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REVIEW

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Acute Immunological Profile and Prognostic Biomarkers of Persistent Joint Pain in Chikungunya Fever: A Systematic Review

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Chikungunya virus infection (CHIKV) increases the risk of persistent arthralgia; however, there is no consistent evidence regarding prognostic biomarkers of progression to chronic arthropathy. This systematic review provides an overview of currently available literature about the potential role of the acute immunologic response in predicting long-term joint pain in patients with a diagnosis of CHIKV. We searched for observational studies using the terms "chikungunya," "cytokines," "biomarkers," and "joint pain" in PubMed/MEDLINE, LILACS, Cochrane Library Plus, and SCOPUS databases, restricting to articles published in English and up to April 2024. PROSPERO registration number: CRD42021279400. Thirty-eight studies were selected for qualitative synthesis with a maximum duration from diagnosis to clinical evaluation of 60 months. The sample sizes ranged from 8 to 346 participants (age range: 0-90 years). We identified an immunologic profile during the acute phase of CHIKV that includes increased levels of proinflammatory cytokines (IFN-α, IFN-γ, IL-2R, IL-6, IL-7, and IL-8), anti-inflammatory cytokines (IL-1Ra and IL-4), chemokines (MCP-1, MIG, and IP-10) and growth factors (VEGF and G-CSF). Only one out of two studies reported differences in cytokine levels during the acute phase, predicting persistent joint pain at 20 months of follow-up. Also, persistence of anti-CHIKV IgG seemed to be a potential prognostic marker. The evidence suggests the existence of an inflammatory response in the acute phase of CHIKV that persists during its chronic phase; however, there is no unequivocal candidate set of biomarkers of progression toward long-term articular sequelae.

INTRODUCTION

Chikungunya, an arthropod-borne disease caused by the Chikungunya virus (CHIKV), is an acute infection associated to the development of rheumatic clinical manifestations that can persist for years [1]. The main long-term consequence of CHIKV infection, the post-CHIK chronic inflammatory rheumatism (pCHIK-CIR), is defined by the persistence of joint and extra-articular symptoms for more than 3 months after the onset of CHIKV disease or the development of specific immune-mediated inflammatory pathology during follow-up [2]. The frequency of persistent rheumatic manifestations ranges from 17% to 53%, a wide variation partially explained by the heterogenicity of clinical definitions and follow-up times at which they were implemented [3-6].

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Abbreviations: CHIKV, Chikungunya virus infection; pCHIK-CIR, post-CHIK chronic inflammatory rheumatism.

Keywords: Chikungunya fever, biomarkers, arthralgia, chronic pain, cytokines

Although the first epidemic of CHIKV occurred in Tanzania in 1952 [7], the emergence of this infection in Europe and America is recent. Also, understanding the pathogenesis of persistent rheumatic manifestations is still limited, consistent with Chikungunya being classified as a neglected disease by the World Health Organization (WHO) [8]. It has been suggested that CHIKV may persist in immune-privileged niches such as the synovial tissue, contributing directly to its damage and the progression toward the chronic phase of the disease; however, there is no consistency in the finding of viral RNA or its proteins in macrophages from synovial fluid obtained from patients afflicted by the infection [9,10].

Alternatively, it has been postulated that the immune response, ie, the production of cytokines, chemokines, and growth factors induced by CHIKV, could be associated with chronic articular disease persistence [11-13]. Lee et al. were the first to demonstrate elevated levels of interleukin-8 (IL-8), IFN-γ-induced protein 10 (IP-10), monokine-induced by IFN-γ (MIG), and monocyte chemoattractant protein (MCP-1) in one patient with CHIKV infection [14] a finding inconsistently replicated by other authors [10,15-24] owned in part to the limited availability of longitudinal studies in clinical settings. This systematic review aims to establish an immunological profile of acute CHIKV disease based on the available evidence; also, to identify and critically appraise the evidence on prognostic biomarkers of the immune response in relation to persistent arthralgia, one of the most commonly and consistently reported symptoms of chronic articular compromise, post-CHIKV infection.

MATERIALS AND METHODS

This systematic review was conducted following the AMSTAR (A Measurement Tool to Assess Systematic Reviews) instrument [25] and the reporting of results following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines [26]. Review protocol was registered in the PROSPERO international register of systematic reviews (CRD42021279400). The AMSTAR and PRISMA checklists of this review can be found in Appendix A: Supplementary material I and II.

Data Sources and Search Strategy

PubMed/MEDLINE, LILACS, Cochrane Library Plus, and SCOPUS databases were searched for observational studies published in any language, since their inception to April 2024. We used the search terms "chikungunya," "cytokines," "biomarkers," "arthralgia," and "joint pain." We also checked the abstract books of the meetings of the American Society of Tropical Medicine and Hygiene (ASTMH) from 2011 to 2023 and the reference lists of the identified articles to supplement electronic searching.

Eligibility Criteria

We included observational studies, which evaluated the relationship between markers of immunological response and acute phase of CHIKV disease or arthralgia in patients with a diagnosis of CHIKV infection. Studies published in a language different from English were excluded as well as those in which markers were measured in a biological matrix different from serum or plasma, or after an *in vitro* stimulation of human cells.

Study Selection

Studies identified through the search strategy were recorded in an Excel spreadsheet and the duplicated records were removed. We screened all the titles and abstracts based on the eligibility criteria (authors: AL-P, RMGR). Discrepancies were resolved by consensus and if necessary, a third reviewer was consulted to reach a final decision. After retrieval of full-text articles, one author again checked eligibility. Figure 1 shows the flow chart of the study.

Data Extraction

The following information was retrieved by one author, for each study: author name, study design, country, publication year, patient age, sample size (prevalent or incident cases and controls, if applicable), maximum disease duration, the test used for CHIKV diagnosis and type of biological matrix used for the measurement of markers' concentrations.

Bias Risk Assessment

Two authors independently assessed the methodological quality of the eligible observational studies using the Newcastle-Ottawa Scale (NOS). According to the NOS criteria for selection (four points at most), comparability (two points at most), and the adequacy of outcome measures (three points at most), a maximum of nine points could be awarded. The risk of bias was categorized based on the obtained score as follows: high-risk (0-3), intermediate-risk (4-6), and low-risk (7-9).

RESULTS

Study Selection

The search strategy identified 1213 articles; however, after removing 625 duplicates and excluding 536 non-eligible articles based on the screening of their titles and abstracts, we retrieved and reviewed 52 full-text articles of which 38 studies were selected for qualitative

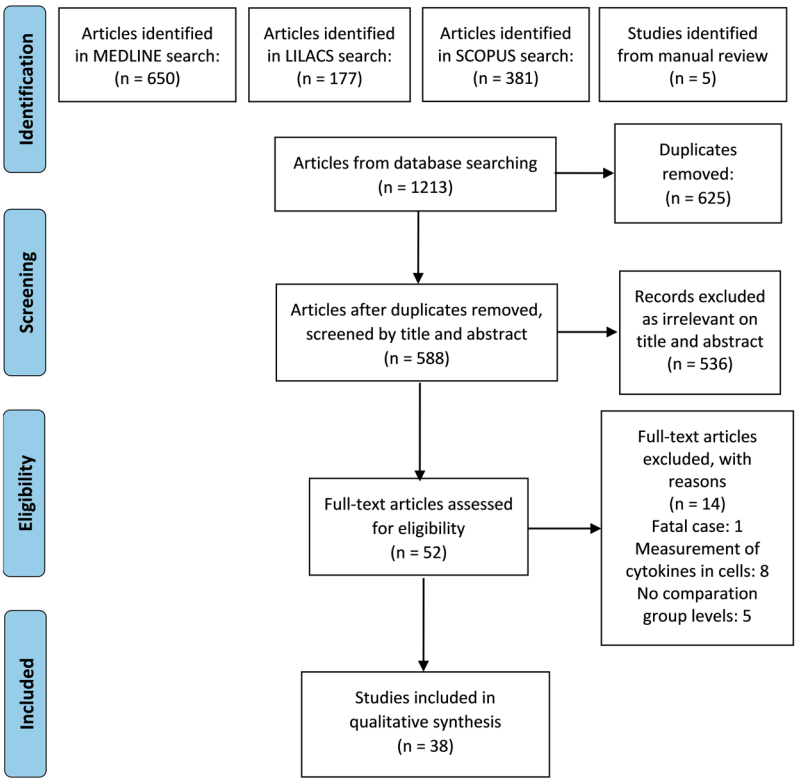

Figure 1. **Search flow chart**.

synthesis (Figure 1).

Studies' Characteristics and Risk of Bias

Among the included studies, 52.6% were conducted in Asia, 31.6% in America, 13.2% in Europe, and 2.6% in Africa with a predominance of case-control and cohort designs (55.3% and 34.2%, respectively; Table 1). The sample sizes ranged from 8 to 346 participants (age range: 0 to 90 years old), and the maximum duration from diagnosis to clinical evaluation was 60 months. CHIKV infection was defined as a positive result of a reverse transcription-polymerase chain reaction (RT-PCR) test to detect the CHIKV genome (n=8), an enzyme-linked immunosorbent assay (ELISA) to detect IgM or IgG anti-CHIKV (n=13) or both (n=17). The most often evaluated biomarkers were IL-6 (n=21), tumor necrosis factor-alpha (TNF-α; n=19), interferon-gamma (IFN-γ; n=17), IL-8 (n=17), and IL-10 (n=17), with most studies (71.1%) using serum as a biological matrix for biomarker quantification. In relation to risk of bias, most of the studies (73.5% and 23.5%) were classified as at intermediate- and low-risk according to the NOS (Table 2).

Profile of Immune Response in the Acute Disease

Twenty-seven studies (71.1%) evaluated immune response markers during the acute phase of CHIKV infection. Figure 2 shows a heat map of 37 immune mediators from at least two studies, indicating comparisons among CHIKV cases and healthy controls (HC). We observed higher levels of proinflammatory cytokines (IFN-α, IFN-γ, interleukin 2 receptor (IL-2R), IL-6, IL-7, and IL-8) and anti-inflammatory cytokines (IL-4 and the IL-1 antagonist receptor (IL-1Ra)) as well as chemokines (MCP-1, MIG, and IP-10) and growth factors (vascular endothelial growth factor (VEGF), and granulocyte colony-stimulating factor (G-CSF)) in the acute phase of CHIKV infection compared with HC. This pattern persisted regardless of the precedence of cases (Asia or America), except by IL-4, and IFN-α, which were not included in the pattern of studies from America. The clinical laboratory parameter, C-reactive protein (CRP) was reported to increase in the acute phase of CHIKV infection compared with HC [10,21,22,27-30]. Moreover, recently three molecules were reported as potential markers of acute CHIKV infection: IL-27 [31], galectin 9 (GAL-9) [32], and high mobility group box 1 protein (HMGB1) [33].

Biomarkers of Persistent Joint Pain

The duration of arthropathy symptoms among CHIKV cases ranged from 3 months [15,29,34] to 60 months [35]. Concurrent evaluation of biomarkers be-

Table 1. Characteristics of the Included Studies

Author's name, pub year	Study design	Country	Patient age (years)	Sample size (n)		Maximum disease duration	CHIKV diagnosis	Sample
				Cases	Controls			
Hoarau, 2010 [10]	Cohort	France	19-90	32	8	12 months	PCR & serology	Serum
Chaaithanya, 2011 [36]	Cohort	India	25-55	22	6	10 months	Serology	Serum
Kelvin, 2011 [18]	Cohort	Italy	No data	50	10	12 months	PCR & serology	Serum
Chow, 2011 [29]	Cohort	Singapore	23-67	30	8	2-3 months	PCR	Plasma
Chopra, 2012 [28]	Cohort	India	15-24	225	49	24 months	Serology	Serum
Lohachanakul, 2012 [20]	Cohort	Thailand	20-54	35	27	1 month	PCR & serology	Plasma
Moro, 2012 [41]	Cohort	Italy	0-60	250	0	12 months	Serology	Serum
Kam, 2012 [34]	Cohort	Singapore	23-67	30	0	2-3 months	Serology	Plasma
Gérardin, 2013 [40]	Cohort	France	15-65	346	0	24 months	Serology	Serum
Venugopalan, 2014 [30]	Cohort	India	Adults	110	80	1 month	Serology	Serum
Chang, 2018 [39]	Cohort	Colombia	Adults	242	0	20 months	Serology	Serum
Nayak, 2020 [42]	Cohort	India	15-77	72	0	20 months	PCR & serology	Plasma
Jacob-Nascimento, 2021 [63]	Cohort	Brazil	14-50	253	81	>3 months	PCR & serology	Serum
Alves de Souza, 2022 [15]	Cohort	Brazil	28-66	78	10	3 months	PCR & serology	Plasma
Chirathaworn, 2010 [64]	Case-Control	Thailand	No data	28	20	13 days	PCR & serology	Serum
Wauquier, 2011 [24]	Case-Control	Gabon	Adults	69	30	1 week	PCR	Plasma
Chirathaworn, 2013 [16]	Case-Control	Thailand	2-84	46	20	13 days	PCR & serology	Serum
Schilte, 2013 [38]	Case-Control	France	Adults	20	22	36 months	PCR	Serum
Kashyap, 2014 [17]	Case-Control	India	35-85	8	5	11 days	PCR & serology	Serum
Reddy, 2014 [65]	Case-Control	India	21-80	48	37	3 months	PCR & serology	Plasma
Rojas, 2015 [43]	Case-Control	Colombia	Adults	73	0	1 month	Serology	Serum
Dutta, 2017 [66]	Case-Control	India	9-76	173	157	>8 days	PCR & serology	Serum
Chattopadhya, 2017 [27]	Case-Control	India	5-65	30	30	No data	Serology	Serum
Banerjee, 2018 [67]	Case-Control	India	Adults	40	25	2 weeks	PCR & serology	Plasma
Tanabe, 2019 [68]	Case-Control	Brazil	7-82	29	21	5 days	PCR & serology	Serum
Cavalcanti, 2019 [31]	Case-Control	Brazil	41-69	45	49	5 months	Serology	Serum
Ninla-Aesong, 2019 [35]	Case-Control	Thailand	No data	93	30	60 months	Serology	Serum
Sánchez-Arcila, 2020 [23]	Case-Control	Brazil	Adults	33	37	<8 days	PCR	Serum

Cavalcanti, 2020 [32]	Case-Control	Brazil	41-69	44	49	5 months	Serology	Serum
Krishnan, 2021 [19]	Case-Control	India	22-65	16	10	14 days	PCR & serology	Plasma
Rocha, 2022 [33]	Case-Control	Brazil	15-89	80	32	<19 days	PCR & serology	Serum
Liu., 2022 [69]	Case-Control	Brazil	18-66	40	13	6 months	PCR	Serum
Babu, 2023 [70]	Case-Control	India	12-70	196	24	1 month	PCR & serology	Serum
Restrepo, 2022 [22]	Case-Control	Colombia	18-15	83	10	3 months	PCR & serology	Serum
Dhenni, 2021 [71]	Cross-sectional	Indonesia	1-78	32	4	<9 days	PCR	Serum
Ng, 2009 [21]	Case series	Singapore	22-65	10	9	<10 days	PCR	Plasma
Chopra, 2014 [72]	Case series	India	No data	70	80	>6 weeks	Serology	Serum
Sepúlveda-Delgado, 2017 [37]	Case series	Mexico	27-64	10	0	12 months	PCR & serology	No data

tween 6 and 12 months post-CHIKV infection showed higher IL-6 levels and lower eotaxin, HGF, IL-5, and Regulated on Activation, Normal T-Expressed and Secreted (RANTES) levels in cases with persistent joint pain as compared to recovered cases [29,36,37] (Table 3). Moreover, studies with longer disease duration (36 and 60 months) found higher concentrations of IL-1α and matrix metalloproteinases 1 and 3 (MMP-1 and MMP-3) among cases with persistent joint pain as compared to recovered cases [35,38].

About prognosis, only one out of two studies [10,39] reported cytokines levels measured during the acute phase predicting persistent joint arthropathy at 20 months of follow-up (Table 4) [39]. Although Hoarau et al. also observed lower IL-4 and IL-13 levels in the early days of CHIKV infection in patients with persistent arthralgia at 12 months of follow-up, such differences were not statistically significant [10]. Also, CRP levels were higher in the first 5 days in chronic cases compared to the recovered case (60.2±59.7 vs 11.3±10.1 mg/l) [10].

Several studies evaluated anti-CHIKV IgG levels as biomarkers of chronic arthropathy [34,40-43]. The TELECHIK study reported an association between higher IgG levels and increased risk of persistent rheumatic pain at 24 months of follow-up (OR=6.2; 95 CI%: 2.8-13.2) [40]. Rojas et al. observed associated higher IgG levels with a positive squeeze test in the sub-acute phase (OR=1.1; 95% CI: 1.01-1.12) [43]. An early antibody response, indicated by increased IgG3 levels, could clear the virus faster and protect against persistent arthralgia [34]. Notably, the early appearance of neutralizing antibodies (irrespective of the isotype) during the febrile phase of CHIKV infection increased the risk of developing chronic arthritis [42].

DISCUSSION

In this systematic review we identified an immunologic profile characterized by a set of increased biomarkers during the acute phase of the infection by CHIKV that includes proinflammatory cytokines (IFN-α, IFN-γ, IL-2R, IL-6, IL-7, and IL-8), anti-inflammatory cytokines (IL-1Ra and IL-4), chemokines (MCP-1, MIG, and IP-10) and growth factors (VEGF and G-CSF). IL-27, GAL-9, and HMGB1 also could contributed to such profiles, while IL-6, IL-4, IgG, and CRP emerged as potential prognostic biomarkers of chronic complications.

Previous systematic reviews provide context for our findings. Teng et al. [44], reported a similar acute immune response profile in CHIKV infection but found no changes in VEGF and IL-8 levels. They observed increased concentration of IL-2, IL-10, IL-12, IL-15, IL-17, IL-18, monocyte chemoattractant protein 1α and β (MIP-1α and MIP-1β) and the basic fibroblast growth factor (FGF-β).

Table 2. Newcastle-Ottawa Risk of Bias Assessment

Author's name	Study design	Selection	Comparability	Exposure	Score	Risk of bias
Hoarau, 2010 [10]	Cohort	3	0	1	4	Intermediate
Chaaithanya, 2011 [36]	Cohort	3	0	2	5	Intermediate
Kelvin, 2011 [18]	Cohort	3	0	2	5	Intermediate
Chow, 2011 [29]	Cohort	2	0	2	4	Intermediate
Chopra, 2012 [28]	Cohort	3	0	2	5	Intermediate
Lohachanakul, 2012 [20]	Cohort	4	0	0	4	Intermediate
Moro, 2012 [41]	Cohort	4	1	3	8	Low
Kam, 2012 [34]	Cohort	2	0	0	2	High
Gérardin, 2013 [40]	Cohort	4	1	2	7	Low
Venugopalan, 2014 [30]	Cohort	3	0	1	4	Intermediate
Chang, 2018 [39]	Cohort	4	1	2	7	Low
Nayak, 2020 [42]	Cohort	4	0	2	6	Intermediate
Jacob-Nascimento, 2021 [63]	Cohort	4	2	1	7	Low
Alves de Souza, 2022 [15]	Cohort	3	0	1	4	Intermediate
Chirathaworn, 2010 [64]	Case-Control	2	0	3	5	Intermediate
Wauquier, 2011 [24]	Case-Control	3	0	3	6	Intermediate
Chirathaworn, 2013 [16]	Case-Control	1	0	3	4	Intermediate
Schilte, 2013 [38]	Case-Control	4	1	3	8	Low
Kashyap, 2014 [17]	Case-Control	1	0	3	4	Intermediate
Reddy, 2014 [65]	Case-Control	1	1	3	5	Intermediate
Rojas, 2015 [43]	Case-Control	4	1	3	8	Low
Dutta, 2017 [66]	Case-Control	3	2	3	8	Low
Chattopadhya, 2017 [27]	Case-Control	2	1	3	6	Intermediate
Banerjee, 2018 [67]	Case-Control	2	1	3	6	Intermediate
Tanabe, 2019 [68]	Case-Control	1	0	3	4	Intermediate
Cavalcanti, 2019 [31]	Case-Control	3	1	2	6	Intermediate
Ninla-Aesong, 2019 [35]	Case-Control	4	1	3	8	Low
Sánchez-Arcila, 2020 [23]	Case-Control	2	0	3	5	Intermediate
Cavalcanti, 2020 [32]	Case-Control	2	1	2	5	Intermediate
Krishnan, 2021 [19]	Case-Control	1	1	2	4	Intermediate
Rocha, 2022 [33]	Case-Control	3	0	3	6	Intermediate
Liu, 2022 [69]	Case-Control	3	0	3	6	Intermediate
Restrepo, 2022 [22]	Case-Control	1	0	3	4	Intermediate
Babu, 2023 [70]	Case-Control	3	0	2	5	Intermediate

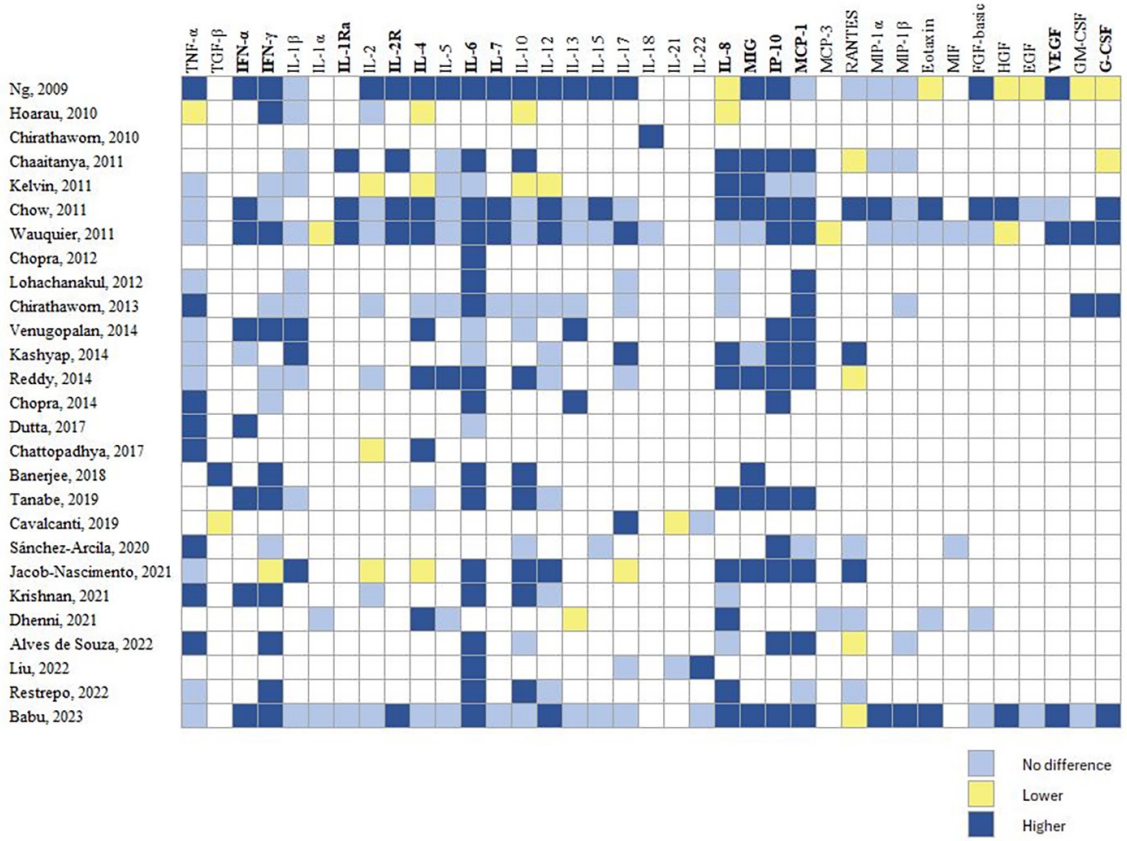

Figure 2. **Heat map of immune profile reported in the acute phase of chikungunya infection**.

These differences may be attributed to the genetic background of the populations in the studies reviewed by Teng et al. which mostly originated in Asia and Europe [45,46]. Ferreira et al. revealed higher biomarker concentrations in severe acute infection or chronic cases; however, their quantitative synthesis focused on IL-6, CRP and TNF-α showing no significant differences [47]. Our results partially agree with those from Ferreira et al. in relation to their qualitative finding of increased levels of IL-6, IL-8, MCP-1, IL-1α, GM-CSF, IL-1Ra, MIP-1α, and MIP-1β; however, these results cannot be directly contrasted because Ferreira et al. did not report separate data for patients evaluated during acute infection from those with chronic disease and considered as control group a mix of healthy individuals and recovered patients.

As the first line of defense against viral infections, IFN-α induces an antiviral state to restrict the infection and promotes the expression of class I major histocompatibility complex (MHC I) on infected cells. This favors the recognition and destruction of infected cells by CD8+ T cells [48]. Our results are in accordance with previous reports showing high levels of IFN-α and a predominance of CD8+ T cells during the early stages of CHIKV infection [24]. We also found increased concentrations of IFN-γ, IP-10 and MIG among CHIKV infection cases compared to healthy controls. IFN-γ is crucial for maintaining the Th1/Th2 balance and induces these chemokines, which recruit macrophages, monocytes, NK cells, and T cells to the affected joints [14,36].

Elevated MCP-1 levels during the acute stage of CHIKV infection could attract monocytes to infection sites, correlating with increased CD14+ and CD14+CD16+ monocyte subpopulations [49]. In addition, MCP-1 stimulates monocytes differentiation into osteoclasts, which could partially explain early joint pain in CHIKV patients [50]. Likewise, IL-6 and IL-8 may contribute to the differentiation of monocyte into osteoclasts [50], and IL-7 may stimulate T cells to secrete osteoclastogenic cytokines [51]. Furthermore, IL-8 and VEGF may be relevant to CHIKV pathogenesis. IL-8 contributes to joint inflammation by attracting neutrophils and T cells to the inflammatory site [52,53], while VEFG, a proinflammatory growth factor, has been reported at high levels during the acute and chronic phase of the Mayaro virus infection and detected in synovial fibroblasts cultures from rheumatoid arthritis patients [54,55].

Other molecules, whose production was elevated during the acute phase of CHIKV infection but for which no replication studies are available, could also contribute to the pathogenesis of long-term disease's complications.

Table 3. Concurrent Markers of Persistent Joint Pain in Chikungunya Infection

Study	Outcome	Sample size (n)		Evaluation time of markers and outcome (months)	Contrast of markers' concentrations (Recovered patients as reference group)		
		Chronic	Recovered		Higher	Lower	Non difference
Chow, 2011 [29]	Joint pain	4	26	3	IL-6, GM-CSF	Eotaxin, HGF	
Restrepo, 2022 [22]	Musculoskeletal disorder	28	55	3		IL-10, MIP-1	IFN-γ, TNF-α, IL-6, IL-12, IL-8, MCP-1, RANTES
Sepúlveda-Delgado, 2017 [37]	Joint pain	6	4	3 and 12	IL-6, RF		CRP
Chaaithanya, 2011 [36]	Joint pain	10	6	10	IL-1β, IL-1Ra, MCP-1, IL-6, IL-8, G-CSF, MIP-1α, MIP-1β	IL-5, RANTES	IL-2R, IL-10, IP-10, MIG
Schilte, 2013 [38]	Joint pain	20	22	36	IL-1α		GM-CSF, IFN-γ, IL-1β, IL-10, IL-12, IL-15, IL-17, IL-18, IL-1RA, IL-2, IL-23, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, MCP-1, MIP-1 α, MIP-1β, RANTES, TNF-α, TNF- β, IP-10
Ninla-Aesong, 2019 [35]	Joint pain	63	30	60	MMP-1, MMP-3	TNF-α	IL-4, IL-10, IL-12, IL-6, IL-1β, IL-8, IL-17A, RANTES, IFN-γ, TGF- β, MCP-1

Table 4. Predictor Markers of Persistent Joint Pain in Chikungunya Infection

Study	Follow-up (months)	Outcome	Sample size (n)		Time of markers evaluation	Contrast of markers' concentrations (Recovered patients as reference group)		
			Chronic	Recovered		Higher	Lower	Non difference
Hoarau, 2010 [10]	12	Joint pain > 1 joint, > 3 months	9	6	5 days	CRP		TNF-α, IL-8, IL-6, IFN-γ, IFN-α, IL-12, IL-4, IL-13
Chang, 2018 [39]	20	Joint pain	121	121	Acute phase		IL-1β, IL-6, TNF-α, IL-10, IL-12, IL-13, IL-17, IL-2, IL-4, IL-5	

One of these is IL-27 [31] which might play both, proinflammatory and anti-inflammatory roles. In the first case, IL-27 downregulates the regulatory T cells but stimulates the Th1 and Th17 response, whereas in its anti-inflammatory role IL-27 stimulates the production of IL-10 by T cells and decreases the Th17 response [31]. IL-27 also induces a strong IFN-independent antiviral response to CHIKV in monocyte-derived macrophages by activating JAK-STAT signaling pathway and inducing IFN-stimulated genes in the antiviral state [56,57]. GAL-9 is another relevant molecule to CHIKV infection pathogenesis, with higher levels among CHIKV infection cases compared to healthy controls and is associated with the duration of morning stiffness [31]. Increased levels of GAL-9 have also been observed in rheumatoid arthritis patients, which suggests that the Galectin family is implicated in the processes of osteoclast genesis and inflammatory bone destruction [58]. On the other hand, HMGB1, which is secreted by infected or active immune cells to potentiate the proinflammatory response, has been associated with viremia in CHIKV infection as proposed as a disease biomarker [59]. Furthermore, HMGB1 has been observed in the synovial fluid of rheumatoid arthritis cases, contributing to cartilage and bone destruction [59]. While increased CRP levels have been found in CHIKV cases, CRP does not seem to have a prognostic role for the persistence of articular manifestations of CHIKV [60]. Interestingly, Hoarau et al. reported that early CRP levels discriminated between patients with persistent joint pain and those who recovered at 12 months of follow-up [10], suggesting that CRP might be a simple and affordable prognostic marker of disease progression, a finding that requires replication.

When contrasted against patients who recovered from CHIKV infection, those with persistent clinical findings showed higher levels of IL-6 at 3, 10, and 12 months post-onset of infection. Similarly, MCP-1 remains elevated after 10 months of follow-up in patients with arthralgia compared to those without [36]. As mentioned before, MCP-1 and IL-6 are related to bone degradation [50], with IL-6 inducing its own production and upregulating MCP-1 expression [48]. On the other hand, matrix metalloproteinases (MMP-1, MMP-3, MMP-9, and MMP-13) which degraded the extracellular matrix in cartilage may also contribute to chronic arthralgia in CHIKV infection [61]. Ninla-Aesong et al. proposed that the activation of MMP-1 and MMP-3, secondary to the increase of Th1 markers (IL-6, IL-8, IL-1β, MCP-1, and TNF-α) [35], might partially contribute to the pathogenesis of chronic arthralgia in Chikungunya.

Although the body of evidence suggests an important contribution of immune mechanisms in the pathogenesis of the long-term articular complications in CHIKV infection, its prognostic potential remains poorly developed. In fact, only two prospective studies have evaluated the association between acute-phase biomarkers and the development of relevant clinical outcomes [10,39]. However, they reported conflicting results for TNF-α, IL-6, and IL-12 due to the differences in study design. Hoarau et al. found no association between cytokine levels and persistent joint pain, possibly due to their smaller sample size and shorter follow-up [10]. In contrast, Chang et al. conducted a nested case-control study, with 242 age- and gender-matched CHIKV infection cases, assessing joint pain by telephone survey at 20 months [39].

The titers of anti-CHIKV IgG antibodies also have been related to persistent joint pain [40,41,43]. Experimental and epidemiological studies suggest that pre-existing neutralizing antibodies are associated with a lower risk of both symptomatic and asymptomatic CHIKV infections [62]. Consistently, a Colombian cohort study observed that a positive test for anti-CHIKV IgG antibodies doubled the likelihood of having a symptomatic infection compared to a negative result (preliminary data). In relation to IgG subtypes, Kam et al. suggest that a strong and rapid response of the IgG3 subtype could clear the virus faster and potentially protect against the development of persistent joint pain after CHIKV infection [34].

The available evidence is consistent with the existence of an inflammatory response from the acute phase to the chronic phase of CHIKV infection; however, there is no clear profile of prognostic biomarkers for long-term sequelae, specifically arthralgia. This might be partially explained by the high heterogeneity among studies regarding eligibility criteria for cases and controls (sociodemographic composition, geographical origin, comorbidities, etc.), candidate biomarkers, timing of measurements, definition of outcomes, and follow-up durations. Most of the studies in this review had a high risk of bias, mainly attributable to the lack of adjustment for relevant covariates. Moreover, some studies in this review are reported as cohorts, only two prospectively evaluated the relationships between exposure(s) and outcomes. Finally, our search strategy might be considered as highly sensitive (ie, selected terms, databases, and checking reference lists) and we do not expect that publication bias due to the omission of publications in languages other than English has significantly impacted our conclusions.

CONCLUSIONS

The evidence suggests the existence of an inflammatory response in the acute phase of CHIKV that persists during its chronic phase; however, there is no unequivocal candidate set of biomarkers of progression toward long-term articular sequelae. This may be due to the heterogeneity of the studied populations, the definition of outcomes, and the timing for quantification of biomarkers

during disease.

Given the current gaps in understanding CHIKV pathogenesis, further research should focus on key areas such as synovial membrane biopsies and predisposing genetic factors could provide insights associated with chronic disease. Since alphavirus target synovial fibroblast and cause arthritis, new studies could investigate the expression of biomarker discussed in this review on these cells. Additionally, evaluating polymorphisms in genes encoding JAK-STAT pathway components, IFNs, or toll-like receptors like TLR3/8, IL-27 receptor, should be informative. These approaches could enhance our understanding of disease mechanisms and inform more effective diagnostic and therapeutic approaches.

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Appendix A: Supplement I and II

AMSTAR 2: a critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both

1. Did the research questions and inclusion criteria for the review include the components of PICO?

For Yes:
- ☑ Population
- ☑ Intervention
- ☑ Comparator group
- ☑ Outcome

Optional (recommended)
- ☐ Timeframe for follow-up

☑ Yes
☐ No

2. Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?

For Partial Yes:
The authors state that they had a written protocol or guide that included ALL the following:
- ☑ review question(s)
- ☑ a search strategy
- ☑ inclusion/exclusion criteria
- ☑ a risk of bias assessment

For Yes:
As for partial yes, plus the protocol should be registered and should also have specified:
- ☐ a meta-analysis/synthesis plan, if appropriate, *and*
- ☐ a plan for investigating causes of heterogeneity
- ☐ justification for any deviations from the protocol

☐ Yes
☑ Partial Yes
☐ No

3. Did the review authors explain their selection of the study designs for inclusion in the review?

For Yes, the review should satisfy ONE of the following:
- ☐ *Explanation for* including only RCTs
- ☐ OR *Explanation for* including only NRSI
- ☐ OR *Explanation for* including both RCTs and NRSI

☐ Yes
☑ No

4. Did the review authors use a comprehensive literature search strategy?

For Partial Yes (all the following):
- ☑ searched at least 2 databases (relevant to research question)
- ☑ provided key word and/or search strategy
- ☑ justified publication restrictions (e.g. language)

For Yes, should also have (all the following):
- ☑ searched the reference lists / bibliographies of included studies
- ☐ searched trial/study registries
- ☑ included/consulted content experts in the field
- ☑ where relevant, searched for grey literature
- ☑ conducted search within 24 months of completion of the review

☐ Yes
☑ Partial Yes
☐ No

5. Did the review authors perform study selection in duplicate?

For Yes, either ONE of the following:
- ☑ at least two reviewers independently agreed on selection of eligible studies and achieved consensus on which studies to include
- ☐ OR two reviewers selected a sample of eligible studies and achieved good agreement (at least 80 percent), with the remainder selected by one reviewer.

☑ Yes
☐ No

AMSTAR 2: a critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both

6. Did the review authors perform data extraction in duplicate?

For Yes, either ONE of the following:
- ☐ at least two reviewers achieved consensus on which data to extract from included studies
- ☐ OR two reviewers extracted data from a sample of eligible studies and achieved good agreement (at least 80 percent), with the remainder extracted by one reviewer.

☐ Yes
☑ No

7. Did the review authors provide a list of excluded studies and justify the exclusions?

For Partial Yes:
- ☑ provided a list of all potentially relevant studies that were read in full-text form but excluded from the review

For Yes, must also have:
- ☑ Justified the exclusion from the review of each potentially relevant study

☑ Yes
☐ Partial Yes
☐ No

8. Did the review authors describe the included studies in adequate detail?

For Partial Yes (ALL the following):
- ☑ described populations
- ☑ described interventions
- ☑ described comparators
- ☑ described outcomes
- ☑ described research designs

For Yes, should also have ALL the following:
- ☐ described population in detail
- ☐ described intervention in detail (including doses where relevant)
- ☐ described comparator in detail (including doses where relevant)
- ☑ described study's setting
- ☑ timeframe for follow-up

☐ Yes
☑ Partial Yes
☐ No

9. Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?

RCTs

For Partial Yes, must have assessed RoB from
- ☐ unconcealed allocation, *and*
- ☐ lack of blinding of patients and assessors when assessing outcomes (unnecessary for objective outcomes such as all-cause mortality)

For Yes, must also have assessed RoB from:
- ☐ allocation sequence that was not truly random, *and*
- ☐ selection of the reported result from among multiple measurements or analyses of a specified outcome

☐ Yes
☐ Partial Yes
☐ No
☑ Includes only NRSI

NRSI

For Partial Yes, must have assessed RoB:
- ☑ from confounding, *and*
- ☑ from selection bias

For Yes, must also have assessed RoB:
- ☑ methods used to ascertain exposures and outcomes, *and*
- ☑ selection of the reported result from among multiple measurements or analyses of a specified outcome

☑ Yes
☐ Partial Yes
☐ No
☐ Includes only RCTs

10. Did the review authors report on the sources of funding for the studies included in the review?

For Yes
- ☑ Must have reported on the sources of funding for individual studies included in the review. Note: Reporting that the reviewers looked for this information but it was not reported by study authors also qualifies

☑ Yes
☐ No

AMSTAR 2: a critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both

11. If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?

RCTs

For Yes:

- ☐ The authors justified combining the data in a meta-analysis
  - ☐ AND they used an appropriate weighted technique to combine study results and adjusted for heterogeneity if present.
  - ☐ AND investigated the causes of any heterogeneity

☐ Yes
☐ No
☑ No meta-analysis conducted

For NRSI

For Yes:

- ☐ The authors justified combining the data in a meta-analysis
  - ☐ AND they used an appropriate weighted technique to combine study results, adjusting for heterogeneity if present
  - ☐ AND they statistically combined effect estimates from NRSI that were adjusted for confounding, rather than combining raw data, or justified combining raw data when adjusted effect estimates were not available
  - ☐ AND they reported separate summary estimates for RCTs and NRSI separately when both were included in the review

☐ Yes
☐ No
☑ No meta-analysis conducted

12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?

For Yes:

- ☐ included only low risk of bias RCTs
- ☐ OR, if the pooled estimate was based on RCTs and/or NRSI at variable RoB, the authors performed analyses to investigate possible impact of RoB on summary estimates of effect.

☐ Yes
☐ No
☑ No meta-analysis conducted

13. Did the review authors account for RoB in individual studies when interpreting/ discussing the results of the review?

For Yes:

- ☐ included only low risk of bias RCTs
- ☐ OR, if RCTs with moderate or high RoB, or NRSI were included the review provided a discussion of the likely impact of RoB on the results

☐ Yes
☑ No

14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?

For Yes:

- ☐ There was no significant heterogeneity in the results
- ☐ OR if heterogeneity was present the authors performed an investigation of sources of any heterogeneity in the results and discussed the impact of this on the results of the review

☐ Yes
☑ No

15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?

For Yes:

- ☐ performed graphical or statistical tests for publication bias and discussed the likelihood and magnitude of impact of publication bias

☐ Yes
☐ No
☑ No meta-analysis conducted

AMSTAR 2: a critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both

16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?

For Yes:

- ☐ The authors reported no competing interests OR
- ☐ The authors described their funding sources and how they managed potential conflicts of interest

☐ Yes
☐ No

To cite this tool: Shea BJ, Reeves BC, Wells G, Thuku M, Hamel C, Moran J, Moher D, Tugwell P, Welch V, Kristjansson E, Henry DA. AMSTAR 2: a critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both. BMJ. 2017 Sep 21;358:j4008.

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Prospero (ID = CRD42021279400).
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	No Apply
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	No Apply

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	No Apply
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	No Apply
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	5
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Table 2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	No Apply
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	No Apply
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	No Apply
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	No Apply
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	14
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	14
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	15

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097