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Short communication

Chikungunya fever: How accurate is the clinical-epidemiological diagnosis compared to the gold standard of molecular and serological laboratory diagnosis?

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ABSTRACT

Objective: To evaluate the accuracy of the current World Health Organization' (WHO) Chikungunya fever (CHIKF) clinical-epidemiological case definition against the gold standard of laboratory diagnosis.
Methods: This was a prospective study of patients seeking medical care at an Emergency Department in the metropolitan area of Rio de Janeiro, Brazil, from January to June 2018. Clinical features were recorded. Screening for CHIKF was performed using the RT-qPCR and ELISA-IgM antibody assay. Clinical features of CHIKF RT-qPCR/IgM positive cases were compared with those with other febrile illnesses.
Results: 27,900 ED visits were recorded, of which 172 (0.61 %) patients were screened for arboviral illness. The prevalence of laboratory-confirmed CHIKF (Lab-CHIKF) was 110/172 [64 %]. Chikungunya virus RNA was detected in 92/172 (53.5 %) patients, while in 18/80 (10.5 %), only IgM was positive. Compared to CHIKV-negative subjects, patients with CHIKF presented much earlier after the onset of symptoms (2 [1–4] vs. 3.5 [2.5−5], $p = 0.007$), and more frequently reported arthritis (61.8 % vs. 33.9 %, $p < 0.0001$), arthralgia (96.4 % vs. 79 %, $p < 0.0001$), and conjunctivitis (35.5 % vs. 16.1 %, $p = 0.007$). After adjustments for other clinical predictors, arthritis/arthralgia [aOR: 6 (95 % CI 1.8–19.7)] and the presence of conjunctivitis [aOR: 2.85 (95 % CI 1.30−6.24] were positively associated with lab-CHIKF. The sensitivity, specificity, positive predictive value, and negative predictive value of the WHO CHIKF clinical case definition was 96.3 %, 20.9 %, 68.3 % and 76.4 %, respectively, and accuracy was 0.69 [AUC: 0.69 (95 % CI 0.61−0.75)].
Conclusion: The WHO case definition needs to be improved for better accuracy, especially in areas in epidemics in areas with co-circulation of arboviruses.

1. Introduction

Chikungunya virus (CHIKV), transmitted by the *Aedes* (*Stegomya*) mosquitoes, has spread globally and constitutes a serious threat to various tropical but also temperate areas of the world [1,2].

In 2013, the first autochthonous case of chikungunya fever (CHIKF) in the Americas was reported in St Martin [3]. By 2015, autochthonous cases had been described in Brazil, Colombia and Venezuela, and many other countries [4]. CHIKF may result in acute and long-term poor quality of life due to joint pain and disability, as well as consequent to neurological complications, especially in the elderly and in the newborn. Clinical diagnostic confusion between dengue and Chikungunya may be historical, as arthritis may be an essential feature in both conditions [5]. Diagnostic criteria are crucial, both in resource-limited and non-resource limited scenarios, for adequate care, especially in large outbreaks. We evaluated the accuracy of the current World Health

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Organization' (WHO) CHIKF clinical-epidemiological case definition [1], that is, that of a patient with "acute onset of fever >38.5 °C and severe arthralgia/arthritis not explained by other medical conditions AND who is residing in or has visited epidemic areas, having reported transmission within 15 days prior to the onset of symptoms", against the gold standard of laboratory diagnosis, which is at least one of the following tests in the acute phase: virus isolation, presence of viral RNA by RT-PCR; presence of virus specific IgM antibodies in single serum sample collected in acute or convalescent stage or a four-fold rising of IgG titers in samples collected at least three weeks apart. The comparison of clinical features with RT-PCR and IgM and IgG antibodies was done in a cohort of patients with suspicion of arboviral illness seeking care in an urban emergency department in Rio de Janeiro, Brazil, from January to June 2018, a scenario where other arboviruses co-circulate and the initial clinical presentation often does not allow distinction between different viral aetiologies of infection. We emphasize that the studied area has a tropical climate which is warm and rainy especially from the months of January to April, and therefore favors vector proliferation and transmission of arboviruses.

2. Methods

The participants of the study were patients, predominantly adult, seeking medical care at the Emergency Department (ED) located in a general hospital in Duque de Caxias, metropolitan area of Rio de Janeiro, Brazil, from January to June 2018. Patients eligible for the study had a clinical diagnosis of arboviral disease, regardless of gender and age. Individuals with a clear source of infection (eg pneumonia, sinusitis) and those who refused to participate were excluded. Clinical information, duration of symptoms (DOS), and routine physical examination were recorded. A single whole blood sample was collected on the day of enrolment, and serum samples were stored at −80 °C for further laboratory analysis. RNA extraction was performed using the QIAamp® Viral Mini Kit (QIAGEN, Valencia, CA, USA). Screening for CHIKV was performed using the reverse transcriptase reaction assay, followed by real-time PCR (RT-qPCR). The primers and probes used were synthesized in a Primetime qPCR® (IDT, Integrated DNA Technologies) strategy, according to Lanciotti et al. [6]. RT-qPCR assays were performed using the TaqMan® Fast Virus 1-Step Master Mix kit (Thermofisher Scientific, Waltham, MA, USA) on 7500 Real-time PCR System (Applied Biosystems, Foster City, CA, USA). XGEN kits (Biometrix, Brazil) were used to detect the presence of CHIKV-specific IgM and IgG antibodies following the manufacturer's instructions.

Our algorithm for the laboratory diagnosis of CHIKV was based on the current WHO guidelines. First, we performed CHIKV RT-PCR in acute sera in all patients in the cohort, then, for those with a negative molecular assay, we screened for CHIKV IgM and IgG antibodies. We considered a laboratory confirmed case that with both RT-PCR and IgM detection, or that with either a reactive RT-PCR or IgM positive result.

Demographic and clinical features of CHIKV RT-PCR/IgM positive cases (laboratory-confirmed CHIKF cases) were compared to those of patients with other febrile illnesses. Differences in proportions were tested by applying the chi-square test, and medians were compared using the Mann-Whitney-Wilcoxon test. Odds ratio (OR) was used as the association measure to identify the clinical features related to CHIKF against the laboratory diagnosis of CHIKV. Explanatory variables with a marginal association with the outcome in the univariate analysis were included in the binary regression model. Stepwise logistic regression was done with forwarding selection to identify the prediction model, with a significance level of 5 %. We then evaluate the suspected case definition of CHIKV, according to WHO. Measures of accuracy, sensitivity, specificity, positive predicted value, and negative predictive value and their corresponding 95 % confidence intervals were computed. The performance indicators were estimated, taking into account true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN) results. Sensitivity was calculated as TP/(TP + FN), specificity as TN/(TN + FP), positive predictive value as TP/(TP + FP), and negative predictive value as TN / (FN + TN). Concurrently, likelihood ratio (LR) and accuracy were also computed to estimate the clinical case definition. Positive LR (PLR) for the presence of a case definition pattern was calculated as [(Sensitivity) / (1-Specificity)]; negative LR (NLR) for the absence of a case definition pattern was calculated as [(1-Sensitivity)/(Specificity)]. Accuracy was defined as the proportion of all combinations that were consistent with a correct result according to a positive CHIKV molecular and/or serological status. This latter indicator was calculated as the (TP + TN) ratio for the number of positive molecular and serological tests. All estimates were expressed as percentages with their corresponding 95 % confidence intervals (CI).

3. Results

During the study period, 27,900 ED visits were recorded, of which 172 (0.61 %) patients were screened for arboviral illness after their consent/assent to participate. The prevalence of laboratory-confirmed CHIKV infection was 110/172 [64 % (95 CI 56–71)], and 62/172 (36 %) had another acute febrile illness. Overall, participants had a mean age of 39 (±15.5, range: 11–73) years, females predominated (52.3 %), and the majority presented within three days after onset of illness (65 %). Among CHIKV-infected patients, CHIKV RNA was detected in 92/172 (53.5 %) patients, while in 18/80 (10.5 %), only IgM was positive (Fig. 1).

A comparison of clinical features for patients with CHIKV and other acute febrile illness (AFI) is shown in Table 1. Patients with CHIKV presented much earlier after onset of symptoms (2 [1–4] *vs*. 3.5 [2.5−5], $p = 0.007$) and reported more frequently arthritis (61.8 % *vs*. 33.9 %, $p < 0.0001$), arthralgia (96.4 % *vs*. 79 %, $p < 0.0001$), and conjunctivitis (35.5 % vs. 16.1 %, $p = 0.007$) compared to CHIKV-negative subjects, respectively. After adjustments for other clinical predictors, arthritis/arthralgia [aOR: 6 (95 % CI 1.8–19.7)] and the presence of conjunctivitis [aOR: 2.85 (95 % CI 1.30−6.24] were positively associated with laboratory-confirmed CHIKV infection.

One hundred and six patients with laboratory confirmed CHIKV met the current WHO clinical-epidemiological definition of CHIKF. The sensitivity, specificity, positive predictive value, and negative predictive value was 96.3 % [90.9–99.0], 20.9 % [11.6–33.1], 68.3 % [65.4–71.1] and 76.4 % [52.5–90.5], respectively. When we evaluated the WHO definition of CHIKV in our setting, this clinical classification adjudicated 69 % (95 % CI: 61.7–75.9) of subjects accurately with respect to laboratory confirmation. These figures were obtained after calculations based on our results as presented in Table 2.

4. Discussion

Clinical diagnostic criteria were studied regarding CHIKF in order to distinguish it from other illnesses [7–11], especially dengue, which is hyper-endemic in Brazil. This is an important issue to consider in areas in which different arboviruses circulate, as medical decisions need to be made based on clinical impression, rather than laboratory confirmed diagnosis. We therefore set out to study the sensitivity and specificity of clinical diagnostic criteria in CHIKF compared to the standard confirmed cases. In our study, fever was observed in 89 % of all patients with laboratory confirmed CHIKF. Severe joint paint is a regular feature of symptomatic CHIKF, and was seen significantly more frequently in our patients, where 81 (73.6 %) of laboratory confirmed CHIKF cases had moderate to severe arthralgia compared to 24 (38.7 %) of other AFI, with an OR of 4.42. van Genderen et al. [12], studying 180 outpatients in an outbreak of CHIKF in Suriname in 2014, found that approximately 70 % of 121 RT-rPCR positive CHIKF patients had moderate to severe pain; besides, arthralgia was significantly more common in the RT-rPCR positive vs the RT-rPCR negative (84.4 % vs 65.7 %, p = 0.02).

Despite the frequency and severity of joint pain, in our study, the WHO criteria for probable CHIKF (acute onset of fever >38.5 °C and

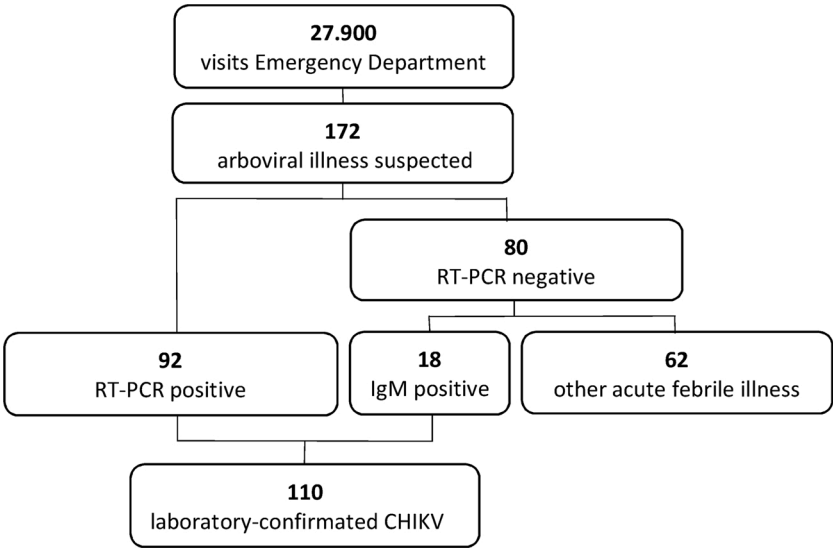

Fig. 1. Laboratory-confirmed Chikungunya fever cases in a cohort of patients with suspected arboviral disease seeking care at an Emergency Department in Rio de Janeiro, Brazil, January–June 2018.

Table 1
Clinical features at study enrollment by chikungunya diagnosis in patients with suspicion of arboviral disease seeking care at an Emergency Department in Rio de Janeiro, Brazil, January–June 2018.

	CHIKF N = 110	All other AFI N = 62	OR [95 % CI]
Characteristics			
Age (years), mean ± SD	40 (15.2)	37 (16)	
Female – n (%)	54 (49)	36 (58)	0.70 [0.37–1.30]
DPO, median [IQR]	2 [1,2,3, 4]	3.5 [2.5–5]	–
Fever > 38.5 °C	98 (89)	21 (33.8)	**15.94 [7.18–35.40]**
Rash – n (%)	37 (33.6)	20 (32.3)	1.06 [0.54–2.06]
Myalgia – n (%)	78 (70.9)	45 (72.6)	0.92 [0.46–1.84]
Pruritus – n (%)	16 (14.5)	14 (22.6)	0.58 [0.26–1.29]
Mild arthralgia – n (%)	25 (22.7)	25 (40.3)	0.43 [0.22–0.85]
Moderate/severe arthralgia – n (%)	81 (73.6)	24 (38.7)	**4.42 [2.27–8.59]**
Arthritis – n (%)	68 (61.8)	21 (33.9)	**3.16 [1.64–6.06]**
Conjunctivitis – n (%)	39 (35.5)	10 (16.1)	**2.85 [1.30–6.24]**
Headache – n (%)	87 (79.1)	47 (75.8)	1.20 [0.57–2.53]
Hemorrhagic manifestations – n (%)	3 (2.7)	1 (1.6)	1.71 [0.17–16.80]

AFI stands for acute febrile illness; CHIKF = chikungunya fever; DPO = days after onset of illness; IQR = interquartile range; OR = odds ratio; SD = standard deviation.

Table 2
Classification of Chikungunya fever cases according to the WHO clinical definitions and to laboratory confirmed diagnosis.

	Laboratory CHIKV positive	Laboratory CHIKV negative	Total
WHO clinical definition positive	106	49	155 (90.1 %)
WHO clinical definition negative	4	13	17 (9.9 %)
Total	110 (63.9 %)	62 (36.1 %)	172 (100 %)

WHO = World Health Organization; CHIKV = Chikungunya virus.

severe arthralgia/arthritis not explained by other medical conditions in the epidemiological context of transmission) had 69 % accuracy. In the study by Sissoko et al. [7], the WHO criteria "fever + arthralgia" was present in 83.4 % of 318 CHIKV antibody confirmed cases and in 86.9 % of 107 laboratory negative cases, that is, it was unable to discriminate clinically the patients. Thiberville et al. [9], in an outpatient investigation similar to our study, proposed a refinement of the score, using wrist and metacarpophalangeal joint arthralgia, absent or minor myalgia and more frequent lymphopenia as the best predictors of CHIKF, which reached 87 % PPV in their population of acutely ill 18–60 years old. Lee et al. [8], in a study of hospitalized adults, compared 117 patients with RT-PCR confirmed CHIKF with 917 patients with RT-PCR dengue confirmed ones, and found that myalgia and arthralgia were more common in CHIKF, while platelet counts below 118 × 109/L were the best predictor of dengue.

Our CHIKF confirmed cases presented with conjunctivitis twice more often compared to other AFI (35.5 % vs 16.1 %, respectively, aOR of 2.85); the frequency of conjunctivitis is similar to that published by de Souza Costa et al. [13] where it occurred in 15/40 (37.5 %) of patients with laboratory confirmed CHIKF studied in the nearby city of Rio de Janeiro in 2016. In a recent series of 95 laboratory confirmed CHIKF from Mexico [14], conjunctivitis was present in 15 % of cases. Conjunctivitis was considered an important diagnostic criterion for Zika virus infection [15], but again overlap of clinical features are prominent.

We emphasize CHIKV viremia is short-lived, and the majority of the clinical manifestations are virus-induced immunopathology. Conversely, CHIKV antibodies cross-react with other alphaviruses that manifest with similar clinical manifestations and cause several outbreaks in Latin America (i.e., Mayaro virus, Una virus, Eastern equine encephalitis, and Venezuelan equine encephalitis virus) [13]. Fortunately, during the study period, the local state surveillance system did not report any other alphaviruses in the region, so we are much confident that the serology profile presented in our cohort reflects current CHIKV infection.

In an area with the circulation of dengue, Zika and Chikungunya viruses, patients with CHIKF presented earlier than those with other arboviral illnesses, more often had severe arthralgia/arthritis and conjunctivitis. However, specificity of the WHO criteria for CHIKF was low, and accuracy was less than expected. A refinement of criteria is crucial and should be actively sought for.

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Ethical approval

The Research Ethics Committee of Universidade do Grande Rio approved the study (CAAE 54544316.3.0000.5283).

CRediT authorship contribution statement

Hury Hellen Souza de Paula: Conceptualization, Methodology, Investigation, Writing - original draft. **André Frederico Martins:** Investigation, Writing - original draft. **Raphael Rangel das Chagas:** Investigation, Writing - original draft. **José Moreira:** Validation, Formal analysis, Data curation. **Renato Santana de Aguiar:** Investigation, Writing - review & editing, Visualization. **Cristiane da Cruz Lamas:** Writing - review & editing, Visualization, Supervision. **Sergian Vianna Cardozo:** Conceptualization, Resources, Writing - review & editing, Supervision, Project administration.

Declaration of Competing Interest

None.

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References

[1] World Health Organization (WHO), Guidelines on Clinical Management of Chikungunya Fever. [Accessed on 2017 Oct 14] [Internet], Available:, 2008 http://www.wpro.who.int/mvp/topics/ntd/Clinical_Mgnt_Chikungunya_WHO_SEARO.

[2] A.M. Powers, C.H. Logue, Changing patterns of chikungunya virus: re-emergence of a zoonotic arbovirus, J. Gen. Virol. 88 (2007) 2363–2377, https://doi.org/10.1099/vir.0.82858-0.

[3] M. Henry, L. Francis, V. Asin, K. Polson-Edwards, B. Olowokure, Chikungunya virus outbreak in Sint Maarten, 2013–2014, Rev. Panam. Salud Publica 41 (2017) e61.

[4] Pan American Health Organization (PAHO), Number of Reported Cases of CHIK Fever in the Americas by Country or Territory 2013-2014, and 2015 [Accessed on 2020 Jan 14] [internet], Available:, 2016 http://www.paho.org/hq/index.php?option=com_topics&view=readall&cid=5927&Itemid=40931&lang=en.

[5] G. Kuno, A Re-Examination of the history of etiologic confusion between dengue and Chikungunya, PLoS Negl. Trop. Dis. 9 (11) (2015), https://doi.org/10.1371/journal.pntd.0004101 e0004101.

[6] R.S. Lanciotti, O.L. Kosoy, J.J. Laven, A.J. Panella, J.O. Velez, A.J. Lambert, et al., Chikungunya virus in US travelers returning from India, Emerg. Infect. Dis. 2007 (13) (2006) 764–767, https://doi.org/10.3201/eid1305.070015.

[7] D. Sissoko, K. Ezzedine, A. Moendandzé, C. Giry, P. Renault, D. Malvy, Field evaluation of clinical features during chikungunya outbreak in Mayotte, 2005–2006, Trop. Med. Int. Health 15 (2010) 600–607, https://doi.org/10.1111/j.1365-3156.2010.02485.x.

[8] V.J. Lee, A. Chow, X. Zheng, L.R. Carrasco, A.R. Cook, et al., Simple clinical and laboratory predictors of Chikungunya versus dengue infections in adults, PLoS Negl. Trop. Dis. 6 (9) (2012) e1786, https://doi.org/10.1371/journal.pntd.0001786.

[9] S.-D. Thiberville, V. Boisson, J. Gaudart, F. Simon, A. Flahault, et al., Chikungunya fever: a clinical and virological investigation of outpatients on Reunion Island, South-West Indian Ocean, PLoS Negl. Trop. Dis. 7 (1) (2013) e2004, https://doi.org/10.1371/journal.pntd.0002004.

[10] L. Godaert, F. Najioullah, L. Bousquet, T. Malmontet, B. Fournet, R. Césaire, et al., Do two screening tools for Chikungunya Virus infection that were developed among younger population work equally as well in patients aged over 65 years? PLoS Negl. Trop. Dis. 11 (1) (2017) https://doi.org/10.1371/journal.pntd.0005256 e0005256.

[11] Cavalcanti LPDG, Farias ABGL, K.A.F. Barreto, A.M. Siqueira, G.S. Ribeiro, R.R. A. Freitas, et al., Chikungunya case classification after the experience with dengue classification: how much time will we lose? Am. J. Trop. Med. Hyg. (2019) tpmd190608.

[12] F.T. van Genderen, I. Krishnadath, R. Sno, M.G. Grunberg, W. Zijlmans, M. R. Adhin, First chikungunya outbreak in Suriname; clinical and epidemiological features, PLoS Negl. Trop. Dis. 10 (4) (2016) e0004625, https://doi.org/10.1371/journal.pntd.0004625.

[13] M.C. de Souza Costa, L.M. Siqueira Maia, V. Costa de Souza, A.M. Gonzaga, V. Correa de Azevedo, L. Ramos Martins, J.H. Chavez Pavoni, F. Gomes Naveca, R. Dezengrini Slhessarenko, Arbovirus investigation in patients from Mato Grosso during Zika and Chikungunya virus introdution in Brazil, 2015–2016, Acta Trop. 190 (2019) 395–402, https://doi.org/10.1016/j.actatropica.2018.12.019.

[14] R. Danis-Lozano, E.E. Díaz-González, Kd C. Trujillo-Murillo, S. Caballero-Sosa, J. Sepúlveda-Delgado, et al., Clinical characterization of acute and convalescent illness of confirmed chikungunya cases from Chiapas, S. Mexico: a cross sectional study, PLoS One 12 (10) (2017) e0186923, https://doi.org/10.1371/journal.pone.0186923.

[15] J.U. Braga, C. Bressan, A.P.R. Dalvi, G.A. Calvet, R.P. Daumas, N. Rodrigues, et al., Accuracy of Zika virus disease case definition during simultaneous Dengue and Chikungunya epidemics, PLoS One 12 (6) (2017) e0179725, https://doi.org/10.1371/journal.pone.0179725.