Dengue Fever Virus (DENV)

Arbeitskreis Blut, Untergruppe «Bewertung Blutassoziierter Krankheitserreger»

Introduction

The name of the disease is said to be derived from the Portuguese word dengue which means fastidious or vain, which would describe the disturbed gait of an infected person suffering from muscle and bone pain (see also English: ‘dandy’). Dengue virus and dengue haemorrhagic fever virus are synonyms. The dengue fever virus (DENV) is a member of the genus of the 53 flaviviruses and ranks among the 27 species of flavivirus which can be transmitted by mosquitoes. The significance of arboviruses was summarised in a paper published in 2004 [1]. This paper addresses the subject of whether DENV is one of the threatening viruses that might become significant in transfusion-medicine in the near future.

1. Current Knowledge about the Pathogen

Probably the oldest report consistent with the clinical course of DENV infection originates from China and dates back to the year 992 [2]. Historical reports on the so-called ‘breakbone fever’ date back more than 200 years, 1779 from Batavia, Indonesia, and Cairo, Egypt. The first dengue fever epidemic reported in an English-speaking country occurred in Philadelphia, USA, in 1780. Further cases followed in 1934 in Florida and in 1945 in New Orleans [3]. Major epidemics with haemorrhagic shock (dengue fever virus haemorrhagic shock syndrome – DHSS) occurred in Australia in 1897, in Greece in 1928, and in Formosa in 1921. The long intervals of 10–40 years between the epidemics in the same region were shortened due to the increasing trade after World War II. Besides, the rapid urbanization in South East Asia led to hyperendemics [2]. The propagation of Aedes aegypti and other Aedes species by modern means of transport, and in particular, shipping, led to a rapid spread of Aedes mosquitoes and at the same time DENV in all tropical regions of the world, so that the number of yearly infected people is assumed to be 50–100 million, that of hospitalisations around 500,000, and that of infected patients with complications 250,000 [4].

Urban and Sylvatic Cycle

Dengue fever is a typical zoonosis the host of which is essentially the monkey; however, after transmission to humans and the presence of the appropriate mosquito population, animal hosts are no longer required. All old-world and new-world monkeys are infectable with DENV and develop clinical signs of infection as well as viraemia. The significance of other animals as a reservoir for the proliferation of DENV is unclear. New-born mice can be infected with DENV, whereas adult guinea pigs, rabbits, hamsters, chickens, bamboo rats, and lizards cannot. Mice can only be infected in the lab using high doses. Neurotropism develops in these mice with increasing passage [5]. In Mexico, DENV antibodies were identified in 1 fruit-eating and 3 insect-eating bats, while DENV-2 (Dengue fever virus serotype 2; serotypes see section 1.1) was detected in 2 fruit-eating and 1 insect-eating bat. These bats were DENV-antibody negative [6].

Apart from Aedes aegypti and Aedes albopictus, DENV reproduces in Aedes africanus, Aedes leuticephalus, Aedes opok, Aedes taylori, Aedes furcifer, and other species [7] so that other Aedes species, too, can serve as vectors.

The sylvatic cycle occurs in monkeys, and possibly also other smaller mammal species. Various Aedes species are involved in the cycle, which have become accustomed to the habitat of primates. Rain forests are the preferred reservoir in tropical regions. In West Africa, this also applies to gallery forests, so that epidemics also occur in savanna regions [7]. The sylvatic and urban cycles are interrelated via the mosquito vector [8]. The urban cycle is maintained by Aedes aegypti and Aedes albopictus strains which have become accustomed to the human habitat. They lay their eggs in small water reservoirs (sewage water, drinking water supplies, plastic waste), in which the larvae hatch after several days at temperatures >25 °C and develop into mosquitoes. The vertical transmis-
Transmission Pathway

Aedes mosquitoes are essential natural vectors. Transmission by other mosquito species should be possible. Ticks are epidemiologically insignificant for transmission. Replication of the virus in the mosquito essentially depends on the external temperature. Increased temperatures elevate the virus concentration in the mosquito, thus increasing the transmission rate. However, mosquitoes die at very high temperatures and low humidity, which presents limits to transmissibility. Other factors determining the replication of the vector include relative humidity, rain, dryness, wind and storm, as well as conditions for deposition of eggs and larvae development [8].

1.1 Characteristics of DENV

1.1.1 Virus

DENV is one of the medically significant flaviviruses which also include other arboviruses such as West Nile Virus (WNV), Japanese Encephalitis Virus, Yellow Fever Virus, and Tick-Borne Encephalitis Virus. The enveloped DENV has a diameter of approximately 50 nm (fig. 1) with an electron-tight capsid of around 30 nm containing the single-stranded positive-sense RNA. The lipid envelope contains the E-protein (envelope; mw 50,000) which carries the essential antigenic determinants and the M-protein (membrane; mw 8,000) which is formed from the pre-M protein (prM; mw 18,000) by proteolytic cleavage [9]. The prM is required for adhesion and maturation of the virus on the membrane of the endoplasmatic reticulum. The E-protein forms dimers in the membrane and is flexible by rotation so that different epitope regions can be presented on the surface in mammal and mosquito cells. This flexibility, which is partly associated with formation of trimers, facilitates the fusion of virus envelope and cell membrane and can also be found in immature viruses as described for the DENV serotype 2 (DENV-2) [10]. Non-infectious empty virus particles, too, can be released by cells [11]. DENV can be replicated in many primary and permanent cell culture cells of e.g. humans, monkeys, hamsters, or mosquitoes [8].

1.1.2 DENV Serotypes 1 to 4

DENV is distinguished in 4 serotypes, DENV-1 to 4, which are further subdivided into genotypes. Among the serotypes, DENV-1 and 3 are more closely related to each other than to DENV-2 and finally DENV-4.

1.1.3 Virus Receptor and Replication

Adherence of DENV to the host cell, usually to macrophages or dendritic cells, partly also endothelial cells, occurs via the C-type lectin, DC-SIGN (ICAM-3) and via the carbohydrate chain of the E-protein [14]. Other membrane proteins such as

Fig. 1. Transmission electron microscopic picture of DENV, with a size of around 30 nm in diameter. The thick stained round envelope may be seen. The photograph is courtesy of Dr Regine Allwin, Institute of Medical Virology, University Frankfurt/M.

DENV-1

Genotype 1 of DENV-1 seems to be less virulent for humans.

DENV-2

Four pathogenic genotypes of DENV-2 circulate: the sylvatic West-African, American, South-East Asian, and Malayan-Indian DENV-2. DENV-2 is highly pathogenic for humans.

DENV-3

DENV-3 is subdivided into 4 genotypes. The American genotype has the lowest epidemiological propagation potential, since the virus replicates in mosquitoes only to a minor extent [12]. The South-East Asian and Indian genotypes of DENV-3 were responsible for the major epidemics which occurred on both subcontinents and Sri Lanka in 1989/90.

DENV-4

Three genotypes of DENV-4 are known of which the Malayan genotype has the lowest pathogenic significance, whereas the 2 other genotypes have also spread in the 2 Americas. Six amino acid exchanges in the prM, E, NS4B, or NS5 regions can be sufficient to cause a change in virulence [13]. In the E-protein, intramolecular links between the amino acids 204Arg, 261His, and 257Glu in mutants are responsible for the change in virulence. The age of the common precursor, from which the currently known 4 DENV serotypes developed in primates, is calculated as roughly 500 years (range 100–1,500 years) [4, 7]. The age determination is based on the calculation of mutations in the nucleic acid sequence of different DENV strains isolated at known time points at particular locations. The calculation performed raises the question whether the mutations found all developed in humans, in animals, or by recombination. If the calculation is correct, DENV has a large adaptation potential for future epidemics and further hosts. Various DENV can form intragenotypic variants [8], which extends the variability of the DENV strains.

Dengue Fever Virus (DENV)
1.1.4 Genome

The genome of DENV is typical of flaviviruses with a 5' non-encoding region (NCR) and 3' UTR (untranslated region) on the flanks, between which the regions of core, prM, E, and the nonstructural proteins are located. The genes for the nonstructural proteins encode the proteins NS1, NS2A and NS2B, with NS3 consisting of protease and helicase, NS4B and NS5 containing methyltransferase and the RNA-dependent RNA polymerase (fig. 2). The 10 proteins are cleaved from the polyprotein precursor protein [9]. The methyltransferase, which influences the 5'-cap structure, can be inhibited by ribavirin [22, 23].

1.2 Infection and Infectious Disease

Transmission usually occurs via a mosquito bite of the Aedes species. After an incubation period of typically 2–7 days, the DENV-specific symptoms occur, which include sudden high fever, head and limb ache, retrobulbar pain, and severe muscle pain. The latter was the reason why the disease is also called break-bone fever. After a few days, a maculopapular exanthema (fig. 3) manifests itself beginning on the torso and spreading out into the extremities and the face. Petechiae may also occur, in particular, at the site of the mosquito bite. In the first 2 weeks, infected individuals show extreme pathological signs, which subside 1 week after occurrence of the exanthema. The fever course is typically biphasic (saddle-shaped) with the second peak starting as soon as the exanthema appears. Fever of up to 41 °C is not unusual (fig. 4). The diagnosis is confirmed by detection of IgM and IgG antibodies, and viraemia is confirmed via nucleic acid testing (NAT; see section 1.4). The following diseases are distinguished, depending on virulence of DENV and clinical symptoms: Dengue fever, Dengue haemorrhagic fever (DHF), and Dengue shock syndrome (DSS). DHF was described in the Philippines in 1950. It occurred above all in children who had probably previously

αβ-integrin, GRP78, and CD14 facilitate phagocytosis of the DENV particles. In addition, heparan sulphate is involved in the adhesion [15]. The integrin-binding site in the E-protein domain III concerns the RGD (arginine, glycine, aspartic acid) motive of encephalitic flaviviruses [16] which is also present in other viruses, such as rhinoviruses. DENV-1 and DENV-2 are fixed to the laminin receptor GRP78 [17] which is also expressed on liver cells. DENV is absorbed via the immunoglobulins bound to the particle (immunophagocytosis) on Fe-receptor-carrying cells [18]. The capsid is released from the phagocytosed virus in the phagosome at low pH. During reshaping of the virus envelope, the E-protein dimers dissociate into monomers and then form trimers. A high cholesterol content of the membrane accelerates fusion [19]. The 2 heat shock proteins, 70 and 90, accelerate the uptake of DENV in cholesterol-rich membranes [20]. DENV replicates in cytoplasm without cell nucleus involvement. Maturation occurs on the endoplasmatic reticulum. The immature virus buds into the endoplasmatic reticulum where it envelopes itself with lipid membrane of the cell, and is then transported in the tubes of the endoplasmatic reticulum to the cell membrane, where it is released in its mature form [21].
been infected by a different DENV serotype. DHF can evolve into DSS, which seldom occurs with first-time infections. With DHF, only minor bleeding events with manifestation of petechiae may be present, but it may also display severe pathological signs with transition to DSS or DHF/DSS, combined with a drop in blood pressure, cyanosis, hepatomegaly, and partly gastrointestinal bleeding. DSS lasts 1–2 days and either takes a lethal course or leads to complete recovery.

1.2.1 Pathogenesis

Essentially, monocytes and macrophages are involved in the pathogenic effect of DENV. At least a part of the DENV in the macrophages is destroyed after phagocytosis by these cells. If antibodies are present which do not sufficiently neutralize the DENV, a part of the viruses is ingested by macrophages via immunephagocytosis, and replicates in these cells. Other cells which carry the Fc gamma receptor are also infected. When the virus is released, immune complexes are formed which activate the complement system and lead to damage of the capillaries with haemorrhage if the complexes adhere to endothelial cells. DSS manifests itself depending on the degree of intravascular activation of the coagulation cascade and damage to the endothelial layer. The damage to the cells is aggravated further by the activation of different cytokine cascades (known as cytokine storm) and by NF-kappa B and partly gastrointestinal bleeding. DSS lasts 1–2 days and either takes a lethal course or leads to complete recovery.

Development of DHF and DSS depends on the following factors [28]:

i) Antibodies: Binding but not sufficiently neutralising antibodies, known as enhancing antibodies. They are present especially if an infection with another DENV serotype had occurred before, or, for instance, in children, if residual maternal antibodies are present. Antibody enhancement is favoured by precursor membrane (prM) antibodies, which are cross-reactive between the 4 serotypes but not neutralising [29].

ii) Age: Complications hardly occur after the age of 12 years. Repeated exposure to all DENV serotypes in endemic areas is probably the reason for having established sufficient immunity by then.

iii) Ethnical background: Caucasians and Asians have more severe courses of the disease than Africans.

iv) State of nutrition: A good state of nutrition improves the body’s defence mechanisms.

v) DENV infection sequence: If DENV-1 is followed by DENV-2, severe courses occur more frequently than if for instance DENV-4 is followed by DENV-2.

vi) DENV serotype: DENV-2 seems to show the highest virulence. As described in 1.1, major differences in virulence also exist within one DENV serotype.

For 1% of the patients with DFH or DSS, the infection is lethal. The lab parameters of the DENV infection are thrombocytopenia, low haemoglobin, haemolysis parameters, and an increase in transaminases with liver cell decay. Time-dependent IgM and IgG antibodies can also be detected. The NAT on DENV-RNA is positive with the occurrence of fever. No clinical symptoms occur in DENV-infected rhesus monkeys [30]. This is a possible indication that the adaptation between DENV and rhesus monkey occurred a very long time ago. In other primates, DENV causes the typical clinical symptoms but no neurological disorders. Mice develop immunity after a short period of time (see section 1), so that they are not a suitable animal model for virulence assays.

1.3 Epidemiology

The presence of the 4 DENV serotypes has been described worldwide in tropical, partly also subtropical, zones since 2006. Endemic areas of DENV essentially correspond to the areas of geographical distribution of the Aedes mosquito and the lower primates that live in these areas (fig. 5) ([31]; WHO: http://gamapserver.who.int/mapLibrary/). DENV regions include Asia, Oceania, Australia, Africa, as well as Central and South America. Since 1990, Dengue fever has also been endemic in Argentina, Nepal, Mexico, and Hawaii. In 2008/2009, a major epidemic occurred in Bolivia with more than 7,250 cases. Another typical characteristic of Dengue fever is that it occurs in cycles, which is due to the distribution of variant serotypes, the infection rate of mosquitoes, and immunity in lower primates and humans. In zones with an urban cycle, the immune status of humans, the ambient temperature, and the...
of whom 55 (24%) had DENV antibodies, while in the control hospitalised patients with fever were examined in Dhaka, out a DENV infection can also take an asymptomatic course. Assumed sufficient specific of DENV-antibody ELISA, these results indicate that a DENV infection can also take an asymptomatic course.

During the DENV epidemic in Bangladesh in 2000, 225 hospitalised patients with fever were examined in Dhaka, out of whom 55 (24%) had DENV antibodies, while in the control group of 184 blood donors, only 1 (0.5%) was antibody-positive [36].

The occurrence of Dengue fever could be reduced by the eradication of the vector. The prevention of the spreading of the mosquito fails due to the fact that stagnant water is available for depositing the egg in the vicinity of human accommodation worldwide (e.g. non-degradable plastic containers filled with water or end-of-life tyres). Further factors favouring the occurrence of DENV infections include the resistance of mosquitoes to insecticides, a shortage of ventilators and mosquito nets for the prevention of mosquito bites among the poor population, and the non-availability of a vaccine.

Pursuant to Section 7 German Infection Protection Act (Infektionsschutzgesetz, IfSG), haemorrhagic fever must be reported by name (Section 7.47), which means that this obligation also refers to the direct and indirect detection of DENV. Imported DENV infections are so far the only source of DENV in Central Europe. In Austria, altogether 93 cases were verified between 1990 and 2005. A total of 43% of the cases displayed exanthema and 22% lymphadenopathy, and in addition thrombocytopenia, leukopenia and an increase in transaminases were noted. None of the infections were of lethal outcome [45]. In France, around 12–28 infections were registered per month between 2001 and 2006. This figure was higher in 2001/2002, since an epidemic took place in the French overseas Départements (West-Indies and Guyana) [46]. In Sweden, 30–60 cases became known per annum between 2004 and 2008. About 75% of these cases had become limited in the Netherlands [42]. In September 2010, the first non-unexpected, autochthonous DENV infection was reported in a 64-year-old man in Nice, which was followed by a second case only a few days later. Both viruses were DENV-2 and showed the same nucleic acid sequence [43]. Furthermore, a DENV infection was confirmed in a German person, which had been acquired in Croatia. The nucleic acid sequence of the DENV acquired in Croatia diverged from the DENV found in the French cases [44].
transmissions of DENV by mosquito bite have so far not been reported in Germany, but they could occur in the same way as they did in France and Croatia.

1.4 Detection Methods and Their Significance

The following commercial tests are available for virus detection using NAT and antibody diagnostics.

1.4.1 Antibody Detection

In serological diagnostics, the pronounced cross-reactivity between the different flaviviruses, leading to limited specificity, must be taken into account [1]. First IgM antibodies are detectable about 1 week after the occurrence of clinical symptoms, i.e. around 2 weeks after infection by the mosquito bite. The IgM tests have so far not been sufficiently validated, and tend to produce false-positive results [34]. For IgG tests, the sensitivity values are given between 21–99% and specificity values between 77–99% [48]. The rate of false-positive results is, among other factors, influenced by concomitant malaria and previous DENV infections [48]. A neutralisation test can be used for the differentiation of further flavivirus species [49]. Domains II and III of the envelope E-protein are epitopes suitable for the specific detection of DENV-2 [50, 51]. NAT polymerase chain reaction (PCR) is the better method to identify DENV double infections than ELISA [52]. Antibodies and double infections can also be tested via capillary blood absorbed on filter paper (dry blood spot) [53]. ELISA results should be confirmed by means of Western blot with purified virus or a neutralisation test. Out of the stained bands in the Western blot, those of the NS1, E, and prM proteins are the most informative ones [54].

1.4.2 Antigen Detection

NS1 protein is secreted from infected cells in large quantities, and is therefore suitable for antigen detection. For this purpose, an ELISA is available which contains monoclonal antibodies against NS1 protein [55]. Another commercial test uses NS1 in a capture ELISA and reaches a sensitivity of 90% at a specificity of 99.5% [56]. A similarly configured test yielded a sensitivity of 63% and a specificity of 98% in 253 samples in Thailand [57]. Commercial tests are available which recognize the 4 DENV serotypes with a sensitivity of 77% and a specificity of 98% [58]. The NS1 antigen test is usually only positive during the first DENV infection [59]. The NS1 antigen test can only be interpreted together with the DENV IgM and IgG results [58].

1.4.3 Virus Detection

At the time of primary infection, increase in temperature and detectability of the virus by means of NAT coincide. Viraemia can last up to 1 or 2 weeks. During secondary infection, the virus can be detectable in the blood 2–3 days before the rise in temperature. The viraemia in this case usually only persists 2–5 days [4]. Mosquito cells extracted from Ae. albopictus are suitable for virus cultivation [60–62]. Further cells are Vero cells or, for example, primary human pulmonary epithelial cells and pulmonary carcinoma cells [63]. DENV 2 can be replicated in mouse macrophages J774 [64]. DENV influences the cell metabolism if cultivated in HepG2 cells [65]. All 4 DENV serotypes produced viruses with high genetic stability after transfection of Vero cells or MRC5 cells with a c-DNA clone [66].

1.4.4 Genome Detection

Different papers describe the detection of DENV genome by real time(RT)-PCR [67, 68]. Suitable primer binding sites are located in the core and envelope section. A Taqman RT-PCR in the NS5 region has been developed in order to detect all DENV serotypes; the test showed a sensitivity of 90% and a specificity of 100% in samples from Malaysia [69]. RNA could be detected in 89% of the blood samples of patients from Puerto Rico with clinically detectable acute DENV infection using the transcription mediated amplification (TMA) test [70]. High sensitivity can also be achieved if the 3' UTR region is used as target sequence [71]. DENV serotypes can be differentiated by nested PCR by amplifying the core region (sensitivity 76%, specificity 100%) at a sensitivity for the detection of DENV-1 of 10 copies, and for DENV-2 to 4 of 100 copies [72, 73]. Finally, a test has been developed in which EDTA whole blood was used as starting material for RT-PCR [74]. After RT-PCR amplification, different flaviviruses can be identified by mass spectrometry [75]. The concentration of DENV virus in the blood correlates with the severity of the disease. For values > 10^9 genome equivalents/ml, there is a risk of DHF development of approximately 90% [76]. Values of 10^9 genome equivalents/ml can be found in plasma [76, 77].

### Table 1. Reported DENV cases in Germany (RKI Epidemiologisches Bulletin, 27/2007, completed)

<table>
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<th>Year</th>
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<tr>
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<td>595</td>
</tr>
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<td>2011</td>
<td>110 (up to May)</td>
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2. Blood and Plasma Donors

The risk of transmission of DENV via the blood is still rated as low, even though 2 cases have been reported from Hong
of the DENV infection can take an asymptomatic to lethal course, i.e. a short-term viraemic phase may not be detected in clinically healthy blood donors. A major part of the endemic areas of Dengue fever overlaps with those where malaria is endemic. A possibly acquired DENV infection should be overcome after deferral based on malaria during a stay in these endemic areas for 6 months. From that point of view, exclusion criteria for the prevention of transfusion-associated transmission of malaria are also effective for DENV. A chronic DENV infection with the presence of the virus of more than 6 months, as was described for West Nile fever, has so far not been reported for a DENV infection [87]. Australia, where DENV is endemic in the north of the country, has minimised the risk of transmission by blood through epidemiological surveillance and donor selection, even though DENV screening tests have so far not been used [88].

2.3 Donor Testing and Significance

Based on the epidemiological situation, the testing of donors or donations for DENV is currently not required in Germany.

2.4 Donor Interviews

Donors are interviewed about their travels to tropical regions and general symptoms of inflammation as well as fever. They are not specifically interviewed about DENV symptoms, since, based on the epidemiological data situation, this is not required to prevent DENV transmissions.

2.5 Donor Information and Counselling

Specific counselling on Dengue fever is available in the infectious diseases centres or institutes for tropical medicine. For blood donation centres, this is currently not necessary.

3. Recipients

3.1 Prevalence and Incidence of Blood-Associated Infections in Recipient Populations

If recipients in Germany have not been in DENV endemic regions, they do not have antibodies against DENV and are therefore susceptible to DENV infection. Recipients from endemic regions might have acquired antibodies, depending on the region and the prevalence of DENV. These antibodies can be neutralising, cross-reactive, or enhancing, depending on the serotype and infection course.
3.2. Immune Status (Resistance, Existing Immunity, Immune Response, Age, Exogenous Factors)

The acute DENV infection is normally overcome in 2–3 weeks. The patient’s age and an iatrogenic immune suppression may slow down the process of recovery. The so-called immune enhancement, i.e. enhanced pathogenesis by insufficient immune defence has so far only been reported in children in endemic regions. Specific antiviral treatment is not available, so that the course of recovery cannot be influenced. Natural resistance against DENV is not known, and is not expected either, since several receptors are present for the entry of DENV into the cell (see section 1.1 Characteristics of DENV).

3.3 Severity and Course of the Disease

In a quarter of infected individuals, the DENV infection displays a severe course which leads to hospitalisation in 1% due to the general symptoms with high fever [89]. Five to 10% of the hospitalised individuals develop DHF, and around 1% develop DSS. The death rate is below 1% of the hospitalised individuals [85]. Laboratory parameters for the severity of the infection include the degree of thrombocytopenia and the frequency of the elevation of transaminases [89]. Since capillary damage with DHF is partly viral, partly autoimmunological, therapeutic intervention designed to alleviate the symptoms cannot be specific. Complications of the DENV infection are partly due to involvement of the nervous system [90, 25], partly due to haemorrhage [91], and very frequently due to myositis [92]. A specific hyperimmunoglobulin is not available. If it would be available, it should have to be used on a serotype-specific basis (see section 3.4 Therapy).

3.4 Therapy and Prophylaxis

3.4.1 Therapy

A specific therapeutic product against DENV or a different flavivirus is not available. Treatment occurs on a symptomatic basis by reducing the fever and supplying the patient with liquid. In vitro, iminosugars, which inhibit glucosidase I and II on the endoplasmatic reticulum, show antiviral activity against DENV, WNV, and bovine viral diarrhoea virus (BVDV) [93]. Receptor blockade is possible in vitro via the zosteric acid (p-sulfoxy-cinnamin acid) [94]. As is the case with hepatitis C virus, the inhibition of the NS3 protease reduces polyprotein cleavage which is an essential step in DENV replication [95]. Experimental immunoglobulin therapy involves administration of antibodies, including monoclonal antibodies, specifically directed against a DENV serotype, by which the pathogenic effect of DENV can be stopped in the mouse [96]. A cross-neutralising monoclonal antibody was generated which binds to the ED3 domain of the E-protein and neutralises all 4 DENV serotypes [97]. However, an ‘immune enhancement’ can also be created by administering monoclonal antibodies in vivo and in vitro on K562 cells [98].

3.4.2 Prophylaxis

Avoiding mosquito bites is the most effective prophylaxis, also against DENV infection, similar to other infectious agents transmitted by mosquitoes. In many regions where DENV is transmitted, other arboviruses, plasmodia, and microfilaria, can also be transmitted by mosquitoes. Another prophylactic activity is the elimination of the mosquitoes’ breeding grounds as mentioned above.

3.4.3 Vaccination

A vaccine against DENV is not yet available. Developing such a vaccine is characterised by 2 problems: the first one involves defining suitable cross-neutralising epitopes for all DENV serotypes, and the second one involves conferring vaccine-induced immunity to such a level and long-lasting synthesis that no ‘immune enhancement’ is created during exposure with any given DENV serotype. An overview of the possibility of a vaccination against DENV for travellers was published in 2008 [99]. Rhesus monkeys can be partly protected by a tetravalent vaccine in which the E-protein is integrated into an adenovirus vector. Vaccinated animals develop low viraemia following exposure [100]. Depending on the serotype, the immune response in rhesus monkeys can be 70–100% and reach a protective effect of 50–80% [101]. An enhancement of the T-cell immune response has been reached by a polytope vaccine [102]. Recombinant attenuated virus with a deletion in the 3’ UTR region was able after 4 vaccine shots to protect rhesus monkeys against DENV-2 [103]. Balb/c mice could be protected against DENV infection by means of an NS1 protein containing a DNA vaccine [104]. A vaccine with attenuated DENV which can no longer enter the cell has also been produced as a potential vaccine against DENV [105]. Recombinant tetravalent DENV vaccine, produced in drosophila cells, confers protective immunity in mice and monkeys [106].

3.5 Transmissibility

3.5.1 Blood Transfusion

So far 2 papers report DENV transmission by blood transfusion: 1 paper has been published reporting on 3 transfusion-associated DENV transmissions in Singapore in 2008 [107]. The donor was a 52-year-old man; the 3 recipients of red blood cells, fresh frozen plasma, and platelets all had signs of a DENV infection. The predominant symptom was myalgia. Another transmission by blood transfusion was reported from Hong Kong [108]. Among 126 hospitalised cases with DENV infection between 1998 and 2005, the latter was the only case in Hong Kong, which occurred by blood transfusion. The risk of a transmission via the blood outside the endemic regions
has been estimated as very low in the USA [78, 81]. Regarding transplants, up to now, 1 DENV transmission with development of DHF has been reported in a recipient after kidney transplantation in 2005 [109].

3.5.2 Needle Stick Injury
Small quantities of blood as in the 4 described transmissions by needle stick injury are sufficient for DENV transmission, if the index patient is in the acute viraemic phase [110–113]. Clinical signs of infection can be observed after about 1 week. The exanthema can be seen after approximately 2 weeks, and after approximately 3 weeks, the symptoms of the DENV infection will have subsided. No haemorrhage occurred in any of the primary infections caused by a needle stick.

3.6 Frequency of Administration, Type and Amount of Blood Products
A transmission of DENV by blood or blood products has so far not been reported in Germany. The deferral of the donors 6 months after a stay in a tropical region because of the risk of malaria on the one hand and the deferral for 4 weeks following febrile diseases on the other minimize the risk of transmission. The low number of transfusion-associated DENV infections worldwide does not allow an estimation of the transmission risk based on the frequency of the applications and the quantity administered.

4. Blood Products

4.1 Infectious Virus Load of the Starting Material and Test Methods
There is currently no infectious virus load for DENV in blood or plasma collected in Germany. Asymptomatic carriers with protective DENV antibodies are not infectious, therefore antibody testing is not required (see section 4.2). Tests for the detection of DENV (RNA-NAT or the less sensitive antigen test), and DENV antibodies (ELISA) are described in section 1.4. Even though the risk of transmission of imported DENV infections can almost entirely be ruled out linked to the above described donor referral for travellers, presence of DENV in imported plasma for fractionation cannot be ruled out. Testing for DENV, however, is not required, since the inactivation procedures should be also effective against DENV.

4.2 Methods for Removal and Inactivation of the Infectious Agent
DENV could be completely removed or inactivated from spiked plasma by protein fractionation and subsequent inactivation by pasteurisation or solvent detergent treatment [114]. If the individual steps of the methods are added on to each other, a cumulative reduction factor for DENV-2 can be reached of more than 10 log_{10} for albumin, and of more than 14 log_{10} for an immunoglobulin product. With regard to inactivation, DENV behaves like other enveloped viruses. Due to the request that products from plasma must undergo at least 1 virus inactivation step during the manufacturing procedure, there is no risk of DENV transmission for these products, even if the starting material should contain a high viral burden.

4.3 Feasibility and Validation of Procedures for Removal/Inactivation of the Infectious Agent
Similar to other flaviviruses, DENV has been rated as biosafety level 3. Work with DENV must be carried out using the appropriate safety preconditions [115]. So far, only DENV infections transmitted by percutaneous, but not aerogenous exposure have been described. Since DENV can be replicated in different cultured cells from humans and monkeys, for example Vero cells, a sufficient amount of virus can be manufactured to spike intermediate steps in the manufacture of blood products. Validation of the virus reduction could be performed using characterised DENV strains of all serotypes. Validation of DENV, however, is not required by the specification of the Committee for Medicinal Products for Human Use (CHMP) (Note for Guidance on Virus Validation Studies, CPMP/BWP/268/95), since the data for the inactivation of model viruses are equivalent [116].

4.3.1 Inactivation
DENV can be inactivated by pasteurisation and solvent-detergent treatment [114]. DENV is also inactivated by methylene blue/light treatment [117]. No inactivation data are published for treatment with psoralen/amotosalen (Intercept®, Cerus, Concord, CA, USA) and riboflavin. In plasma [118] and in platelet concentrates [119], WNV, a flavivirus which is very similar to DENV, is inactivated by treatment with amotosalen. Heat treatment of serum at 56 °C for 30 min inactivates 10^6 pfu/ml WNV completely [120]. A burden of WNV-RNA was found in plasma from the USA, while it was absent in pools from Asia and Europe [119]; in conclusion, the burden of plasma pools with DENV should be low.

5. Assessment
DENV causes yearly around 50–100 million infections worldwide, of which around 500,000 lead to hospitalisations. DENV is thus the most frequent arbovirus infection transmissible to humans by mosquitoes. Lower primates and possibly other animals constitute the animal reservoir for
DENV. It is unlikely, that this zoonotic agent can be eradicated. Essentially, children and adolescents in tropical endemic areas, who do not yet possess sufficient immunity against the 4 DENV serotypes, are the human reservoir. In the case of insufficient immunity, DENV infection can lead to lethal DHF or DSS via immune enhancement. During primary infection, DENV is transmissible by blood for around 4–21 days following a mosquito bite. Specific antiviral treatment of the DENV infection or a vaccine are so far not available, even after DENV being accepted and addressed as a global problem [121]. The DENV genome is highly variable by mutation and recombination. DENV is able to replicate in primates at 37 °C and in the Aedes mosquito at ambient temperature. *Ae. aegypticus* and *Ae. albopictus* are the most frequent vectors for the transmission of DENV. These mosquitoes have spread over the entire Mediterranean region and to Central Europe. A human reservoir which would initiate the urban cycle for DENV does so far not exist in Central Europe. Apart from 3 cases in the year 2010, no autochthonous transmissions have been reported from South and Central Europe. Epidemiological studies should be performed to record the imported DENV infections in Germany, as well as surveillance whether autochthonous infections are occurring in Germany in view of a continued change in climate and spread of the vector. No additional action is currently required to prevent a possible DENV transmission by blood transfusion, beyond the mandatory requirement to defer donors for 4 weeks following febrile disease and for 6 months following a stay in a malaria-endemic region.

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**References**


Arbeitskreis Blut


