Arthritis Associated with Alphavirus Infections: Chikungunya

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Abbreviations

BFV	Barmah Forest virus
CCL2	Chemokine (C-C motif) ligand 2
CHIKV	Chikungunya virus
CLIP	Cross-linking immunoprecipitation
CPE	Cytopathic effect
CTLs	Cytotoxic T lymphocytes
CXCL10	C-X-C motif chemokine 10
GM-CSF	Granulocyte-macrophage colony-stimulating factor
MAYV	Mayaro virus
NF-κB	Nuclear factor-kappa B
ONNV	O'nyong-nyong virus
RER	Rough endoplasmic reticulum
RRV	Ross River virus
SINV	Sindbis virus
VEEV	Venezuelan equine encephalitis virus
WEEV	Western equine encephalitis virus
WHODAS II	World Health Organization Disability Assessment Schedule, version 2

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Introduction

Alphaviruses are a genus of enveloped, single-stranded RNA viruses that belong to the *Togaviridae* family, along with other viruses like dengue, yellow fever, West Nile, and Zika. They are arboviruses, so called by the mechanism of transmission to humans: arthropod-borne viruses. All are transmitted in zoonotic cycles and have entered to human-human cycles involving *Aedes* spp. mosquitoes, *Aedes aegypti*, and occasionally, *Aedes albopictus* [1–4].

Alphaviruses are distributed around the world and produce diverse human diseases including febrile rash, encephalitis, and arthritis. They are classically referred to as "Old World" and "New World" viruses. "Old World" group includes viruses that are related to rheumatic diseases, and chikungunya virus (CHIKV) is the most relevant, but Ross River virus(RRV), Mayaro virus (MayV), O'nyong-nyong virus (ONNV), Barmah Forest virus (BFV), and Sindbis virus (SINV) can also produce occasional outbreaks [5–7]. "New World" viruses refer to the group of viruses that in the Americas had produced fatal encephalitic diseases, in which Venezuelan equine encephalitis virus (VEEV) and Western equine encephalitis virus (WEEV) are the most relevant [8, 9]. Although "Old World" and "New World" viruses are a public health concern, this chapter focuses only in "Old World" alphaviruses, mainly CHIKV. Since 2004 there is growing information regarding the association of alphaviruses and acute and chronic arthritis that has become a focus of research from the molecular, cellular, immunogenetics, clinical, treatment, and prevention. The chapter summarizes the most important evidence regarding these aspects.

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Epidemiology of Arthritogenic Alpha Viruses

Alphaviruses are widespread across all continents and in general have the potential to disseminate because of the adaptations of vectors, especially to climate and climate change [10, 11]. Arthritogenic alphaviruses have been globally reported, and in the last two decades, the world has experienced large epidemics due to CHIKV, SINV, RRV, MAYV, and ONNV that produced a high public health and socioeconomic burden [2, 10, 12].

CHIKV is present around the world and is the most relevant arthritogenic alphavirus due to its capacity to generate large epidemics. This virus was isolated in 1952 in Tanzania (before Tanganyika), and the largest registered epidemic took place in 2004–2011 and was transmitted by *Aedes albopictus*. The epidemic that began in Kenya and spread across South East Asia reached an estimated 1.4–6.5 million cases. Italy was the first European country that reported autochthonous cases in 2007 and France in 2010 [3, 10]. Prior to 2013, the virus only circulated in Africa, Asia, Europe, and Australia. In December 2013, the first local transmission of CHIV was reported in Saint Martin island and thereafter spread to other countries, reaching almost all the continent in 2017 [13, 14].

RRV is an endemic virus of Australia and Pacific islands, where the most common is a widespread arboviral disease, causing thousands of cases each year [15]. In the last two decades, RRV outbreaks have increased in Australia, accounting for more than 70% of notifications of mosquito-borne diseases and generating a significant annual cost for the health-care systems. The increase of cases due to RRV has been attributed to many factors, including climate, mosquito density, and individual risk factors [16].

ONNV is an endemic virus for Africa. It was first identified in East Africa between 1959 and 1962 epidemics, in where more than two million cases were reported. After 1962, no more cases were documented, until it reemerged in Uganda in 1996, with attack rates ranging from 3% to 29%; more recent outbreaks have been reported in Liberia and Chad during 2003–2004 [17].

SINV is found in Europe, Asia, Africa, and Australia. In endemic areas, serologic prevalence can reach 39%. It was first isolated from mosquitoes during an epidemic of febrile illness in the district of Sindbis, Egypt, in 1952; the serological tests in cases of rash and arthritis suggested that SINV was the causative agent. In 1961–1962, it was isolated from human tissues (blood sample, skin). Although more outbreaks occurred in South Africa during 1963–1964, it spread to Europe in the same years, suggested by serological studies performed in Israel, Italy, and Finland. In 1974, another outbreak occurred in South Africa, Sweden, and Finland. In the last three decades, more outbreaks have occurred, mainly in South Africa and Northern Europe. The last outbreak was reported in 2013 in Sweden [18, 19].

MAYV is an endemic virus for South America. It was first identified during an outbreak of acute febrile illness in Trinidad in 1954. More outbreaks have been reported in Brazil, Bolivia, Venezuela, Surinam, Guyana, and Peru. The last outbreak registered occurred in Venezuela in 2010 [10, 20].

Immunopathogenesis of Arthritis Associated with Alphavirus Infections

Derived from the lessons of recent epidemics, especially those due to chikungunya in the Americas after 2013, the knowledge of pathogenesis of arthritis associated with alphavirus infection has evolved enough to better understand the mechanisms underlying chronic arthritis after CHIKV infection. The presence of new technologies such as deep sequencing has allowed the characterization of gene regulation involved in the control of infections and symptoms and the long-term immune response. However, despite all the existing information, there are still questions to answer, especially those related to the induction of autoimmune mechanisms that produce rheumatoid arthritis. Herein we summarize the most important findings focused on the pathogenesis of arthritis associated with alphavirus infections with emphasis on the virus and host factors [14].

Viral Factors

There are mainly three viral factors that could influence the development of arthritis associated with alphavirus infection: viral load, evasion of immune responses, and induction of autophagy. Viral load correlates with the presence and intensity of symptoms; the higher the viral load, the higher the organ damage. Studies are consistent that CHIKV-infected patients with higher viral loads developed chronic arthritic symptoms [21].

Evasion of immune responses is another mechanism by which the virus could be responsible for the development of chronic arthritis. In animal models, CHIKV can survive in macrophages for a long time using diverse mechanisms of evasion and establishing chronic infections [22]. Another mechanism of evasion of immune responses includes the neutralization of antibodies via genetic mutations or cell-to-cell transmission. For example, in CHIKV, there are strains that evade domains of neutralizing antibodies, inducing persistence of the virus and impeding clearance [23, 24].

Induction of autophagy has been observed in alphavirus infection, especially in CHIKV, which activates pathways of stress in organelles like endoplasmic reticulum with the consequent autophagy induction, enhancing the viral RNA replication [25]. This phenomenon observed also in other viruses like human immunodeficiency virus, mycobacterium, and parasites is due to the mimicry of viral proteins

with host protein motifs that interact with other proteins in pathways that conduce to an explosion of the cellular infrastructure [26, 27].

Host Factors

A recent meta-analysis reported that nearly 50% of patients that became infected with CHIKV did not recover fully after 3 months of infection [28]. Based on this data, it is logical to assume that not only the viral factors are involved in the immunopathogenesis of arthritis; there are many host factors that play a critical role in the resolution of symptoms or the progression of arthritic symptoms and development of chronic arthritis, including autoimmune arthritis. These factors include innate immune response, host proteins, adaptive immune response, osteoblasts, cytokines, chemokines, growth factors, and genetic factors, especially those related to the main histocompatibility complex [29].

After CHIKV enters the body through an infected mosquito bite, it reaches the dermic microvasculature; replicates primarily in leukocytes, the liver, and the spleen; and disseminates to other organs like muscle, bones, and synovial tissue, a situation that generates a rapid and intense inflammatory response that correlates with the symptoms of an acute phase of infection [14].

According to animal models, innate immune responses are the first line of defense. Monocytes, macrophages natural killer (NK) cells, and dendritic cells drive the initial response. There is evidence showing that arthritis is related to upregulation of gene associated with macrophage recruitment and activation, generating a cascade of inflammatory mediators, prostaglandins, and interleukins that results in tissue damage and arthritis [2, 30, 31]. Gardner et al. [32], who replicated an animal model of CHIKV-induced arthritis, demonstrated that infection of mice with two different isolates resulted in (1) development of clearly foot swelling that was preceded by mononuclear viremias and (2) prolific infiltrate of mononuclear cells in muscular tissues with the subsequent subcutaneous edema, clear signs of arthritis with lymphocyte infiltration, and disruption of synovial membrane.

The presence of monocytes, macrophages, and NK cells, as the main components of inflammatory infiltrate in animal models of alphavirus-induced arthritis, shows that innate immune responses play a role in the pathogenesis of arthritis. In fact, there is evidence showing that in patients with demonstrated alphavirus-induced arthritis, macrophages and NK cells can be isolated from synovial exudates [33].

It seems that the symptom generated by the acute viremia (first 5–7 days) is primarily controlled by innate immune responses through IFN-alpha/beta and with the participation of monocytes, macrophages, and NK cells. Once acute viremia and inflammatory responses generated by the virus and their products drop, the symptoms generally disappear; nevertheless, due to epidemics outcomes, almost all research is focused on investigating the mechanisms involved in the persistence of symptoms beyond the acute phase of the disease. At this time, it is not fully explained whether these chronic symptoms that are different in each patient and can last for months to years are due to host responses only or have a contribution from the virus intrinsic characteristics. There is evidence that supports that arthralgia and arthritis are due to inflammatory responses induced by virus replication within tissues after acute viremia, a situation that has been demonstrated at least for CHIKV. The mechanisms by which alphaviruses can persist for a long period in tissues despite strong T-cell and IFN alpha/beta responses seem to be related to the capacity of the virus to evade neutralization and T-cell responses through the shutdown of major histocompatibility molecules synthesis, a situation that limits antigen presentation [15, 22, 34].

On the other hand, host response to infection has been related to the persistence and progression of arthritic symptoms beyond the acute phase. Most studies have reported that there are pro-inflammatory cytokines secreted during acute and chronic phases that are the same with those associated with autoimmune arthritis, like rheumatoid arthritis, and one theory states that alphavirus infection could trigger autoimmune responses that could explain part of the clinical picture of some chronic arthritis observed in infected patients; nevertheless this is not a consistent feature on published reports [35, 36].

Studies that have focused on host responses instead of viral persistence conclude that immune responses could be the responsible factors of chronic arthritic symptoms. Diverse cytokine profile has been characterized in chronic symptoms after CHIKV infection like IL-6, IFN α/β, CCL2, GM-CSF, IL-7, IL-12, IL-13, IL-17, and CXCL10 upregulation that seems to be related to the persistence of symptoms. Although that information is growing, at this moment it is not consistent and coherent with the clinical evolution of patients [37, 38].

More recently, Chang et al. explored the differences in cytokine profile in acute CHIKV infection between patients with and without chronic arthritic symptoms and demonstrated that robust cytokine response during acute infection was correlated with less incidence of chronic joint pain. Although these authors found differences between cytokine response between subjects with and without chronic symptoms suggesting that cytokine response is necessary to clear the virus from the body, two important limitations are observed: (1) there were no serial measurements of cytokine profile to make multiple comparisons at different times to elucidate the relationship of chronic symptoms and cytokine serum levels; (2) the term "chronic arthritis" was used in patients who referred symptoms only by phone call instead of being evaluated in person to confirm or rule out the presence of true arthritis. This same group reported that there was no evidence of CHIKV virus in synovial fluid of patients with chronic arthritis suggesting that viral persistence

and local replication are not responsible for chronic arthritis and maybe host autoimmune response better explains the chronic symptoms and the response to immunomodulatory treatments [39–41].

To this regard, and trying to provide objective information about the rheumatic manifestations related to CHIKV infection on acute and chronic phase and taking into account the lack of objective information about the presence or absence of true arthritis after CHIKV infection, our group followed ten patients with confirmed CHIKV infection for 1 year (monthly visits) in an attempt to characterize clinically and biochemically the evolution from acute to chronic phase. We used objective tools like Disease Activity Index WHODAS II score to evaluate the self-reported disability, joint exploration to evidence synovitis, and serial measurements of inflammatory biomarkers and rheumatoid factors. In that study, we reported that more than 50% of patients persist with disability and arthritic symptoms beyond the acute phase; all patients presented elevation of inflammatory biomarkers in the acute phase, especially interleukin 6. Interestingly we observed positivity of rheumatoid factor in the acute phase in all patients, drop of levels over time in patients without chronic symptoms and persistence of positivity in patients with chronic symptoms, and persistence of high levels of interleukin 6 in patients with chronic symptoms. After 1 year of follow-up, no case was consistent with the diagnosis of rheumatoid arthritis (RA). Of those ten patients, two presented true arthritis after follow-up, and four presented only arthralgia. We consider that clinical evaluation, joint exploration, and serial measurements are essential to really define the presence or absence of true arthritis because of the implications in terms of classification and therapeutic approaches [42].

Genetic Susceptibility of Arthritis Associated with Alphavirus Infections

Genetic susceptibility of the host may play a critical role in both the infection and the development and progression of arthritic symptoms. Specific polymorphisms of the human leukocyte antigen (HLA) that is known to predispose subjects to develop autoimmune arthritis may be related with CHIKV-induced arthritis. The MHC class II alleles HLA-DRB1∗01:01 and HLA-DRB1∗04:01 are involved in the pathogenesis of RA by recognizing citrullinated peptides and consequently activation and clonal expansion of autoreactive CD4+ T cells [43, 44].

HLA CLASS I Disease Mechanism

HLA class I molecules present endogenous antigens, such as those derived from viruses and intracellular bacteria, for recognition by the immune system. This process involves ubiquitination of endogenous cytosolic proteins and then degradation into short 8–16 amino acid peptides, optimal for HLA class I binding. These are subsequently transported into the endoplasmic reticulum where they bind HLA class I molecules, before exiting the RER and being transported to the cell surface. HLA class I presented antigen is then recognized by CD8+ T cells and natural killer (NK) cells. Once CD8+ T cells become activated, functional effectors T lymphocytes (CTLs) are produced which possess lytic capabilities and also play a role in generating CD8+ T memory cells, acting as part of both the innate and adaptive immune responses. Activated NKs act before clonal expansion and differentiation of CD8+ T cells and compliment the CTL response. They act as one of the first lines of innate immune defense by producing cytokines, including interferons, which aid in the recruitment of additional cells to the site of inflammation and also produce cytokines and chemokines that have a cytolytic activity aiding cell destruction [45].

HLA class I molecules play a role in presenting endogenous antigens, including those derived from viruses, which have been proposed to be key triggers for arthritis. Viral antigens may trigger arthritis through molecular mimicry and via acting as superantigens. Molecular mimicry occurs when viral antigens that are similar to self-antigens activate autoreactive T-cells that can cross-react with self-antigen generating autoimmunity. Some viral antigens could also act as superantigens, producing a strong non-specific immune response that then cross-reacts attacking and damaging tissues in the body [46, 47]. Viruses can also alter HLA class I and II expression, potentially leading to greater antigen presentation to CD8+ T cells, with certain alleles more prone to viral manipulation. During viral infection soluble HLA levels, involved in regulating the immune response, have also been shown to be increased in RA patients, the level of which is dependent on HLA allele present.

HLA Class II Disease Mechanism

Exogenous peripheral antigens are internalized via antigen presenting cells (APC) and are degraded into 13–18 amino acid residue peptides, in the increasingly acidic compartments of the endocytic pathway. HLA class II molecules are synthesized in the rough endoplasmic reticulum (RER) where they associate with the invariant chain (Ii) to prevent endogenous peptide binding. The HLA class II molecule is then routed to the endocytic pathway, where it is degraded, leaving a short fragment of the Ii class II-associated invariant chain peptide (CLIP) bound, which is then exchanged for peptide. The HLA class II peptide complex is then transported to the cell surface for recognition by CD4+ Th cells, which determine whether an immune response is mounted. If

an immune response is mounted, CD4+ Th cells will activate naive B cells to produce antibodies, or in the case of self-antigens autoantibodies and aid in macrophage recruitment.

The highest-risk alleles, belonging to the DR4 group, have higher affinity to a polar residue such as T or S in the P6 pocket, where it can form a hydrogen bond with DR-β13H (histidine at 13th position of beta chain), while DR1 (and presumably DRB1∗0901 and ∗1001) prefer adenine over tyrosine or serine, possibly because the phenylalanine at DR-β13 makes the pocket more hydrophobic [43, 48]. Six of the alphaviruses known to infect humans carry T or S at pocket P6 suggesting a possible mechanism of disruption of tolerance. Of these, CHIKV is one with the highest serologic prevalent in humans and endemic in regions with high prevalence of DR4 alleles, such as Latin-American countries like Mexico and Ecuador [49, 50]. Host genetics haplotype HLA-DRB1∗11 and HLA-DRB1∗11-HLADQB1∗03:01 are associated with resistance to CHIKV infection, and HLA-DRB1∗04-HLA-DQB1∗03:02 are susceptible to CHIKV infection. Also, HLA-DRB1∗04 or HLADRB1∗01 alleles were present in 66.6% of CHIKV-infected patients with RA [28].

Table 11.1 Clinical manifestations of chikungunya fever

Virus	Alfavirus (RNA virus)
Vector	*Aedes aegypti* and *Aedes albopictus*
Incubation	3–7 days(1–12)
Appearance of symptoms	4–8 days (2–12)
The virus causes a febrile illness associated with	
Fever	Sudden +39 ° C 76–100% continuous or intermittent
Arthralgia/arthritis∗	(87%)
Back pain	(67%)
Headache	(62%)
Cutaneous rash	(50%)
Severe forms are rare; symptoms usually remit 7–10 days ∗Asymmetric intense and debilitating more frequently hands and feet, swelling associated with tenosynovitis	
Atypical manifestations	
Skin: hyperpigmentation, ulcers or aphthous, generalized erythema, vesicular-amphilophus lesions, desquamation of palms, vasculitis, liquinoid eruptions	
Lung: pneumonia	
Gastrointestinal: nausea and vomiting, acute hepatitis	
Neurologics: encephalitis, meningoencephalitis Guillain-Barre, cerebellar syndrome, mental confusion, convulsions	
Eyes: conjunctivitis, optic neuritis, episcleritis, rhinitis, uveitis	
Hematological: lymphadenopathy, thrombocytopenia	
Complication: Non-frequent Pain for months or years	

Clinical Manifestations of Alphavirus Infection

CHIKV infection could lead to fever, arthritis, encephalitis, myelopathy, peripheral neuropathy, myeloneuropathy, myopathy, and sometimes death. Chronic disorders post-CHIKV are nonspecific polyarthralgia, rheumatoid arthritis-like illness, undifferentiated inflammatory arthritis, soft tissue rheumatism, seronegative spondyloarthritis, or psoriatic arthritis (PsA) [28]. After incubation period (2–6 days), the symptoms begin as fever (more than 90%, lasting 1 week), myalgia (90% usually lasting between 7 and 10 days), polyarthralgias/polyarthritis (95%, lasting from weeks to months), and erythema (50%, 1 week). The chikungunya fever is divided into an acute phase (less than 10 days), subacute (between 10 and 90 days), and chronic (more than 3 months); the symptoms are continuous or relapsing-recurrent. Symptoms of subacute and chronic disease are distal polyarthritis, non-arthritic polyarthralgia, oligoarthritis in previously affected joints, subacute hypertrophic tenosynovitis, peripheral vascular disorders, depressive symptoms, fatigue, and weakness (Table 11.1) [42, 51, 52].

There are atypical manifestations of chikungunya fever, such as skin manifestations: hyperpigmentation, aphthous ulcers, transient nasal erythema, generalized erythema, vesicular-amphilophus lesions, desquamation of palms, vasculitis, lichenoid eruptions, renal failure that can be triggered by the use of non-steroidal anti-inflammatory drugs (NSAIDs), nephritis, pneumonia, nausea and vomiting, acute hepatitis (associated with the use of paracetamol or previous alcoholism), encephalitis, meningoencephalitis, Guillain-Barre, cerebellar syndrome, mental confusion, convulsions, conjunctivitis, optic neuritis, episcleritis, rhinitis, uveitis, lymphadenopathy, and thrombocytopenia [52, 53] (Table 11.1).

Chronic Arthritis

The clinical manifestation of rheumatic disorders post-CHIKV infection can be divided into three groups: (1) true arthritis, including seronegative and seropositive arthritis, (2) spondyloarthritis, and (3) undifferentiated polyarthritis. Arthralgia without arthritis is the most common manifestation of chronic inflammation post-CHIKV infection [53, 54].

According to carried out studies, arthritis is benign; however, between 10% and 30% arthritis can be persistent up to 3 years after infection and resembles RA and in some cases with the presence of positive rheumatoid factor, with bone erosions and presence of the human leukocyte antigen (HLA DR 04 and HLA DR 01) in a manner similar to that observed in RA. Because the cytokines secreted during CHIKV infection are the same found in RA, CHIKV may be considered to trigger the onset of RA in genetically predisposed individuals. However, it is necessary to demonstrate the presence and

participation of autoimmune processes in arthritis induced by alphaviruses [54].

Risk Factors for Chronic Arthralgia/Arthritis

There are some clinical factors and biomarkers associated with an elevated risk of progression to post-CHIKV chronic disease, such as older age, symmetrical distribution of arthralgia, initial severe joint pain, female gender, and previous osteoarthritis [55–61]. DAS-28 and WHODAS-II score at diagnosis have been associated with increased risk of progression to chronicity [42]. Some biomarkers have also been found as predictors of chronicity, e.g., high level of interleukin-6 and ferritin [42, 55].

Diagnosis

The diagnosis of alphavirus, especially CHIKV, is based on clinical, epidemiological, and laboratory criteria. However, CHIKV infection may be definitely confirmed only by laboratory methods such as detection of viral RNA or by identification of the specific anti-CHIKV antibodies [62–65]. The viremia of CHIKV lasts between 5 and 7 days and is the period in which IgM antibodies are detected 3–8 days after the symptoms and persist for 1–3 months. IgG is observed from 4 to 10 days after onset of symptoms and persists for years. Another method for diagnosis is molecular biology (real-time PCR). Some biomarkers have been investigated as predictors of chronicity, and it was found that in the initial phase the C reactive protein (CRP), the erythrocyte sedimentation rate (ESR) and interleukin-6 (IL6) were found increased. The ESR and IL-6 could predict chronicity from the moment of diagnosis [42]. The non-specific laboratory abnormalities observed during an early stage of chikungunya fever are leukopenia with lymphopenia, thrombocytopenia, or elevated aminotransferases levels [65] (Table 11.2).

Table 11.2 Diagnosis of chikungunya

Molecular and serological tests	Viral RNA: analysis with reverse transcription-polymerase chain reaction; RT-Anti CHIKV antibodies: ELISA IgG, IgM
Leukopenia	++
Neutropenia	+
Lymphopenia	+++
Thrombocytopenia	<100,000
VSG and C-reactive protein	Increased
Interleukin −6	Increased
+++High intensity: 70–100% of patients ++Medium intensity: 40–69% of patients ++Medium intensity: 10–39% of patients	

Treatment of Chikungunya Arthritis

During the acute phase of chikungunya, joint and muscle pain predominates, and analgesics and antipyretics are recommended: acetaminophen at a dose of 500–750 mg every 4–6 h, without exceeding the maximum daily dose of 4.0 g, due to the risk of hepatotoxicity. Tramadol hydrochloride 50–100 mg orally should be used every 6 h. In cases of severe pain, you can combine analgesics with opiates. Hydration and absolute rest are crucial components of the patient's integrative approach [66].

Pharmacological treatment in the chronic phase: the persistence of clinical manifestations for more than 3 months from the onset of symptoms is considered a chronic phase. Arthralgia is mild in some of these patients, which means that the disease is in true regression. On the contrary, in a percentage of patients (20–30%), intense inflammatory manifestations are observed, many of which adequately meet the criteria of the American College of Rheumatology to be classified as RA and the treatment must be with drugs modifying the disease such as hydroxychloroquine, methotrexate, sulfasalazine, and even biological therapy [67, 68]. However, there is little evidence of their efficacy from large clinical trials. Chopra et al. [69] studied the effectiveness of chloroquine and inflammatory cytokine response in patients with early persistent musculoskeletal and arthritis post-chikungunya; the results showed no advantage of meloxicam over the symptoms. Recently, the results of the combination of triple DMARDs therapy (methotrexate, sulfasalazine, and hydroxychloroquine) vs monotherapy with methotrexate in chronic persistent chikungunya arthritis were informed. The triple therapy was superior to monotherapy with hydroxychloroquine with a higher percentage of patients achieving EULAR clinical response and low disease activity. Nevertheless, none of the patent remission was observed [70]. Regarding biological therapy, there is a report of 21 cases of RA following CHIKV fever [71]. Based on the experimental model, there are some perspectives for future treatment of post-chikungunya chronic arthritis especially with biological therapy developed for rheumatoid arthritis, such as tocilizumab abatacept, tofacitinib, etc. [72].

Prevention

As in other diseases transmitted by mosquitos, it is important to have the following recommendations for the prevention of CHIKV [73]:

General Recommendations

- Wear clothes that cover most of the body
- Do not expose yourself to the bite of the mosquitoes

- Use mosquito repellent
- Use a canopy or cloth that covers your bed completely
- Install mosquito nets on doors and windows
- Prevent garbage from accumulating
- Do not leave containers where water accumulates
- Constantly wash water containers, as well as water tanks and cisterns
- Use larvicides in containers to eliminate mosquito larvae
- Use special insecticides to eliminate the mosquito in its adult phase

Vaccines for Chikungunya

Chikungunya fever has reemerged since 2004 to cause millions of cases. Because CHIKV exhibits limited antigenic diversity and is not known to be capable of reinfection, a vaccine could serve to both prevent disease and diminish human amplification during epidemic circulation. Owing to the lack of licensed vaccines and antiviral therapeutics, the primary response to CHIKV outbreaks is vector control. However, *A. aegypti* and *A. albopictus* populations continue to expand because of factors such as insecticide resistance and poor infrastructure, lack of education, and uncontrolled urban development. Thus, a vaccine still provides the best hope for limiting CHIKV infections and spread [74].

Vaccines as in other diseases constitute a fundamental pillar to eradicate these viral diseases; however, in these viral infections (dengue and chikungunya), they are not yet consolidated [75].

Perspectives

Chikungunya virus infection has been described so far in patients from 45 countries, including travelers. Therefore, this infection is considered an epidemic of acute disease, with low mortality, but with persistent and disabling chronic arthritis [76].

In addition to the clinical manifestations described previously, it is important to mention other extra-articular manifestations described in the early stages of the infection, such as myocarditis, cardiac arrhythmias, sepsis, and septic shock. During widespread CHIKV epidemics, excess mortality has been reported in newborns and the elderly [77].

In relation to the transition from acute to the chronic stage, it is important to mention that CHIKV RNA antigen has been found in the synovial tissue at 18-months post-CHIKV infection in a single subject [21]. In contrast, in 22 months after acute infection has not been identified CHIKV RNA or proteins in the synovial fluid of CHIKV arthritis patients suggesting that viral persistence may not be a requirement for persistent joint pain [40]. However, the analysis of the synovial fluid is different from that of the synovial membrane analysis. Therefore it is necessary to investigate the synovial membrane of these patients, in order to find evidences of CHIKV RNA.

Recently, a comprehensive review of the literature on CHIKV infection was conducted [78]. According to this review, the chronological analysis of epidemics of infection with this virus shows cycles of emergency and re-emergence of this infection on all continents. This is due to mutations in the viral genome that allows it to adapt to new vectors and survive at colder temperatures. Therefore the health authorities should remain alert to new outbreaks of CHIKV infection.

Vertical transmission of CHIKV infection has been described in humans. Evidence of CHIKV has been found in saliva and semen from infected patients. Therefore, the possibility of sexual transmission of CHIKV should be investigated [79, 80].

Importation into non-endemic areas of CHIKV infection by travelers returning from endemic areas is high risk. Epidemics can be controlled if health authorities take strict protection measures for travelers and develop vector attenuation programs. CHIKV should be suspected in returning travelers presenting with fever and severe polyarthralgia [81–83].

Regarding serological tests for viral infection, there is evidence of cross-reaction of CHIKV infection with other alphavirus antibodies; therefore, it is necessary to have a highly specific and sensitive test that is a gold standard to diagnose CHIKV infection [84, 85].

One of the most relevant aspects related to morbidity and mortality from this infection is the comorbidities of the patient who contracts CHIKV infection. It has been suggested that chronic diseases such as respiratory, cardiovascular, autoimmune diseases, diabetes, etc., present in the patient can be a risk factor to aggravate this infection and turn it into chronic infection [86, 87]. New studies will be needed to demonstrate the association between comorbidities and chronic CHIKV infection.

Atypical clinical manifestations of CHIKV infection have been described such as nasal skin necrosis, various forms of presentation of uveitis until reaching blindness, and acute disseminated encephalomyelitis. In relation to Guillain-Barre syndrome, an increase in this syndrome has been observed during an epidemic of CHIKV infection [88–91]. These changes in the clinical spectrum of CHIKV infection suggest an increase in virulence due to genetic mutations of the virus, more complete epidemiological and clinical reports, or the existence of Zika virus infection. In this regard, changes at the intra-host level, mutational of the E1 of the CHIKV, have been reported, which makes the virus more efficient and with greater capacity for dissemination by vector exchange [78, 92]. CHIKV and other Alphavirus infections are characterized by global inhibition of cellular

transcription and rapid induction of a cytopathic effect (CPE) in cells of vertebrate origin, causing changes in cell morphology, cell lysis, vacuolization, formation of syncytia, formation of inclusion bodies, etc. CHIKV is a highly pathogenic alphavirus representative because it has a nonstructural protein 2 (nsP2) that plays critical roles in both inhibition of transcription and CPE development. In this sense, a mutation of nsP2 has recently been identified that made CHIKV and its replicons incapable of inhibiting cellular transcription and dramatically this mutation decreases CPE. The mutations in nsP2 may be used for the development of new vaccine candidates against alphavirus infections [93].

One question to be clarified is whether CHIKV infection is a risk factor for developing rheumatoid arthritis (RA). A recent study suggests that in certain endemic regions, CHIKV infection may be one of the risk factors for developing RA [94]. These retrospective findings should be studied prospectively, analyzing the interaction between genes and environment that favors CHIKV infection. In this sense, a recent study shows that in early stages of CHIKV infection, the microRNAs of the skin fibroblast cells of mice and humans which are implicated in RA showed differential regulation in CHIKV infection [95]. Previously, Selvamani et al. [96] demonstrated that CHIKV enhances the replication in primary human synovial fibroblasts by modulating the miR-146a expression, suggesting that CHIKV suppresses the antiviral response by modulating the miR146a expression and downregulating the expression of NF-κB activation through a negative feedback loop. Both studies are relevant because they identify new biomarkers of CHIKV infection.

Conclusions

1. In the last 10 years, CHIKV infection has become a diagnostic and therapeutic challenge for rheumatologists from all over the world.
2. Due to the mutations described, the virus epidemic can appear anywhere in the world. Therefore, health authorities and first-contact physicians should be alert, especially in endemic areas where a new outbreak may occur.
3. The progression of an acute infection to the development of a chronic infection, characterized mainly by chronic arthritis, should be investigated, in order to identify both clinical and molecular progression factors to clarify if it is a chronic post-infectious arthritis or a persistent viral infection.
4. The patient with chronic arthritis should be treated by the rheumatologist using the necessary medications to reduce or if possible eliminate joint inflammation, improve the quality of life of the patient, and prevent the progression of disabling arthritis.
5. The interaction between the CHIKV infection (environmental factor) and the immunological/inflammatory response of the host, genetically determined, is the key to understanding the development of chronic arthritis after infection by the virus. These findings will allow the development of new preventive and therapeutic strategies to deal with outbreaks of CHIKV infection.

References

1. Strauss JH, Strauss EG. The alphaviruses: gene expression, replication, and evolution. Microbiol Rev. 1994;58:491–562.
2. Wilder-Smith A, Gubler DJ, Weaver SC, Monath TP, Heymann DL, Scott TW. Epidemic arboviral diseases: priorities for research and public health. Lancet Infect Dis. 2017;17(3):e101–6. https://doi.org/10.1016/S1473-3099(16)30518-7.
3. Weaver SC, Reisen WK. Present and future arboviral threats. Antivir Res. 2010;85:328–45. https://doi.org/10.1016/j.antiviral.2009.10.008.
4. Kraemer MU, Sinka ME, Duda KA, et al. The global distribution of the arbovirus vectors Aedesaegypti and Ae. albopictus. Elife. 2015;4:e08347. https://doi.org/10.7554/eLife.08347.
5. Nsoesie EO, Kraemer MU, Golding N, et al. Global distribution and environmental suitability for chikungunya virus, 1952 to 2015. Euro Surveill. 2016;21(20) https://doi.org/10.2807/1560-7917.ES.2016.21.20.30234.
6. Adouchier S, Smura T, Sane J, Vapalahti O, Kurkela S. Sindbis virus as a human pathogen-epidemiology, clinical picture and pathogenesis. Rev Med Virol. 2016;26:221–41. https://doi.org/10.1002/rmv.1876.
7. Figueiredo ML, Figueiredo LT. Emerging alphaviruses in the Americas: Chikungunya and Mayaro. Rev Soc Bras Med Trop. 2014;47:677–83. https://doi.org/10.1590/0037-8682-0246-2014.
8. Forrester NL, Wertheim JO, Dugan VG, et al. Evolution and spread of Venezuelan equine encephalitis complex alphavirus in the Americas. PLoS Negl Trop Dis. 2017;11:e0005693. https://doi.org/10.1371/journal.pntd.0005693.
9. Forrester NL, Kenney JL, Deardorff E, Wang E, Weaver SC. Western Equine Encephalitis submergence: lack of evidence for a decline in virus virulence. Virology. 2008;380:170–2. https://doi.org/10.1016/j.virol.2008.08.012.
10. Lwande OW, Obanda V, Bucht G, et al. Global emergence of Alphaviruses that cause arthritis in humans. Infect Ecol Epidemiol. 2015;5:29853.
11. Ogden NH, Lindsay LR. Effects of climate and climate change on vectors and vector-borne diseases: ticks are different. Trends Parasitol. 2016;32:646–56.
12. Gould EA, Coutard B, ÇMalet H, et al. Understanding the alpha viruses: recent research on important emerging pathogens and progress towards their control. Antivir Res. 2010;87(2):111–24. https://doi.org/10.1016/j.antiviral.2009.07.007.
13. Chikungunya. Washington, DC. Pan American Health Organization. https://www.paho.org/hq/index.php?option=com_topics&view=rdmore&cid=6918&item=chikungunya&cat=statistics&type=distribucion-geografica-6918&Itemid=40931&lang=es.
14. Weaver SC, Lecuit ÇM. Chikungunya virus and the global spread of a mosquito-borne disease. N Engl J Med. 2015;372:1231–9. https://doi.org/10.1056/NEJMra1406035.
15. Suhrbier A, Jaffar-Bandjee MC, Gasque P. Arthritogenic alphaviruses, an overview. Nat Rev Rheumatol. 2012;8(8):420–9. https://doi.org/10.1038/nrrheum.2012.64.

16. Tong S, Dale P, Nicholls N, Mackensie JS, Wolff R, McMichael AJ. Climate variability, social and environmental factors, and Ross River Virus transmission: research development and future research Needs. Environ Health Perspect. 2008;116(12):1591–7.
17. Rezza G, Chen R, Weaver S. O'nyong-nyong fever: a neglected mosquito-borne viral disease. Pathog Glob Health. 2017;111(6):271–5. https://doi.org/10.1080/204477724.2017.1355431.
18. Adouchief S, Smura T, Sane J, Vapalahti O, Kurkela S. Sindbis virus as a human pathogen-epidemiology, clinical picture and pathogenesis. Rev Med Virol. 2016;26(4):221–41. https://doi.org/10.1002/rmv.1876.
19. Bergqvist J, Forsman O, Larsson P, et al. Detection and isolation of Sindbis virus from mosquitoes captured during an outbreak in Sweden in 2013. Vector Borne Zoonotic Dis. 2015;15:133–40. http://dx.doi.org/10.1089.
20. Vasconcelos PF, Calisher CH. Emergence of human arboviral diseases in the Americas, 2000-2016. Vector Borne Zoonotic Dis. 2016;16(5):295–301. https://doi.org/10.1089/vbz.2016.1952.
21. Hoarau JJ, JaffarBandjee MC, KrejbichTrotot P, et al. Persistet chronic inflammation and infection by Chikungunya arthritogenic alphavirus in spite of a robust host immune response. J Immunol. 2010;184:5914–27.
22. Labadie K, Larcher T, Joubert C, et al. Chikungunya disease in nonhuman primates involves long-term viral persistence in macrophages. J Clin Invest. 2010;120:894–906.
23. Hawmann DW, Fox JM, Ashbrook AW, et al. Pathogenic chikungunya virus evades B cell responses to establish persistence. Cell Rep. 2016;16:1326–38.
24. Lee CY, Kam Y-W, Fric J, et al. Chikungunya virus neutralization antigens and direct cell-to-cell transmission are revealed by human antibody-escape mutants. PLoS Pathog. 2011;7:e1002390.
25. Krejbich-Troto P, Gay B, Li-Pat-Yuen G, et al. Chikungunya triggers an autophagic process which promotes viral replication. Virol J. 2011;8:432.
26. Davis FP, Barkan DT, Eswar N, et al. Host pathogen protein interactions predicted by comparative modeling. Protein Sci. 2007;16:2585–896.
27. Paixão ES, Rodrigues LC, Costa MDCN, Itaparica M, Barreto F, Gérardin P, Teixeira MG. Chikungunya chronic disease: a systematic review and meta-analysis. Trans R Soc Trop Med Hyg. 2018;112(7):301–16. https://doi.org/10.1093/trstmh/try063.
28. Amdekar S, Parashar D, Alagarasu K. Chikungunya Virus-induced arthritis: role of host and viral factors in the pathogenesis. Viral Immunol. 2017;30(10):691–702. https://doi.org/10.1089/vim.2017.0052.
29. Nakaya HI, Gardner J, Poo YS, et al. Gene profiling of chikungunya virus arthritis reveals significant overlap with rheumatoid arthritis. Arthritis Rheum. 2012;64:3553–63.
30. Suhbier A, Linn LA. Clinical and pathologic aspects of arthritis due to ross river and other alphaviruses. Curr Opin Rheumatol. 2004;16:374–9.
31. Dupuis Maguriga L, Noret M, Brun S, et al. Chikungunya disease: infections associated markers from the acute to the chronic phase of arboviral-induced arthralgias. PLoS Negl Trop Dis. 2012;6:e1446.
32. Gardner J, Anraku I, Le TT, et al. Chikungunya virus arthritis in adult wild type mice. J Virol. 2010;84:8021–32.
33. Chen W, Foo SS, Sims NA, Herrero LJ, Walsch NC, Mahalingam S. Arthritogenic alphaviruses: new insights into arthritis and bone pathology. Trends Microbiol. 2015;23:35–43. https://doi.org/10.1016/j.tim.2014.09.005.
34. Soden M, Vasudevan H, Roberts B, et al. Detection of viral ribonucleic acid and histologic analysis of inflamed synovium in Ross River virus infection. Arthritis Rheum. 2000;43:365–9.
35. Labadie K, et al. Chikungunya disease in nonhuman primates involves long-term viral persistence in macrophages. J Clin Invest. 2010;120:894–906.
36. Roscoe DM, Ihikawa K, Lyles D. Role of the novo protein synthesis in target cells recognized by cytotoxic T lymphocytes specific for vesicular stomatitis virus. J Virol. 1991;65:6856–61.
37. Chow A, Her Z, Ong EK, Chen JM, Dimatatac F, Kwek DJ, Barkham T, Yang H, Rénia L, Leo YS, Ng LF. Persistent arthralgia induced by Chikungunya virus infection is associated with interleukin-6 and granulocyte macrophage colony-stimulating factor. J Infect Dis. 2011;203(2):149–57. https://doi.org/10.1093/infdis/jiq042.
38. Chirathaworn C, Rianthavorn WN, Poovorawan Y. Serum IL-18 and IL-18PB levels in patients with Chikungunya virus infection. Viral Immunol. 2010;23:113–7.
39. Ng LF, et al. IL-1B, IL-6, and RANTES as biomarkers of chikungunya severity. PLoS One. 2009;4:e4261.
40. Chang AY, Martins KAO, Encinales L, et al. Chikungunya Arthritis mechanisms in the Americas: a cross-sectional analysis of Chikungunya Arthritis Patients Twenty-Two months after infection demonstrating no detectable viral persistence in synovial fluid. Arthritis Rheumatol. 2018;70:585–93.
41. Chang AY, Tritsch S, Reid SP, et al. The cytokine profile in Acute Chikungunya Infection is predictive of chronic arthritis 20 months postinfection. Diseases. 2018;64. Pii: E95. https://doi.org/10.3390/diseases6040095.
42. Sepúlveda-Delgado J, Vera-Lastra OL, Trujillo-Murillo OL, et al. Inflammatory biomarkers, disease activity index, and self-reported disability may be predictor of chronic arthritis after chikungunya infection: brief report. Clin Rheumatol. 2017;36:695–9.
43. Rims C, Uchtenhagen H, Kaplan MJ, Carmona-Rivera C, Carlucci P. Citrullinated aggrecan epitopes as targets of auto-reactive CD4+ T cells in patients with rheumatoid arthritis. Arthritis Rheumatol. 2018;71:518. https://doi.org/10.1002/art.40768.
44. Fujinami RS, Von HMG, Christen U, Whitton JL. Molecular mimicry, bystander activation, or viral persistence: infections and autoimmune disease. Clin Microbiol Rev. 2006;19:80–94.
45. Simmonds M, Gough S. The HLA region and autoimmune disease: associations and mechanisms of action. Curr Genomics. 2007;8:453–65.
46. Moller E. Mechanisms for induction of autoimmunity in humans. Acta Paediatr. 1998;424:16–20.
47. FitzGerald O, Haroon M, Giles JT, Winchester R. Concepts of pathogenesis in psoriatic arthritis: genotype determines clinical phenotype. Arthritis Res Ther. 2015;17:1–11.
48. Hennecke J, Wiley DC. Structure of a complex of the human α/β T cellreceptor (TCR) HA1.7, influenza hemagglutinin peptide, and majorhistocompatibility complex class II molecule, HLA-DR4 (DRA1∗0101 andDRB1∗0401): insight into TCR cross-restriction and alloreactivity. J Exp Med. 2002;195:571–81.
49. Stern LJ, Brown JH, Jardetzky TS, Gorga JC, Urban RG, Strominger JL, Wiley DC. Crystal structure of the human class II MHC protein HLA-DR1complexed with an influenza virus peptide. Nature. 1994;368:215–21.
50. Zúñiga J, Yu N, Barquera R, Alosco S, Ohashi M, Lebedevai T, et al. HLA class I and class II conserved extended haplotypes and their fragments or blocks in Mexicans: implications for the study of genetic diversity in admixed populations. PLoS One. 2013;8(9):e74442.
51. Chopra A, Anuradha V, Lagoo-Joshi V, Kunjir V, Salvi S, Saluja M. Chikungunya virus aches and pains: an emerging challenge. Arthritis Rheum. 2008;58:2921–.21.
52. Lopes Marques CD, Branco Pinto Duarte AL, Ranzolin A, et al. Recommendations of the Brazilian Society of Rheumatology for diagnosis and treatment of Chikungunya fever. Part 1. Diagnosis and special situations. Rev Bras Reumatol Engl Ed. 2017;57 Suppl 2:421–37.
53. Rajapakse S, Rodrigo C, Rajapakse A. Atypical manifestations of chikungunya infection. Trans R Soc Trop Med Hyg. 2010;104:89–96. https://doi.org/10.1016/j.trstmh.2009.07.031. Epub 2009 Aug 27. Review.

54. Economopoulou A, Dominguez M, Helynck B, Sissoko D, Wichmann O, Quenel P, Germonneau P, Quatresous I. Atypical Chikungunya virus infections: clinical manifestations, mortality and risk factors for severe disease during the 2005-2006 outbreak on Réunion. Epidemiol Infect. 2009;137:534–41. https://doi.org/10.1017/S0950268808001167. Epub 2008 Aug 11.
55. Arroyo-Ávila M, Vilá LM. Rheumatic manifestations in patients with Chikungunya infection. P R Health Sci J. 2015;34:71–7.
56. Simon F, Javelle E, Cabie A, et al. French guidelines for management of Chikungunya (acute and persistent presentation). Med Mal Infect. 2015;45:243–63.
57. Schilte C, Staikovsky F, Coudertc T, et al. Chikungunya virus-associated long-term arthralgia: a 36-month prospective longitudinal study. C. 2013;7:e2137.
58. Javelle E, Rivera A, Degasne I, et al. Specific management of post-Chikungunya rheumatic disorders: a retrospective study of 159 cases in Reunion Island from 2006-2012. PloS Negl Trop Dis. 2015;9(3):e000360.
59. Danis-Lozano R, Díaz-González EE, Trujillo-Murillo KDC, et al. Clinical characterization of acute and convalescent illness of confirmed chikungunya cases from Chiapas, S. Mexico: a cross sectional study. PLoS One. 2017;12(10):e0186923. https://doi.org/10.1371/journal.pone.0186923. eCollection 2017.
60. Essckjee K, Goorah S, Ramchurn SK, et al. Prevalence of risk factors for chronic arthralgia and rheumatoid-like polyarthritis more than 2 years after infection with Chikungunya virus. Posgrad Med J. 2013;89:440–7.
61. Sissoko Malvy S, Ezzedine K, et al. Post–epidemic Chikungunya disease on reunion island: course of rheumatic manifestations and associated factors over a 15-month period. PloS Negl Trop Dis. 2009;e:389.
62. Simon F, Javelle E, Oliver M, et al. Chikungunya virus infection. Curr Infect Dis Rep. 2011;13:218–28.
63. Anfansa F, Privacia L, Geurtsvankessel C, et al. Hyperferritinemia is a potential marker of chronic chikungunya: a retrospective study on the Island of Curacao during the 2014-2015 outbreak. J Clin Invest. 2017;86:1–38.
64. Litzba N, Schuffenecker I, Zeller H, et al. Evaluation of the first commercial chikungunya virus indirect immunofluorescence test. J Virol Methods. 2008;149:175–9.
65. Reddy V, Ravi V, Desai I, et al. Utility of IgM ELIS, TaqMan real PCR, reverse transcription PCR, and RT-LAMP assay for diagnosis of Chikungunya fever. J Med Virol. 2012;84:1771–8.
66. Taubitz W, Cramer JP, Kapauna A, et al. Chikungunya fever in travelers: clinical presentation and course. Clin Infect Dis. 2007;45:1–4.
67. Cunha RVD, Trinta KS. Chikungunya virus: clinical aspects and treatment - a review. Mem Inst Oswaldo Cruz. 2017;112:523–31.
68. Runowska M, Majewski D, Niklas K, Puszczewicz M. Chikungunya virus: a rheumatologist's perspective. Clin Exp Rheumatol. 2018;36:494–501.
69. Chopra A, Saluja M, Venugopalan A. Effectiveness of chloroquine and inflammatory cytokine response in patients with early persistent musculoskeletal pain and arthritis following chikungunya virus infection. Arthritis Rheumatol. 2014;66:319–26.
70. Ravindran V, Alias G. Efficacy of combination DMARD therapy vs. hydroxychloroquine monotherapy in chronic persistent chikungunya arthritis: a 24-week randomized controlled open label study. Clin Rheumatol. 2017;36:1335–40.
71. Bouquillard E, Combe E. A report of 21 cases of rheumatoid arthritis following Chikungunya fever. A mean follow-up two years. J Bone Spine. 2009;76:654–7.
72. Miner JJ, Cook LE, Hong JP, et al. Therapy with CTL$-Ig an antiviral monoclonal antibody control chikungunya virus arthritis. Sci Transl Med. 2017;9:3438.
73. Dengue WHO. Guidelines for diagnosis, treatment, prevention and control. Geneva: World Health Organization; 2009. https://www.who.int/tdr/publications/documents/dengue-diagnosis.pdf.
74. Erasmus JH, Shannan L, Rossi SL, Weaver SC. Development of vaccines for Chikungunya fever. J Infect Dis. 2016;214(S5):S488–96.
75. Porta J, Mangala Prasad V, Wang CI, Akahata W, Ng LF, Rossmann MG. Structural studies of Chikungunya virus-like particles complexed with human antibodies: neutralization and cell-to-cell transmission. J Virol. 2015;90:1169–77.
76. Sutaria RB, Amaral JK, Schoen RT. Emergence and treatment of chikungunya arthritis. Curr Opin Rheumatol. 2018;30:256–63. https://doi.org/10.1097/BOR.0000000000000486.
77. Mavalankar D, Shastri P, Bandyopadhyay T, et al. Increased mortality rate associated with chikungunya epidemic, Ahmedabad, India. Emerg Infect Dis. 2008;14:412–5.
78. Mascarenhas M, Garasia S, Berthiaume P, Corrin T, Greig J, Ng V, Young I, Wadell L. A scoping review of published literature on chikungunya virus. PLoS One. 2018;13:e0207554. https://doi.org/10.1371/journal.pone.0207554. eCollection 2018.
79. Gardner J, Rudd PA, Prow NA, Belarbi E, Roques P, Larcher T, et al. Infectious Chikungunya virus in the saliva of mice, monkeys and humans. PLoS One. 2015;10(10):e0139481. https://doi.org/10.1371/journal.pone.0139481. PMID: 26447467.
80. Bandeira AC, Campos GS, Rocha VF, Souza BS, Soares MB, Oliveira AA, et al. Prolonged shedding of Chikungunya virus in semen and urine: a new perspective for diagnosis and implications for transmission. IDCases. 2016;6:100–3. https://doi.org/10.1016/j.idcr.2016.10.007.
81. Yactayo S, Staples JE, Millot V, Cibrelus L, Ramon-Pardo P. Epidemiology of Chikungunya in the Americas. J Infect Dis. 2016;214(Suppl 5):S441–5. https://doi.org/10.1093/infdis/jiw390.
82. Bocanegra C, Anton A, Sulleiro E, Pou D, Salvador F, Roure S, et al. Imported cases of Chikungunya in Barcelona in relation to the current American outbreak. J Travel Med. 2016;23(3) https://doi.org/10.1093/jtm/tav033. Print 2016 Mar. PMID: 26984354.
83. Perret C, Vizcaya C, Weitzel T, Rosas R, Dabanch J, Martínez C. Chikungunya, emerging disease in Latin America. Description of the first cases in Chile. Rev Chil Infectol. 2018;35(4):413–9. https://doi.org/10.4067/s0716-10182018000400413.
84. YiuWing K, KwoonYong P, KaiEr E, LiKiang T, Kaur S, Lee WWL, et al. Sero-prevalence and crossreactivity of Chikungunya virus specific anti-E2EP3 antibodies in arbovirus-infected patients. PLoS Negl Trop Dis. 2015;9(1):e3445.
85. Hassing RJ, Leparc-Goffart I, Tolou H, van Doornum G, van Genderen PJ. Cross-reactivity of antibodies to viruses belonging to the Semliki forest serocomplex. Eur Secur. 2010;(23):15.
86. Torres JR, Leopoldo Códova G, Castro JS, Rodrıguez L, Saravia V, Arvelaez J, et al. Chikungunya fever: atypical and lethal cases in the Western hemisphere: a Venezuelan experience. IDCases. 2015;2(1):6–10. https://doi.org/10.1016/j.idcr.2014.12.002.
87. Larrieu S, Pouderoux N, Pistone T, Filleul L, Receveur MC, Sissoko D, et al. Factors associated with persistence of arthralgia among chikungunya virus-infected travellers: report of 42 French cases. J Clin Virol. 2010;47(1):85–8. https://doi.org/10.1016/j.jcv.2009.11.014. PMID: 20004145.
88. Torres JR, Cordova LG, Saravia V, Arvelaez J, Castro JS. Nasal skin necrosis: an unexpected new finding in severe Chikungunya fever. Clin Infect Dis. 2016;62(1):78–81. https://doi.org/10.1093/cid/civ718. PMID: 26423381.
89. Taraphdar D, Roy BK, Chatterjee S. Chikungunya virus infection amongst the acute encephalitis syndrome cases in West Bengal, India. Indian J Med Microbiol. 2015;33(5 Suppl):153–6.
90. Oliver GF, Carr JM, Smith JR. Emerging infectious uveitis: Chikungunya, Dengue, Zika, Ebola. Clin Exp Ophthalmol. 2019;47:372. https://doi.org/10.1111/ceo.13450.

91. Mahto SK, Gupta PK, Singh A, Meena RC. Atypical neurological manifestations of Chikungunya fever: two case reports. Indian J Crit Care Med. 2018;22:306–8. https://doi.org/10.4103/ijccm.IJCCM_459_17.
92. Muñoz-Medina JE, Garcia-Knight MA, Sanchez-Flores A, Monroy-Muñoz IE, Grande R, Esbjörnsson J, Santacruz-Tinoco CE, González-Bonilla CR. Evolutionary analysis of the Chikungunya virus epidemic in Mexico reveals intra-host mutational hotspots in the E1 protein. PLoS One. 2018;13:e0209292. https://doi.org/10.1371/journal.pone.0209292.
93. Akhrymuk I, Lukash T, Frolov I, Frolova EI. Novel mutations in nsP2 abolish chikungunya virus-induced transcriptional shutoff and make virus less cytopathic without affecting its replication rates. J Virol. 2018;93(4). pii: JVI.02062-18. https://doi.org/10.1128/JVI.02062-18.
94. Paul B, Pariyapurath R. Risk factor assessment of rheumatoid arthritis in North Kerala. Eur J Rheumatol. 2018;5:184–90. https://doi.org/10.5152/eurjrheum.2018.17111.
95. Parashar D, Paingankar MS, More A, Patil P, Amdekar S. Altered microRNA expression signature in Chikungunya-infected mammalian fibroblast cells. Virus Genes. 2018;54:502. https://doi.org/10.1007/s11262-018-1578-8.
96. Selvamani SP, Mishra R, Singh SK. Chikungunya virus exploits miR-146a to regulate NF-κB pathway in human synovial fibroblasts. PLoS One. 2014;9(8):e103624. https://doi.org/10.1371/journal.pone.0103624. eCollection 2014.