the both cheeks. Approximately 25–50% of such infections may be asymptomatic (White & Fenner, 1994; Kudesia & Wreighitt, 2005).

TAC

TAC is described as a precipitous drop of the hemoglobin associated with cessation of reticulocyte production. It is a temporary but potentially life-threatening condition in patients with various forms of chronic hemolytic anemias (Lefrere *et al.*, 1986a) such as SCD (Pattison *et al.*, 1981), α- and β-thalassemia (Lefrere *et al.*, 1986b), iron deficiency anemia, or hereditary spherocytosis (Lefrere & Bourgeois, 1986). The patient presents with severe anemia associated with pallor, weakness and lethargy. The sudden drop of hemoglobin values is due to the disappearance of the erythroid progenitors in the bone marrow and the reticulocytes in the blood. Recovery normally occurs within 1 week but it is maintained by intensive blood transfusions, which are lifesaving in such cases (White & Fenner, 1994).

Infection in pregnancy (hydrops fetalis)

Hydrops fetalis is a condition defined by the presence of generalized fetal subcutaneous tissue accumulation of fluids (edema) in at least two fetal compartments. Other locations of edema can include pleura (pleural effusion), pericardium (pericardial effusion) and abdomen (ascites). The multiple causes of the development of fetal hydrops are divided into immune (*Rh disease*) and nonimmune ones.

Among all nonimmune causes, B19V is of principal importance. As the fetus is highly dependent on the increased erythropoiesis rates and B19V arrests erythropoiesis, aplastic crisis, profound cardiac failure and edema are often developed (Kinney *et al.*, 1988; Van Elsacker-Niele *et al.*, 1989). Cardiac failure may be the result of the severe anemia but may also be associated with myocarditis, which can cause arrhythmias or even cardiac arrest without evidence of anemia, cardiac failure or hydrops (Lamont *et al.*, 2010). Fetal transmission may occur in up to 30% of the cases with maternal infection but is not always associated with congenital defects. It is estimated that fetal loss can occur in ~7–10% of infected women. The greatest risk is between the 11th and 20th week of gestation coinciding with the increased activity of the fetal liver and the shortened half-life of red blood cells (Yaegashi *et al.*, 1998). After the 20th week of gestation, although fetal transmission may occur, it is believed that it is not associated with unfavorable outcome. Evidence also suggests that the asymptomatic infection in pregnancy carries a higher risk of transmission because it can be connected with weak immune response unable to clear B19V replication (Kudesia & Wreighitt, 2005; Bekhit *et al.*, 2009).

Chronic anemia [pure red cell aplasia (PRCA)]

Patients with various types of immunosuppression may not be able to clear B19V effectively, which can result in persistent low-titer viremia accompanied by PRCA and chronic anemia. PRCA has normally been observed when patients with disturbed cell-mediated immunity are infected with B19V, especially patients with acute lymphoblastic leukemia (Weiland *et al.*, 1989), HIV-positive individuals (Sanphasitvong *et al.*, 2005; Watanabe *et al.*, 2010), bone marrow transplant recipients (Cohen *et al.*, 1997) or children with congenital immune deficiencies (Reed *et al.*, 2000). Such patients may develop persistent anemia due to the continuous and uncontrollable B19V replication and the constant involvement of the erythroid progenitors (Soliman *et al.*, 2009). Studies involving large cohorts of HIV-infected patients demonstrated low titers of circulating B19V DNA (~10^5 copies mL^{-1}) but concluded that there was a minimal impact of the virus and the viral genotype for the development of severe anemic conditions (Ferry *et al.*, 2010); however, such a possibility may exist due to the nature of HIV-infection and the side effects of the antiretroviral therapy.

Approximately 5% of adults and 10% of children undergoing chemotherapy for hematological malignancies may be persistently infected with B19V, which can result in severe and even fatal cytopenias (Broliden *et al.*, 2006). Moreover, the presence of B19V can be associated with lymphadenopathy or splenomegaly, which is attributed to the B19V-associated erythroid suppression and immune cell proliferation and can be important for the pathogenesis of leukemic disease. Cases of B19V-associated pancytopenias of the different hematological cells could be misinterpreted as a relapse of the underlying malignancy or drug toxicity (Zaki & Ashray, 2010). At the same time, B19V infection can seriously worsen the course of the neoplastic disease and increase the number of relapses (Jitschin *et al.*, 2010).

B19V-induced cytopenias in patients with hematological malignancies can significantly prolong the periods of unwanted interruption of chemotherapy, and can require additional bone marrow investigations and more erythrocyte and thrombocyte transfusions (Gustafsson *et al.*, 2010; Jitschin *et al.*, 2010). The frequent blood transfusions and bone marrow aspirates are not only associated with higher costs but also imply an additional risk of infections or complications transmitted by the transfusions. For these reasons, measures avoiding iatrogenic or nosocomial spread of B19V infection among patients with hematological malignancies must be taken (Jitschin *et al.*, 2010). The early molecular detection of B19V in bone marrow samples or plasma is a clinically important tool for all children with malignancies, not only because of the early diagnosis and appropriate treatment but also to limit the effects of the associated complications (Broliden *et al.*, 2006).

Arthropathy

Reid *et al.* (1985) were the first to associate B19V and the development of arthritis. Arthralgias and arthritis are described as major symptoms of B19V infection in adults (Cassinotti *et al.*, 1995). It is supposed that the antibodies developed against B19V in such cases are deposited in the synovial fluid of the joints and thus contribute to the pathogenesis of arthralgia. B19V arthralgia is a self-limiting condition but it may recur after several months and may involve different joints (Guillaume *et al.*, 2002).

Virological diagnosis

Diagnostic cytopathology, electron microscopy and immunohistochemistry

The cytopathological abnormalities caused by B19V are characteristic but they are not sufficient for diagnostic purposes. Active B19V infection induces the formation of giant pronormoblasts in the bone marrow. They are characterized by their cytoplasmic vacuolization, ground-glass appearance of the nucleus and clear perinuclear halo. The chromatin is often immature and appears as a thin rim around the viral inclusion. The cytological methods can be useful for cytopathological evaluation of suspected hydrops fetalis (Iwa & Yutani, 1995). B19V can also be detected using electron microscopy in plasma and fetal tissues, particularly in cases of high-titer viremia during acute infection (Pasquinelli *et al.*, 2009). However, electron microscopy is a technically sophisticated method and cannot be used for routine purposes.

Immunohistochemistry can be used for diagnostic purposes but is a time-consuming method. In patients with dilated cardiomyopathy or inflammatory myocardial diseases, when B19V is suspected as a causative agent, immunohistochemistry enables visualization of B19V VP1/VP2 within the myocardium (Pankuweit *et al.*, 2005; Escher *et al.*, 2008). Immunohistochemistry can also be used for the pathological examination of different tissue materials from hydropic fetuses (lungs, liver, thymus, kidneys, heart, placenta) (Landolsi *et al.*, 2009).

Serological methods

The precise diagnosis of recent or past infection with B19V depends on the use of enzyme immunoassays to detect anti-B19V IgM and IgG in plasma. Commercial assays have been developed using expression of B19V capsid proteins (VP1 and VP2) in the baculovirus system (Michel *et al.*, 2008). The general advantage of the baculovirus system is its ability to induce post-translational protein folding, which is necessary for the production of soluble and conformationally complete capsid proteins. This is of critical importance for the sensitivity of the immunoassays because the proteins retain their conformational epitopes and there is less of a risk of false-negative results (Brown *et al.*, 1990).

To confirm acute B19V infection, IgM antibodies must be detected in plasma or serum. These antibodies are synthesized approximately 7–10 days after the high-titer viremia and are directed against linear and conformational epitopes of VP1 and VP2. The IgM response against VP1 conformational epitopes predominates, whereas IgM against the linear VP2 antigen is found less frequently. IgM antibodies against VP1 and VP2 conformational epitopes and VP1 linear epitopes appear at the same time but IgM antibodies against linear VP2 epitopes diminish rapidly. Thus, utilization of VP1/VP2 linear antigens alone for diagnosis of recent B19V infection could give false-negative results (Manaresi *et al.*, 2001). Currently, there is no International Standard for the preparation of B19V IgM. Decline of IgM production is followed by development of IgG antibodies. They have lifelong persistence and protective functions but an important feature is that IgG directed against the linear epitopes of

VP2 fades rapidly. Similarly, antibodies raised against the linear epitopes of VP1 are also observed. This confirms again that for immunological detection of anti-B19V IgG, at least one undenatured antigen is required (Kerr *et al.*, 1999). In contrast to IgM limitations, there is an International Standard for IgG (2nd International Standard 2003; code 01/602), which is a valuable tool for the accurate confirmation of past B19V infection among different laboratories that use variable test systems (Ferguson *et al.*, 1997).

PCR

The first PCR methods for B19V detection in serum and fetal tissues emerged early in 1989 (Salimans *et al.*, 1989). Nowadays there is a wide variety of molecular methods used for B19V detection with a significant number of primers and genome targets. B19V itself evolves rapidly, at more than 10^{-4} per site per year (Shackelton & Holmes, 2006). This suggests considerable B19V variation, which influences the sensitivity of the applied molecular test systems, especially the primers and hybridization probes used. Another point is that B19V can persist in the blood or bone marrow of healthy subjects for long periods and the PCR detection of viral DNA may simply reflect decay of the viral load. Hence, the qualitative detection of B19V DNA by qualitative PCR is not a valid diagnostic assay to confirm recent infection (Simeoni *et al.*, 2010). Moreover, most of the commercial assays detect genotype 1 of B19V rather than genotypes 2 and 3, which are often misdiagnosed or underestimated (Koppelman *et al.*, 2010).

The nucleic acid amplification technology (NAT) assay for B19V therefore requires precise standardization. Well-characterized reference materials are necessary to provide appropriate validation, decrease the ambiguity between the different assays used by laboratories, and establish an internationally accepted standard unit for measurement of B19V DNA (Saldanha, 2001). This problem has now been addressed with the introduction of the *WHO International Standard* for B19V DNA amplification (Saldanha *et al.*, 2002). In 2010, the 1st WHO International Standard for B19V DNA for NAT assays (code 99/800) was replaced by the 2nd WHO International Standard for B19V DNA for NAT assays (code 99/802) with a potency 10^6 IU mL^{-1} (or 10^5 IU per vial) (Baylis *et al.*, 2010). This standardized PCR quantitative control can be applied successfully not only for optimization molecular diagnostic procedures but also for screening of plasma minipools and various blood products, thereby improving the safety of the transfusions and the hematological therapy (Corcoran *et al.*, 2004). The routine B19V genotyping is an important issue for viral diagnosis as it can trace equal sequences in cases transmitted by transfusion (Yu *et al.*, 2010).

The quantification of B19V viral load is important in terms of avoiding contamination of blood donations. Nowadays, most United States plasma fractionators perform minipool B19V NAT screening as a control measure to detect the virus and thereby to exclude donations with a B19V DNA viral load up to 10^6 IU mL^{-1} so that the level of B19V DNA in manufacturing plasma pools prepared for production of plasma derivates does not to exceed 10^4 IU mL^{-1} (Geng *et al.*, 2007). Minipool as opposed to individual specimen testing is performed to reduce the cost associated with B19V detection. However, despite the high sensitivity of the B19V PCR detection systems, the associated cost combined with the extremely high titers of viremia, potentially leading to cross-contamination during screening and the possibility of false-negative PCR due to the sequence differences between genotypes 1, 2 and 3, indicates that alternative strategies must be considered for screening blood donations (Doyle, 2011).

NAT B19V testing systems must detect and quantify adequately all three viral genotypes (US Food and Drug Administration, 2008). An emerging alternative for B19V-sensitive detection may be loop-mediated isothermal amplification, which has been applied successfully for the detection of porcine parvoviruses. It shows higher sensitivity than the TaqMan real-time PCR but its ability to quantify virus adequately is doubtful (Chen *et al.*, 2009).

The use of the different diagnostic procedures during pathogenesis of B19V infection is demonstrated in Fig. 2.

B19V infection in patients with SCD and thalassemia

B19V is a common pathogen worldwide; serological studies indicate that more than 50% of people are infected during childhood but higher prevalence rates are observed among children with SCD in some tropical regions: Brazil (80% among children of 5–15 years) (Amaku *et al.*, 2009) and Australia (55% of the same year range) (Kelly *et al.*, 2000). B19V outbreaks occur repetitively, generally at intervals of 3–4 years (Serjeant *et al.*, 1981). These outbreaks as well as ongoing sporadic cases of B19V infection have a major impact on the development of TAC among patients with various forms of hereditary anemias. TAC in a patient with hereditary anemia is defined as a transient episode of PRCA, with absence of erythroid precursors in the bone marrow and of reticulocytes in the blood circulation. Because of the shortened red cell life span in such patients, a temporal interruption of erythropoiesis leads to a severe fall in hemoglobin and hematocrit levels and, as a consequence, acute life-threatening anemia (Saarinen *et al.*, 1986).

It was discovered in 1981 that B19V causes TAC in children with SCD and it is now clear that almost 70% of all B19V infections in this cohort result in this disease (Serjeant *et al.*, 1981). B19V can trigger acute cessation of erythrocyte production, causing TAC in patients with

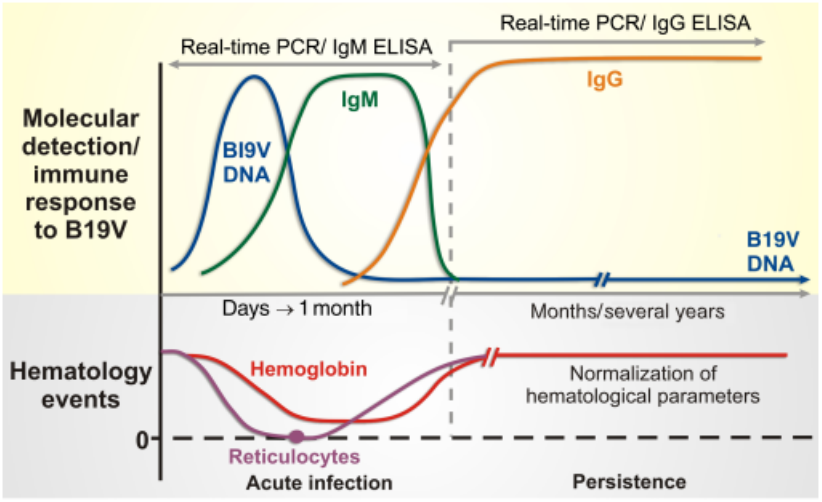

Fig. 2. Main immunological, hematological and diagnostic events during B19V acute and persistent infection. The acute B19V infection is connected with high viral load due to the extensive replication, coinciding with a profound drop of hemoglobin values and disappearance of the reticulocytes from the peripheral blood. The virus is easily detectable by real-time or conventional PCR. Up to 5 days after the acute B19V infection, anti-B19V IgM appear and are detectable by enzyme immunoassays (EIA). After acute viremia, lifelong immunity to B19V is established, consisting of IgG antibodies. They are detectable during an individual's lifetime and have protective functions. B19V DNA is not always cleared from the organism, thus B19V frequently establishes persistent infections. During persistent infection, EIA for anti-B19V IgG as well as the PCR methods for B19V are positive against the background of normal blood parameters.

hereditary anemias (iron deficiency anemia, SCD, thalassemia, hereditary spherocytosis) that already have a shortened red blood cell lifespan (Servey *et al.*, 2007). Manifestations of B19V infection in SCD patients can range from transient and isolated anemia to a life-threatening drop in hemoglobin levels (Biesma & Nieuwenhuis, 1997; Fartoukh *et al.*, 2006). In children with SCD the B19V anemia is characterized by profound reticulocytopenia and frequent splenic sequestration (Yates *et al.*, 2009). Moreover, in patients with SCD, B19V can be a causative agent of massive virally induced bone marrow necrosis, complicated by systemic fat embolism, fungal superinfections (Fartoukh *et al.*, 2006), and even fatal bone marrow embolism. Bone marrow necrosis is a common complication of sickle-cell vaso-occlusion and is frequently found at autopsy in patients dying from such episodes. B19V infection can provoke bone marrow necrosis, triggering embolic syndrome (Godeau *et al.*, 1991; Rayburg *et al.*, 2010). Regarding superinfections, B19V and herpes simplex superinfection were described in a child with life-threatening myocardial dysfunction (Krishnamurti *et al.*, 2007) but the interactions between these two pathogens in aggravating the cardiac functions are not clear.

B19V may be involved in the etiology of myocarditis and acute myocardial infarction in children and adults with SCD (Assanassen *et al.*, 2003; Munro *et al.*, 2003). Unlike enteroviruses that damage the heart muscle via direct lysis of the infected myocytes, B19V does not infect myocytes but infects endothelial cells of small intracardiac arterioles and venules, which results in impairment of myocardial microcirculation with secondary myocyte necrosis during acute infections (Krishnamurti *et al.*, 2007). Another severe clinical condition among patients with SCD is glomerulonephritis accompanied by proteinuria. The involved kidney tissues demonstrate *focal proliferative glomerulonephritis*, progressing to focal or segmental glomerulosclerosis. What is more important, the renal tissue tested by PCR for B19V DNA is positive, whereas the blood is negative. This indicates a direct relationship between B19V replication and kidney involvement but the role of the kidney in supporting the persistent infection is doubtful (Wierenga *et al.*, 1995; Tolaymat *et al.*, 1999). *Acute chest syndrome* (ACS) continues to be a major source of morbidity and mortality among patients with SCD. It is characterized by the presence of pleuritic chest pain, fever, raises on lung auscultation and pulmonary infiltrates. The pathophysiology of this disease remains poorly understood, leading to the descriptive term 'acute chest syndrome' designated by Charache *et al.* (1979). B19V infection is recognized as one of the causes in the development of ACS, as viral replication is often detected in patients with SCD and ACS. Thus, B19V might contribute not only to the development of TAC but also to various spectra of severe conditions including ACS (Lowenthal *et al.*, 1996). Acute B19V infection in SCD patients is also involved in the development of thrombocytopenia associated with hemocytic histiocytosis (transient blood plasmacytosis), which is probably an immunological response to B19V infection (Koduri & Naides, 1996).

There are some reports that B19V can induce *encephalopathy* in patients with β-thalassemia ($S\beta^+$) due to the abnormal immune reaction to B19V with central nervous system (CNS) autoantigens. B19V-induced CNS hypersensitivity vasculitis should therefore be considered in the differential diagnosis of the encephalopathy in patients with thalassemia (Bakhshi *et al.*, 2002). Cerebrovascular complications in close temporal association with aplastic crisis among patients with SCD have also been described. The crude risk of cerebrovascular episodes in the 5-week interval after B19V infection was calculated as 58 times greater than expected, evidence of a causal association between B19V infection and cerebrovascular complications (Wierenga *et al.*, 2001).

The outcome of the TAC episodes in children and adults with SCD is generally benign but most of the patients must be treated by frequent blood transfusions to reduce the risk of circulatory collapse. Furthermore, as B19V induces various complications (acute splenic sequestration, hepatic sequestration, acute chest syndrome, nephritic syndrome, meningoencephalitis and stroke) the development of a vaccine or other prevention strategies that would reduce B19V morbidity and mortality among patients with hemoglobinopathies and SCD in particular is of high priority (Smith-Whitley *et al.*, 2003).

B19V infection in malaria patients and regions endemic for malaria

The high-titer B19V viremia causing a significant drop of the hemoglobin and reticulocytes, could have serious consequences in patients, especially children with underlying malaria or individuals living in malaria regions of the world (Africa, Latin America, South and South-East Asia). Although it is generally accepted that the anemic state in such areas is caused mainly by the malaria, iron deficiency, hookworm (*Ancylostoma duodenale*, *Necator americanus*) infection and SCD, as B19V is a pathogen with significant impact on anemic patients, its importance for aggravating *Plasmodium* sp. infection is notable. Such process could be easily facilitated by the ~3–7-day cessation of erythropoiesis induced by B19V and the dramatic hemolytic potential of malaria (Pasvol, 2006).

Recently, B19V coinfection was reported in a patient with malaria leading to severe anemia, renal failure and hepatosplenomegaly (Ingrassia *et al.*, 2010). However, such coinfections are probably rare given the small number of similar reports: the first describing severe outcome of concomitant B19V-*Plasmodium* sp. infection in two Ghanaian patients (Scarlata *et al.*, 2002) and the second, severe anemia caused by B19V in an Indian boy with *Plasmodium* sp. infection (Gupta & Singh, 2005). Apparently, there is the likelihood of an unfavorable outcome with coinfection, but there are significant inconsistencies between studies examining the relationship between malaria and B19V.

For example, in the archipelago of Papua and New Guinea (malarial endemism) a strong correlation was observed between acute B19V infection and severe anemia, which contributes to the high levels of morbidity and mortality of malaria (Wildig *et al.*, 2006). Similar conclusions were obtained from studies carried out in the Republic of Niger (tropics) (Jones *et al.*, 1990). In contrast, in Malawi (Yeats *et al.*, 1999) and Kenya (Newton *et al.*, 1997), no relationship between the clinical course of malaria and other causes including B19V was observed. One probable explanation could be that Kenya and Malawi are located in a specific geographic region of Africa (Great Rift Valley), where the circulation and the pathological impact of both B19V and *Plasmodium* sp. might show variations. A recent finding that chloroquine and its derivates (medicaments used extensively to treat malaria) exacerbate B19V replication (Bönsch *et al.*, 2010b) and thus anemia poses serious issues regarding the treatment of malaria, the significance of B19V coinfection and patient management. In general, there is a risk of serious complications of B19V-*Plasmodium* sp. coinfection in patients with malaria or individuals living in endemic regions, but their relationship and the contribution to worsening of the anemic state is still a matter of debate.

B19V and blood transfusion

Previously it was thought that the antivirus antibodies in plasma make B19V less infectious and, as a consequence, the transfusion of positive B19V DNA plasma was a safe procedure. This has been contradicted by the discovery of the different viral genotypes, novel human parvoviruses (*PARV4*), the transfusion transmission of B19V in spite of the presence of antiviral antibodies and the severity of the clinical picture in predisposed patients.

B19V is easily transmitted by blood transfusion and therapy with plasma-derived products. Moreover, its viral load in plasma-derived products varies between 2×10^1 and 1.3×10^3 gEq mL^{-1} (Modrow *et al.*, 2010). It is a nonenveloped virus and as a consequence is highly resistant to detergents and organic solvent treatment. The small size of B19V makes its removal by filtration with virus-removal membranes impossible (Yunoki *et al.*, 2004). Liquid–heat treatment, which is considered to be a highly reliable method for virus inactivation, also has an ambiguous effect on B19V, as it can withstand very high temperatures: 100 °C for 30 min after lyophilization (Santagostino *et al.*, 1997). Lipid solvents also have no effect for B19V inactivation (Blumel *et al.*, 2002). Despite all these precautions, B19V can contaminate minipools, coagulation factors and packed red cell concentrates. Such contamination is highly problematic because, depending on the transfused group, the virus may

impact high-risk patients. Despite the widely known fact that B19V infection can be transmitted via contaminated blood, there are at present no strict regulatory requisites regarding B19V contamination of pooled plasma or blood products before product use and transfusion (Corcoran & Doyle, 2004).

In relation to this, the first evidence of blood-transmitted B19V infection was described in 1995 in a 22-year-old patient suffering from thalassemia major, followed by the development of TAC and transitory heart failure (Zanella *et al.*, 1995). Sustained anemia and pure red blood cell aplasia as well as pancytopenia were observed in patients with hematological malignancies and hemolytic diseases after transfusion of B19V-contaminated blood in Japan (Satake *et al.*, 2011). Hypothetical B19V transmission was also reported by Hourfar *et al.* (2011), as B19V sequences detected in transfused patients were identical to the sequences obtained from the red cell concentrates used for their hemotherapy.

According to their importance in transfusion medicine, transfusion-transmitted infectious agents are divided into several groups (August 2009): 'red' – potential for severe clinical outcome; 'orange' – higher priority for the future; 'yellow' – agents of public and regulatory concern; and 'white' – agents which do not appear to need higher prioritization at present. B19V falls into the 'yellow' priority group and is considered an emerging infectious agent in blood transfusion (Stramer *et al.*, 2009). Nowadays, the actual frequency of B19V transmission via blood products is not assessed prospectively and the complete picture of transfusion-transmitted cases could not be assessed. As the group at major risk of B19V infection/reinfection includes patents who have inherited anemias and frequent need of transfusion, it is necessary that routine quantitative PCR diagnostic techniques are implemented, as optimized by the WHO standards, not only in clinical virology laboratories but also in transfusion centers worldwide.

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