

Biomarkers of severity and chronification in chikungunya fever: a systematic review and meta-analysis

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ABSTRACT

Currently, there are no biomarkers for Chikungunya fever (CHIK) in clinical practice that can accurately predict the severity or chronification of the disease. The aim of this study is to evaluate the existing literature on biomarkers related to the severity and chronification of CHIK. In this sense, a systematic review was conducted based on the PRISMA Statement guideline. Articles that described the association of biomarkers with the evolution of the disease (severity or chronification), published until August 20th 2019 were considered eligible. The search was carried out in the PubMed, Scopus, Virtual Health Library (VHL) and Science Direct databases. After searching the databases, 17 articles were added to the review, and after analyzing the articles, several biomarkers were associated with severity, such as increased levels of IL-6, IP-10, IL-1b, MIG, MCP-1, and reduced levels of RANTES and IL-8 or chronification, such as increased levels of IL-6, TNF- α , MCP-1, IL-12, INF- α , IL-13, INF- γ , GM-CSF, CRP, IL-1a, IL-15, Factor VII, IP-10, IL-10, IL-4, IL-1RA, IL-8, MIP-1 α , MIP-1 β , ferritin, MIG, ESR, NO, malondialdehyde, and reduced levels of RANTES, ferritin, eotaxin, HGF, IL-27, IL-17A, IL-29, TGF- β , IL-10, and thiols. IL-6, CRP and TNF- α were included in the meta-analysis to assess the relationship with chronification, although they did not reach statistical significance. It was concluded that several biomarkers showed a relationship with severity and chronification of CHIK; the search for these biomarkers can reveal prognostic factors and important therapeutic targets for the treatment of the disease.

KEYWORDS: Chikungunya virus. Biomarkers. Chronic disease. Severity. Meta-analysis.

INTRODUCTION

Arboviruses such as dengue, yellow fever, zika, West Nile fever and Chikungunya fever (CHIK) have caused several outbreaks throughout history, and their geographical expansion has caused human and financial losses, becoming today one of the major global public health problems. This expansion can be explained by the increase in the global displacement of people, adaptation of vectors and failure to contain epidemics¹.

The Chikungunya virus (CHIKV), originally described in Tanzania², had several isolated outbreaks in Africa, and since 2004, a major epidemic started off the coast of Kenya, and spread to numerous islands in the Indian Ocean, India and Asia³. CHIKV maintained a trend towards geographic expansion, reaching the Americas, having its first record on the continent in 2013⁴.

CHIK fever is transmitted by mosquitoes of the genus *Aedes*. The disease manifests with a sudden onset of high fever, cutaneous rashes and severe arthralgia, arthritis, myalgia, headaches and photophobia. Most of the time, it is self-limited, but

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it can evolve to severe conditions⁵. In addition to its potential severity, CHIK fever can progress to chronification. The most common chronic manifestations are musculoskeletal and long-term rheumatic disease, which lead to a worsening of the patients' quality of life⁶.

In the acute phase of the disease, several cytokines are increased such as interleukin 6 (IL-6), tumor necrosis factor alpha (TNF- α), granulocyte macrophage-colony stimulating factor (GM-CSF), macrophage inflammatory protein-1 (MIP-1), and monocyte chemoattraction protein-1 (MCP-1); while in convalescence, lower levels of these cytokines were observed⁷. However, in clinical practice, there is no cytokine or other type of biomarker routinely used to predict the evolution of CHIK fever to severe or chronic conditions.

Considering the expansion of the disease and its potential to evolve to severe cases and chronification, the identification of biomarkers that can predict the outcome of the disease is of great importance. In this context, this systematic review was conducted to identify the existence of biomarkers related to the severity and chronification of CHIKV fever.

MATERIAL AND METHODS

This systematic review was carried out based on the Preferred Reporting Items for the Systematic Reviews and Meta-Analyses guideline-The PRISMA Statement⁸, seeking to evaluate the hypothesis that biomarkers can predict the evolution (severity or chronification) of the CHIKV fever. This systematic review was registered on the PROSPERO platform under N^o CRD42018094407.

The search for data was carried out in the PubMed, Scopus, Virtual Health Library (VHL), and Science Direct databases including studies published until August 20th 2019 without restricting the initial data collection period, since the objective was to recover the maximum available items.

The search strategy used PubMed and Scopus to select the option "advanced search", "title/abstract" to search for the keyword Chikungunya with the Boolean operator AND associating the keywords marker and markers, in turn linked by the operator OR, in the "All Fields" option. In the Science Direct database the same keywords were used, but the Research Articles filter was added, while in the VHL database, the option "title, summary and subject" was used to search for Chikungunya AND marker OR markers.

After selecting articles in each database, duplicate publications were excluded. The search was carried out initially by two researchers (ASF and NRB) reading the abstracts independently, to avoid the presence of bias in the selection and exclusion of studies. After selection, in

case of disagreement between the two reviewers, a third and fourth (CLO, CSC) researchers defined the relevance of the abstracts, considering the aims of the investigation. To detect any relevant scientific evidence that may not have been identified by the search strategy, a search for articles was also carried out in the reference list of studies and included in the review.

The articles that met the inclusion criteria were read in full. The collected variables regarding the characteristics of the studies were: name of the first author, year of publication, study reference, study location, language, type of study, sample size, age group, gender, outcome, biomarkers used, study result and laboratory method for analyzing the biomarkers.

To assess the quality of the studies included in this review, the Strengthening Reporting of Observational studies in Epidemiology (STROBE) guideline was used. In this methodology, a scale ranging from 0 to 22 is used, and the higher the score the higher the quality⁹.

Meta-analysis

After determining the biomarkers related to chronification and severity, it was decided not to perform a meta-analysis related to severity due to different criteria for defining the term used by each study.

Chronification-related biomarkers, which had data available in more than two studies, were used for meta-analysis, comparing their mean levels and the studies controls.

When relevant data were not found, the authors were contacted by email, and in cases in which there was no success in obtaining the information, the article was excluded from the meta-analysis. After excluding studies with incomplete data, and evaluating the available biomarkers, studies that addressed TNF- α , IL-6 and CRP were included in the meta-analysis. The mean value of the biomarker, its standard deviation, and number of patients in the chronification group and study control group were collected, and meta-analysis was performed to compare the mean difference (MD) of the biomarker between the groups. To create forest plots, we calculated the weighted mean difference (WMD) with 95% confidence intervals (95% CIs). Analyzes were performed using the fixed effects model and the random effects model.

The studies included in the meta-analyses performed the collection of biomarkers at different times during the natural history of the disease (acute or chronic phase) and had different control groups, and to try to control the impact of these, each meta-analysis describes the moment of collection and the control group used. In the case of

IL-6, it was possible to analyze two variations of the meta-analysis, one grouping studies in which sample collections were carried out in both phases (acute and chronic), and one only with studies performed during the chronic phase. The PCR metanalysis covered chronic phase studies and the TNF- α metanalysis used studies carried out during both phases of the disease.

The I^2 index, (I^2) and the chi-square test was performed to test the heterogeneity among the included studies. A P value < 0.05 was considered statistically significant. $I^2 > 50\%$ was considered indicative of a high heterogeneity.

One study presented the means, but did not provide standard deviations, thus we performed the imputation of values using the highest value among the studies that addressed the biomarker to obtain a more conservative result.

Additionally, we performed a meta-analysis to calculate the pooled prevalence with 95% CI to assess the percentage of chronification in studies that followed-up patients from the acute phase and measured the presence of persistent symptoms. Articles that had a loss of follow-up greater than 50% of the sample were excluded to reduce the chance of bias. The R[®] software with the R Studio[®] interface were used to perform all the statistical analyzes through the meta and metafor packages, using metaprop and metacont commands.

RESULTS

The search strategy resulted in the identification of 104 articles in the PUBMED database, 478 in Scopus, 463 in Science Direct and 92 in the VHL, totaling 1,137 manuscripts. In addition, 385 abstracts from the references of selected articles were evaluated to allow them to enter the systematic review. After removing the duplications, 1,281 studies remained, and their titles and abstracts were read resulting in 30 articles selected by two reviewers, independently (ASF & NRB). The divergences were assessed by a third and fourth reviewers (CLO & CSC) who excluded five articles because they were considered outside the context of the guiding question (three did not address biomarkers, two were reviews). In total, 25 articles were read in full and after reading them, eight articles were excluded. Among the articles excluded after full reading, two were case reports, two did not address chronification or severity of the disease, and four did not address biomarkers (Figure 1).

Of the 17 (100%) articles included in the review, 13 (76%) addressed chronification^{10,11,22}, three articles (18%) addressed severity²³⁻²⁵ and one article (6%) addressed both themes²⁶. Eleven cohort studies^{10,12-14,16,17,19,21,22,24,26}, two case-control studies^{15,18} and four sectional studies^{11,20,23,26}

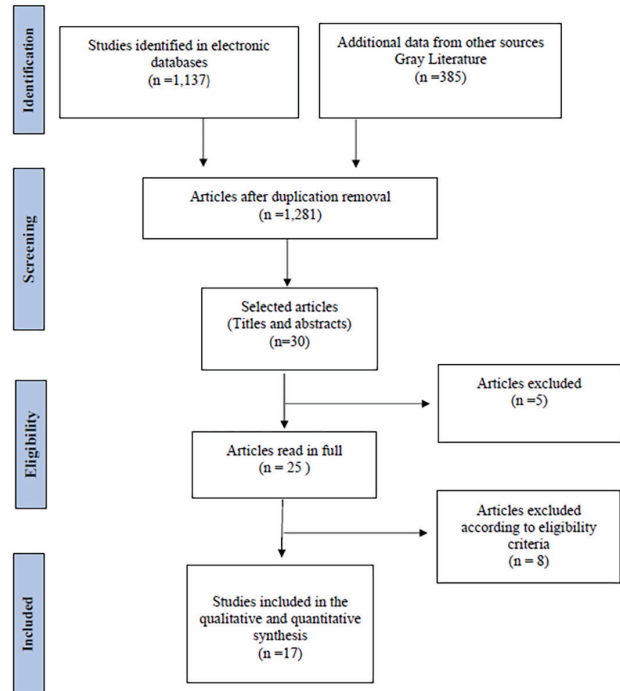


Figure 1 - Flowchart of articles selection based on PRISMA guidelines.

were included. The cohort studies had follow-up times ranging from 1 to 36 months, samples came from India (7 studies)^{10,11,15,17,21,22,26}, Singapore (three studies)^{13,16,23}, France (two studies)^{12,14}, Curacao Island¹⁸, Mexico¹⁹, Italy²⁴, Thailand²⁵ and Brazil²⁰ (one from each country). These publications took place between 2009 and 2019, samples were heterogeneous, the age groups ranged from 19 to 90 years. The proportion of patients of each gender also varied, with the percentage of men ranging from 44% to 100%. (Table 1). The samples size ranged from 10 to 573 participants.

Biomarkers were measured during the acute phase of disease in six studies^{10,18,21,23,25,26}, in the subacute/chronic phase in four articles^{12,13,15,20}, and was measured in both phases in seven studies^{11,14,16,17,19,21,24}. The control group used in each study has also varied, with seven studies using healthy patients^{10,15,17,20,22-24}, two used patients with other febrile diseases^{18,25}, and eight used patients who had CHIK fever, however they had no symptoms in the chronic phase^{11-14,16,19,21,26} (Table 2 and Table 3).

The criterion for defining severity of CHIKV fever has also varied, and two studies considered as a severe CHIKV fever patient those who had a temperature > 38.5 °C or heart rate > 100 beats/minute, or platelet count below 100 x 10⁹ g/L^{23,25}. Another study evaluated the severity of arthritis through a visual analog scale²⁶ and a survey defined the severity from a questionnaire, created by the authors, which quantified the symptoms²⁴. The persistent symptom

Table 1 - Comparative analysis between articles according to the designs of the studies and characteristics of the evaluated populations (n = 13).

Study	Outcome	Location	Study design	Time of follow-up	Age range	Mean age	Gender (M/F)
Win et al. ¹³	Chronification	Singapore	Cohort	1.5 months	20-79	37	75/22
Hoarau et al. ¹⁴	Chronification	France	Cohort	12 months	19-90	60,3	19/30
Chopra and Venugopalan ¹⁵	Chronification	India	Case control	NA	NI	NI	54/74*
Chow et al. ¹⁶	Chronification	Singapore	Coorte	2 to 3 months	23-67	39	26/4
Chaitanya et al. ¹¹	Chronification	India	Sectional	NA	25-55	34.5	NI
Chopra et al. ²²	Chronification	India	Cohort	24 months	NI	NI	226/282***
Schilte et al. ¹²	Chronification	France	Cohort	36 months	NI	NI	73/74***
Reddy et al. ¹⁷	Chronification	India	Cohort	3 months	21-80	40,6	31/17
Venugopalan et al. ¹⁰	Chronification	India	Cohort	1 month	NI	36	44/66*****
Anfasa et al. ¹⁸	Chronification	Curaçao Island	Case control	NA	NI	NI	NI
Sepúlveda-Delgado et al. ¹⁹	Chronification	Mexico	Cohort	12 months	27-68	48	4/6
Banerjee and Mukhopadhyay ²¹	Chronification	India	Cohort	0.33 a 1 month	35-76*	55*	10/15*
Cavalcanti et al. ²⁰	Chronification	Brazil	Sectional	NA	NI	55.2	8/37
Ng et al. ²³	Severity	Singapore	Sectional	NA	22-65	35	10/0
Kelvin et al. ²⁴	Severity	Italy	Cohort	12 months	NI	NI	NI
Lohachanakul et al. ²⁵	Severity	Thailand	Sectional	NA	NI	34.9**	23/39*****
Jain et al. ²⁶	Severity/ Chronification	India	Cohort	3 months	17-69	36	282/291

*Considering only samples with chronic symptoms** Considering only samples with severe symptoms; ***Considering the follow-up of 14 months; **** Estimated value; *****Estimated value considering only samples with chronic symptoms; ***** Considering patient with or without chikungunya; M = Male; F = Female; NI = Not informed; NA = Not applicable

accompanied by most studies to define subacute or chronic disease was arthralgia, but other symptoms such as arthritis, fatigue, myalgia and low back pain were also assessed.

The biomarkers covered in the studies were leukocyte, lymphocyte and platelet count, liver enzymes dosage, serum creatinine, hemoglobin, haptoglobin, ferritin, fibrinogen, VHS, lactic dehydrogenase (LDH), CRP, IL-1, IL-1a, IL-1RA, IL-1b, IL-2, interleukin -3 (IL-3), IL-4, IL-5, IL-6, interleukin-7 (IL-7), IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, IL-18, interleukin-21 (IL-21), interleukin-22 (IL-22), interleukin-23 (IL-23), interleukin-27 (IL-27), interleukin-29 (IL-29), IL-1ra, INF- α , INF- γ , TNF- α , TNF-b, tumor necrosis factor receptor 2 (TNFR2), MCP-1, IP-10, MIG, CC ligand 5 chemokine regulated by expressed activation and secreted by normal T cells (RANTES), eotaxin, GM-CSF, hepatocyte growth factor (HGF), transforming growth factor beta (TGF- β), factor VII, C3, anti-cyclic citrullinated peptide (anti-CCP), rheumatoid factor (FR), anti-nuclear antibody (Anti-ANA), Von Willebrand factor, neopterin, matrix metalloproteinases (MMP), tissue inhibitor of metalloproteinases 1 (TIMP-1), MIP-1 α , epidermal growth factor (EGF), HGF, FGF-b, VEGF, G-CSF, GM-CSF, MIP-1 β , vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), vitamin D carrier protein (DBP), alpha-2-macroglobulin, alpha-1-antitrypsin (AAT), beta-2-

microglobulin (B2M), brain-derived neurotrophic factor (BDNF), stem cell factor antagonist, nitric acid (NO), carbonylated protein, thiols and malondialdehyde (Figure 2).

Chronification was associated with increased levels of IL-6 (6 studies)^{10,11,15,16,19,22}, TNF- α (2 studies)^{10,15}, MCP-1 (3 studies)^{10,11,17}, IL-12¹⁴, INF- α ¹⁴, IL-13¹⁵, INF- γ ¹⁵, GM-CSF¹⁶, CRP¹², IL-1a¹², IL-15¹², Factor VII¹², IP-10¹⁰, IL-10¹⁰, IL-4¹⁰, IL-1RA¹¹, IL-8¹¹, MIP-1 α ¹¹, MIP-1 β ¹¹, ferritin¹⁸, MIG¹⁷, ESR¹⁹, NO²¹, malondialdehyde²¹ and with reduced levels of RANTES^{11,17}, ferritin¹², eotaxin¹⁶, HGF¹⁶, IL-27²⁰, IL-17a²⁰, IL-29²⁰, TGF- β ²¹, IL-10²¹, and thiols²¹ (Figure 2).

Among the increased biomarkers that showed an association with severity were increased levels of IL-6 (2 studies)^{23,25}, IP-10 (2 studies)^{23,24}, IL-1b²³, MIG²⁴, MCP-1²⁵ and reduced levels of RANTES²³, and IL-8²⁵ (Figure 2).

Among the articles included in the meta-analysis, the methodology used in each study to measure biomarkers has greatly differed, using Enzyme Linked Immunosorbent Assays (ELISAs)^{14,15,19} or the Multiplex Kit – Luminex¹⁶ for measuring cytokines, and in the case of measuring CRP one study cited the use of protein electrophoresis¹².

The studies had scores between 19 and 13 on the STROBE scale; the score of 19 was obtained by three

Table 2 - Comparative analysis between articles that addressed chronification according to the sample size, chronification index, collection time and characteristics of the control group (n = 13).

Study	Sample size	Sample with biomarkers in the group with persistent symptoms	Control	Control sample	Collection time in the group with persistent symptoms
Win <i>et al.</i> ¹³	97	14	Cases without chronification	25	Chronic phase
Hoarau <i>et al.</i> ¹⁴	49	6	Cases without chronification*	9	Acute and chronic phase
Chopra and Venugopalan ¹⁵	141	141	Healthy patients	80	Chronic phase
Chow <i>et al.</i> ¹⁶	30	4	Cases without chronification*	26	Acute and Chronic phase
Chaaityanya <i>et al.</i> ¹¹	22	10	Cases without chronification*	6	Chronic phase**
Chopra <i>et al.</i> ²²	509	22***	Healthy patients	NI	Acute and chronic phase
Schilte <i>et al.</i> ¹²	180	20	Cases without chronification	22	Chronic phase
Reddy <i>et al.</i> ¹⁷	48	4	Healthy patients	37	Acute and chronic phase
Venogopalan <i>et al.</i> ¹⁰	509	132	Healthy patients	80	Acute phase
Anfasa <i>et al.</i> ¹⁸	116	116	Febrile patients without CHIK	25	Acute phase
Sepúlveda-Delgado <i>et al.</i> ¹⁹	10	6	Cases without chronification	4	Acute and chronic phase
Banerjee and Mukhopadhyay ²¹	65	25	Cases without chronification*	15	Acute/subacute phase
Cavalcanti <i>et al.</i> ²⁰	45	21 (chronic)/ 24 (subacute)	Healthy patients	49	Subacute/chronic phase
Jain <i>et al.</i> ²⁶	572	40	Cases without chronification	27	Acute phase

*Additional analysis with a control group of healthy patients; **Collection at 10th month; ***Considering the follow-up of 18 to 24 months.

Table 3 - Comparative analysis between the articles that addressed severity according to the sample size, collection time and characteristics of the control group (n = 4).

Study	Sample	Sample with severe CHIK	Control	Control sample	Collection time
Ng <i>et al.</i> ²³	10	5	Healthy patients	9	Acute phase
Kelvin <i>et al.</i> ²⁴	50	14	Healthy patients	10	Acute and chronic phase
Lohachanakul <i>et al.</i> ²⁵	62	15	Febrile patients without CHIK	27	Acute phase
Jain <i>et al.</i> ²⁶	573	40	Cases without severe arthralgia	27	Acute phase

studies^{10,12,23}, 18 points by four other studies^{14,21,22,24}, 17 points by five studies^{11,13,18,19,26}, 16 points by four studies^{15,16,20,25}, and one study obtained 13 points¹⁷; the average of score was 17. No article achieved the maximum score.

Meta-analysis

The assessment of IL-6 levels in the meta-analyzes that grouped studies with patients who evolved to chronicity at any stage of the disease (acute or chronic phase)

(MD = -7.65/95% CI -34.06 - +18 , 17) or only in the chronic phase (MD = -2.90/95% CI -35.74 - +29.94), in both cases there was no statistically significant difference in comparison with healthy patients or patients with a previous history of Chikungunya without chronification. Additionally, the evaluation of CRP in the chronic phase (MD = -2.70/95% CI -16.93-+11.54) and the evaluation of TNF-a at any stage of the disease (MD = -1.91/95% CI -33.19-+37.01) did not show any statistically significant difference when compared to the control group (Figure 3).

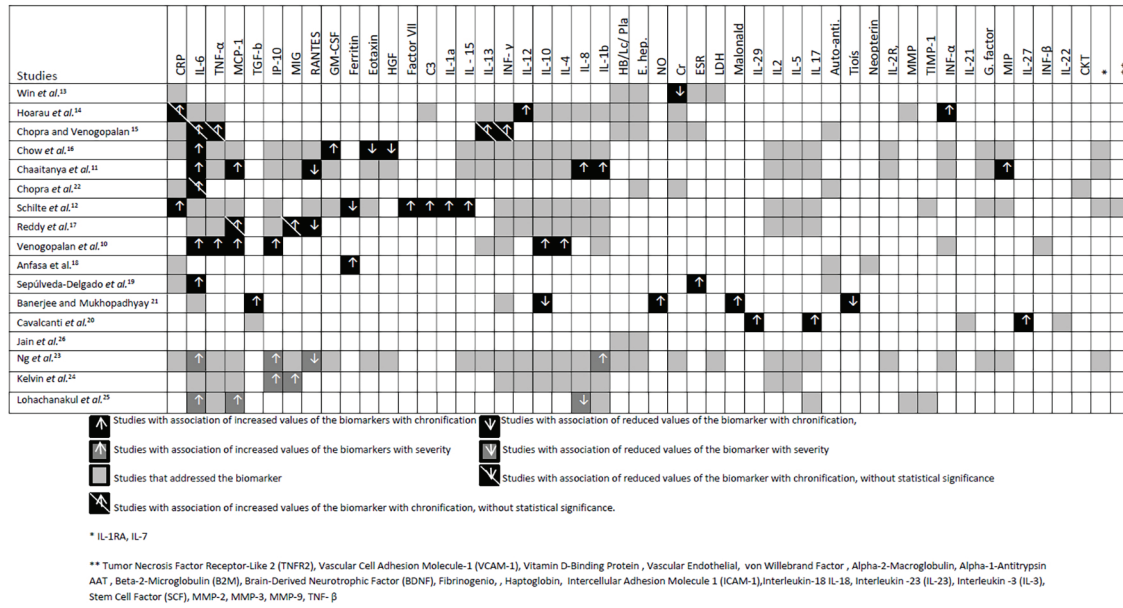


Figure 2 - Chart showing the relationship of the biomarkers addressed with severity or chronification of CHIKV fever according to the authors' assessments.

The proportion of chronification considering only cohort studies that followed patients from the acute phase was 15% (95% CI, 0.08-0.26) of chronification among patients who had CHIK fever (Figure 4).

The heterogeneity was statistically significant in the IL-6 meta-analyzes measured in the chronic phase ($I^2 = 72\%$, $P = 0.03$), in the evaluation of CRP ($I^2 = 74\%$, $P = 0.02$), and in the assessment of the proportion of the chronic ($I^2 = 87\%$, $P = 0.01$). In addition, the meta-analyzes that evaluated IL-6 in any stage of the disease ($I^2 = 57\%$, $P = 0.07$) and in the meta-analysis that evaluated TNF-α ($I^2 = 59\%$, $P = 0.09$) showed high I^2 values.

DISCUSSION

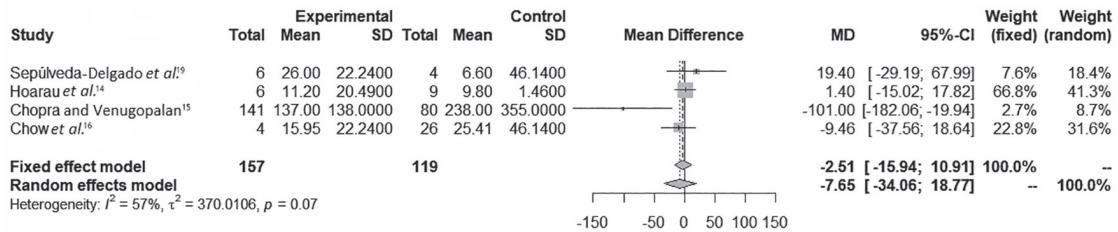
Although the meta-analyzes did not show statistically significant relationships, several studies cited biomarkers related to the severity and chronicity of the disease, especially the main biomarkers associated with chronification, being IL-6, TNF-α and MCP-1. These biomarkers may have a potential use as therapeutic targets or prognostic markers in future studies. Another relevant finding is that other biomarkers widely used and easily accessible in practice, such as liver enzymes, renal function and antibodies did not show a relationship with the evolution of the disease. Research on the topic is still scarce so that it is possible that with the increase in the number of studies and the analysis of larger samples, the statistical associations will become more notorious and other associations will be discovered, proven or refuted.

Many studies evaluated the association with ILs and

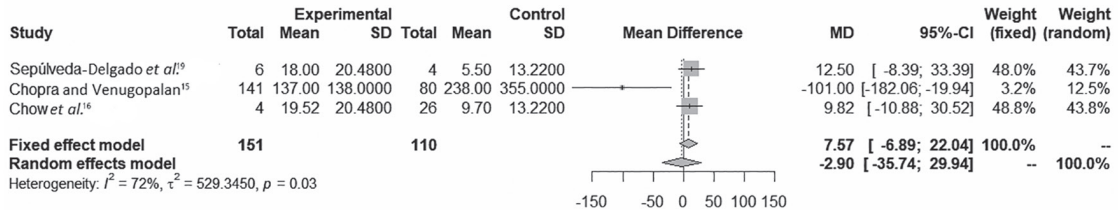
chronification, among them IL-6 was related to chronification by six studies^{10,11,15,16,19,22}, Chopra and Venugopalan¹⁵ evaluated patients with persistent symptoms after one year of illness and observed a marginal increase in IL-6, IFN-γ, TNF-α and IL-13, a finding that according to the authors, may suggest an ongoing immune inflammatory process. Chopra et al.²² found an eight-fold increment of IL-6 values in patients in comparison with the healthy control group and IL-6 remained elevated during the two-year follow-up period; the researcher argued that the increased levels may be related to viral persistence. Sepulveda-Delgado et al.¹⁹ observed patients with high levels of IL-6 at the time of diagnosis and ESR developed subacute or chronic disease, but the study conducted by Hoarau et al.¹⁴ found no differences, in the acute phase, between patients who progressed or not to chronification in most analyzes, but the levels of circulating IL-12 persisted high for months only in the group that evolved to chronicity. In the study conducted by Chow et al.¹⁶ patients with persistent arthralgia had increased levels of IL-6 and GM-CSF, while those who recovered had normal levels. In contrast, patients who did not have persistent arthritis had higher levels of HGF, a hormone that facilitates cartilage repair, and eotaxin, a cytokine that indicates the presence of a local anti-inflammatory response.

Banerjee and Mukhopadhyay²¹ evaluated inflammatory and oxidative stress parameters in patients with CHIK and observed that high levels of IL-6 and INF-γ are related to the presence of reactive oxygen species (ROS). The study also observed that in the group with persistent arthritis, the levels of malondialdehyde (marker of lipid peroxidation), and nitric oxide (NO) were increased in relation to controls, and the levels of thiols (important agents of enzymatic

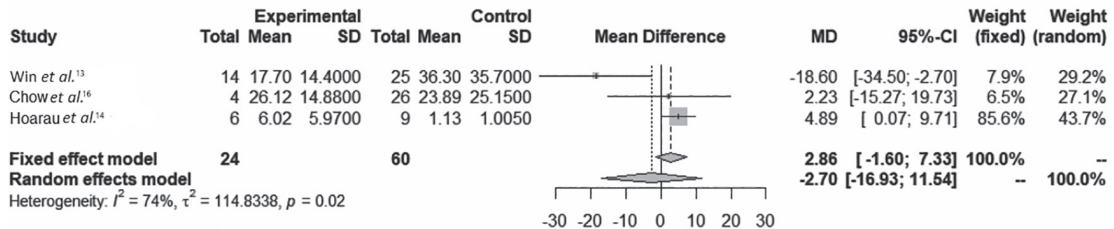
Comparative meta-analysis of the level of IL-6 measured at any stage of the disease between patients who progressed to chronification and patients with a previous history of chikungunya without chronification or healthy people (pg / ml)



Comparative meta-analysis of the level of IL-6 measured in the chronic phase of the disease between patients who progressed to chronification and patients with a previous history of chikungunya without chronification or healthy people (pg / ml)



Comparative meta-analysis of the CRP level measured in the chronic phase of the disease among patients who progressed to chronification and with a previous history of chikungunya without chronification (mg / dL).



Comparative meta-analysis of the TNF-α level measured at any stage of the disease in patients who progressed to chronification compared to patients with a previous history of chikungunya without chronification or healthy people (pg / ml).

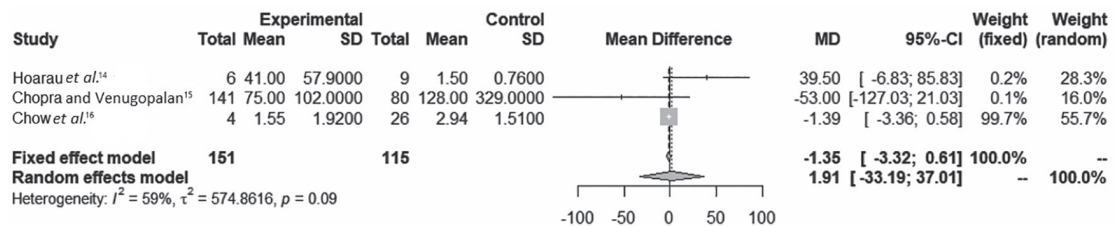


Figure 3 - Comparative meta-analyses of biomarkers related to chronification of CHIKV fever.

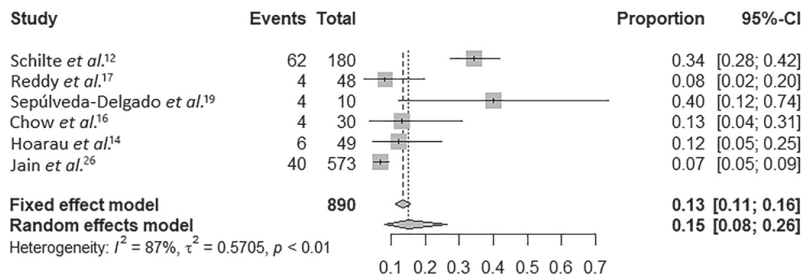


Figure 4 - Meta-analysis on the percentage of chronification among studies used in the systematic review.

activity that can reduce damage caused by the oxidative stress) were reduced in patients with persistent symptoms,

showing a tendency towards a greater oxidative stress in this group of patients. In turn, free radicals continuously

increase the availability of neoantigens, which possibly causes more oxidative damage, including joint damage, and the development of persistent polyarthralgia. Another interesting finding of this research was that the anti-inflammatory interleukin IL-10 was significantly reduced in the group with persistent arthralgia. The authors related these reduced levels to the presence of decreased numbers of regulatory T cells in a patient with polyarthralgia secondary to CHIKV, and in turn these cells are IL-10 producers.

The study conducted by Cavalcanti *et al.*²⁰ compared a patient with CHIK fever in the subacute or chronic phase with healthy people and observed increased levels of IL-27, IL-17A, IL-29 and TGF- β in sick patients. The levels of IL-27 were significantly higher in patients in the chronic phase, when compared to those in the subacute phase, and the authors propose that IL-27 production can be initiated with antigen presenting cells of monocytic/macrocyclic strains, which can act in CHIK fever as reservoirs of the virus or as catalysts in the inflammation process. In addition, IL-27 can also act in a pro-inflammatory way, down regulating the response of regulatory T cells (Tregs) increasing the Th1 or the Th17 responses in the absence of STAT1. In the study by Chaaitanya *et al.*¹¹, the cytokines IL-1RA, IL-6, IL-8, MIP-1a, MIP-1b and MCP-1, showed elevated levels in patients with chronic arthropathy after CHIKV infection compared with healthy patients. The study has also shown that in the acute phase the levels of IL-1RA and IL-6 are high, however the level of IL-6 observed in chronic patients was much higher than those of acute phase patients, indicating its strong association with chronic arthritis in patients infected by CHIKV.

MCP-1 has been related to chronification in three studies^{10,11,17}. Reddy *et al.*¹⁷ reported that patients with persistent arthralgia had high values of IL-1b, IL-4, IL-6, IL-8, IP-10, MCP-1 and MIG during the second week of illness and reduced levels of RANTES. The authors highlighted the chemotactic function of activation and migration of MCP-1 macrophages in the mechanism of induction of joint injury. Venogopalan *et al.*¹⁰ observed that cytokines, particularly TNF- α , MCP-1, IL-4, IL-6 and IL-10 showed maximum levels in the extended symptomatic phase and remained high in recovered subjects. Additionally, they found a relationship between the increase in IL-6, IP-10, CRP levels and the persistence of CHIKV musculoskeletal pain. In addition, an early and intense response of IFN- α , IFN- β and IFN- γ was associated with persistent symptoms; the authors associated this response with a failure of the host to eradicate the virus. Chaaitanya *et al.*¹¹, found that the levels of IL-8, MCP-1, MIP-1a and MIP-1b were much higher in patients with chronic arthritis compared to controls, acute patients and

patients who had fully recovered. In addition, reduced levels of RANTES have also been observed in chronic patients; MCP-1 acted as a chemotactic factor for monocytes²⁷, in turn monocytes and macrophages played an important role in the pathophysiology of arthritis²⁸.

TNF- α has also been correlated to chronification in two studies^{10,15}. TNF- α is a cytokine mainly induced by macrophages, has a chemotactic effect on several leukocyte populations, is involved in the process of inflammation onset, is an important innate immunity component and has also been related to the pathophysiology of rheumatoid arthritis²⁹.

The immune response and the Th1 or Th2 profile manifested by individuals can also be related to the cytokine profile found. Elevated levels of Th2 cytokines have been associated with prolonged musculoskeletal symptoms (IL-4, IL-6, and IL-10)¹⁰. Another factor to be considered is that the high viral load was related to the chronicity of the disease; high viral load was associated by Chow *et al.*¹⁶ with levels of IFN- α , IL-6, IL-12, IL-1RA, IP-10 and MCP-1. Similarly, in the study by Reddy *et al.*¹⁷, MCP-1 strongly correlated and IL-6 moderately correlated with high viral load in patients in the acute phase of CHIKV fever.

The association of CRP with chronification has been found in some studies. In the study conducted by Schilte *et al.*¹², CRP levels tended to be higher in patients with persistent arthritis. Hoarau *et al.*¹⁴ observed that patients who progressed to chronification presented a high level of CRP when compared to healthy controls. Chow *et al.*¹⁶ observed high levels of CRP during the acute phase patients who evolved to chronicity, when compared with those of healthy controls and recovered patients. The authors suggested that CRP, due to its low cost, high availability, and because it is related to increased levels of viral load, could help in the early identification and monitoring of patients with CHIK at risk of serious disease.

Other factors have been considered in some studies, such as creatinine levels and ferritin. Win *et al.*¹³ observed that creatinine was significantly lower in patients with persistent arthralgia, but it was more prominent in women, however women had lower levels of creatinine, which could explain this result. In the same study, there was a tendency of thrombocytopenia and high levels of liver enzymes associated with slow recovery from joint pain. Regarding ferritin, Anfasa *et al.*¹⁸ found an association between increased serum ferritin in the acute phase of the disease and prolonged arthralgia. In addition, they have also associated increased levels of ferritin with increased neopterin, which is a specific marker of macrophage activation. Increased levels of ferritin and neopterin are markers of macrophage activation, and ferritin synthesis

can be triggered by cytokines and iron; the study showed that increased ferritin was associated with the absence of viremia. Macrophages and hepatocytes are producers and secretors of ferritin, in turn the production of this compound can be activated during viral replication. Conversely, Schilte *et al.*¹² found a relationship between low levels of ferritin and the chronification of the disease. The divergences in the findings do not allow a conclusion to be drawn, and further studies are needed to assess the true impact of ferritin in CHIKV fever.

The profile of biomarkers of patients who progressed with persistent symptoms changed when assessed at the beginning of the disease onset compared to the period of prolonged illness. This finding is found in the study of Reddy *et al.*¹⁷, who followed-up four patients with persistent symptoms up to 12 months after the disease onset. The authors observed that although several markers were altered during the acute phase (IL-1b, IL-4, IL-6, IL-8, IP-10, MCP-1, MIG, and RANTES), most returned to values close to normal in the follow-up. However, MCP-1 and MIG were persistently elevated in patients during the chronic phase, while RANTES levels remained low during the acute phase with a tendency to decline even further during the chronic phase.

Schilte *et al.*¹² evaluated 20 patients with chronic arthralgia 36 months after the onset of the disease, who did not have lymphopenia, and the plasma protein levels measured by electrophoresis remained within the normal range, but the CRP levels were significantly elevated in the patients with arthralgia. In addition, the values of factor VII, C3, IL-1a and IL-15 were also high in this phase.

In the present review, around 15% of patients evolved to chronification in the studies evaluated, although the values obtained cannot be extrapolated to other contexts, since the meta-analysis was not designed to specifically evaluate the chronification rate. A similar value is cited in the literature that points to a percentage of around 14% of evolution to chronic arthritis, a symptom evaluated by most studies in this review. On the other hand, considering other types of manifestations of the disease, the persistence rate of nonspecific arthralgia and musculoskeletal symptoms can reach 25%³⁰.

In the acute phase, some markers were related to the severity of the disease. However, it is important to highlight that the severity was classified in a very heterogeneous way in the evaluated studies. Ng *et al.*²³ classified as severe, patients with temperatures equal to or above 38.5 °C, a heart rate > 100 beats/minute or platelets lower than 100×10^9 g/L and found a relationship between severity and increased levels of IL-1b and IL-6 or reduced levels of RANTES. When analyzing these findings, the author

highlighted that IL-1b and IL-6 are pyrogens and may be responsible for the high fever. In addition, IL-1b can mediate the development of arthralgia, since these cytokines are involved in the pathogenesis of several other forms of arthritis, such as rheumatoid arthritis. In turn, RANTES is a T-cell chemokine and its main reservoir in peripheral blood are platelets, which are typically reduced in CHIK infection. CRP was not associated with severity. The study by Ng *et al.*²³ evaluated patients in the acute phase and found high CRP values in 60% of the patients, but did not identify a relationship between the acute phase marker and the severity of the disease.

Lohachanakul *et al.*²⁵ has also used the same previously mentioned criteria, and observed a relationship between high concentrations of IL-6 and MCP-1, in addition to low IL-8 titers with the severity of the disease. The authors point out that IL-6 is a potent pyrogen and could mediate joint pain by sensitizing the nociceptors in the region, in addition to being able to participate in the pain mechanism in local neurons, associated with the inflammatory process. The authors have also highlighted the role of MCP-1 in the induction of monocytes/macrophages, which are involved in the pathophysiology of arthritis, in addition to mentioning that low levels of IL-8 are consistent with severity of disease, since IL-8 fights viral transcription and promotes viral clearance.

Kelvin *et al.*²⁴ classified the severity of patients qualitatively using a questionnaire on the severity of symptoms. They observed that patients with high levels of MIG, IP-10 and IgG had more severe symptoms, these levels had a tendency to decrease, but still remained elevated after six months. The high levels of MIG, IP-10 and IL-6 present in the acute phase are related, by the authors, to a possible consequence of the activation of the T cell adaptive immunity. In addition, they pointed out that MIG and IP-10 are chemokines induced by IFN- γ and they participate in the regulation of monocyte/macrophage migration, memory T cells and NK cells, and can contribute to the persistent immune activation in CHIK disease, leading to chronic symptoms.

Severity-related biomarkers are also studied in other arboviruses, such as dengue, a disease with a clinical presentation similar to CHIK fever. The dengue virus (DENV)-infected endothelial cells produce chemokine and cytokine responses that activate or recruit immune cells to the endothelium. The most serious form of the disease, the hemorrhagic dengue fever, present a 'cytokine storm', with high levels of circulating cytokines and chemokines. Some studies have shown that elevated levels of IL-6, IL-10, IFN- γ , MIF and CCL-4 could be used as potential predictors of severity in dengue. Furthermore, increased levels of IFN- γ , TNF- α , IL-1 β , IL-4, IL-6, IL-7, IL-8, IL10,

IL-13, IL-15, IL-17, IL-18, MIF, CCL2, CCL4, CCL5 and IP-10 have been reported in patients with hemorrhagic dengue fever, when compared to dengue fever. Thus, factors associated with the endothelium, such as the increase in Ang-2, vWF, sVCAM, VEGF and VEGFR1 levels, and decrease in Ang-1, ADAMTS-13 and VEGFR2 levels might serve as biomarkers of severity in dengue. Liver injury is associated with severe dengue disease with the increase in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase, alkaline phosphatase and serum albumin concentrations³¹. There are also reports that CRP levels in the early stages following the onset of fever may be prognostic for severe dengue. Platelets are also mentioned in some studies, and platelet associated IgM was suggested to be a predictor of hemorrhagic dengue fever. Platelets bind to DENV which activates them, this might indicate a coagulation response involving DENV immune complexes formation³². In comparison with the findings in CHIK fever, some interleukins are mentioned in both cases (IL-6 IP-10, IL-1 β), which may correspond to an initial inflammatory phase present in the severe phase of both diseases. The studies included in this review do not report on the inclusion of biomarkers related to the endothelium to assess their relationship with severe forms of CHIK fever; in addition, other increased biomarkers such as liver enzymes, CRP and the number of platelets were not mentioned in the studies as factors related to the severity of CHIKV, however few studies have been carried out on the subject, making possible to find new biomarkers and parallels with other arboviruses according to the advance of research in the area.

It is important to mention that the studies are divergent in many key methodological aspects such as: sample size, length of follow-up time, stage of the disease at the time of blood collection, sample sources and different control groups. In addition, the cohorts show significant follow-up losses, and samples for the study of biomarkers were taken from a very limited number of patients, either because the total sample of the studies was already limited, or because only a small number of patients underwent more in-depth laboratory analysis. The methods of analysis for each biomarker has also differed, although the studies have shown a good correlation between the ELISA method and the Multiplex Cytokine Measurement Kits, often finding very close values³³, a fact that reduces the chances of bias, since these were the main methods cited by the studies.

CONCLUSION

Our study has some limitations. Firstly, we can point out the fact that not all studies were included in the meta-

analysis due to the lack of available data. Despite the attempts to obtain data from the authors, the feedback obtained was low, probably because some articles were published a long time ago or because the authors have changed their electronic mail addresses and information has not been updated, because many of the unexpected or undesirable results were not published and bias of publication is a concern in all systematic reviews.

CHIK fever is a public health problem with the potential for serious complications. To date, there are no widespread exams in the clinical practice of prognostic evaluation, in addition, the study of the pathophysiology of the disease is fundamental for the development of new therapeutic targets. Several biomarkers are cited in the literature related to severity (increased levels of IL-6, IP-10, IL-1 β , MIG, MCP-1 as well as reduced levels of RANTES and IL-8) or chronification (increased levels of IL-6, TNF- α , MCP-1, IL-12, INF- α ¹⁴, IL-13, INF- γ , GM-CSF, CRP, IL-1 α , IL-15, factor VII, IP-10, IL-10, IL-4, IL-1RA, IL-8, MIP-1 α , MIP-1 β , ferritin, MIG, ESR, NO, malondialdehyde, and reduced levels of RANTES, ferritin, eotaxin, HGF, IL-27, IL-17A, IL-29, TGF- β , IL-10 and thiols). The search for these biomarkers can reveal prognostic factors and important therapeutic targets for the treatment of the disease. However, the evaluated studies have shown differences and weaknesses that make it difficult to compare results. In this sense, more standardized studies with larger samples are needed to consolidate these findings.

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