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Secondary lower limbs lymphedema in patients with Chikungunya fever

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Abstract

Chikungunya fever (CHIKF) is an arbovirus characterized by acute fever, myalgia and polyarthralgia. Lymphedema in the lower limbs (LL) was observed in several patients during an outbreak of CHIKF in the state of Pernambuco (Brazil) in 2016. No reports on lymphatic vessels disease due to CHIKF have been described. The aim of the study was to follow lymphatic abnormalities in the LL of 16 patients with CHIKF, using lymphoscintigraphy.

An observational, prospective study with patients in the acute phase of CHIKF (confirmed serological diagnosis) with LL edema submitted to clinical evaluation and lymphoscintigraphy at baseline and after 90 days.

Sixteen patients (81% females) participated in this study. All patients presented with lower limb lymphedema, being 15 (94%) bilateral. Of the 31 limbs affected by lymphedema, 24 (77%) presented abnormalities in lymphatic drainage by lymphoscintigraphy. The delay to visualize pelvic lymph nodes was the most frequent lymphoscintigraphic abnormality, observed in 16 (51,6%) LL. Nine (56%) patients were clinically reevaluated after 90 days, and all 18 LL remained with lymphedema. A second lymphoscintigraphy showed persistent abnormalities in 13 (72%) of the 18 LL.

CHIKF can lead to lymphedema, and lymphedema may persist or progress after 3 months of the acute phases of the disease.

Abbreviations: Ceap Classification = clinical-etiology-anatomy- pathophysiology classification of the chronic venous disorders, Chikf = Chikungunya fever, IgG = immunoglobulin G, IgM = immunoglobulin M, LL = lower limbs, PCR = polymerase chain reaction.

Keywords: Chikungunya fever, lower limb lymphedema, lymphatic abnormalities, lymphatic vessels, lymphoscintigraphy

Author summary

1. Chikungunya fever (CHIKF) is an acute disease accompanied by arthralgia, intense asthenia, myalgia, headache and skin rash. The diagnosis of CHIKF is typically clinical, since the association between acute fever and arthralgia is highly predictive in areas where the disease is endemic or epidemics occur. A definitive diagnosis can be made by detecting the virus by polymerase chain reaction (PCR) or through serological test by detecting immunoglobulin M (IgM) and immunoglobulin G (IgG).
2. The most common vascular manifestations are peripheral arterial disorders and the Raynaud phenomenon but lymphedema in the lower limbs (LL) was observed in several patients during an outbreak of CHIKF in the state of Pernambuco (Northeastern Brazil) in 2016. For the first time, lymphoscintigraphic abnormalities were demonstrated in patients with CHIKF and LL edema. CHIKF can lead to lymphedema, and lymphedema may persist or progress after three months of the acute or subacute phases of the disease.

1. Introduction

Chikungunya virus is an alphavirus transmitted by the *Aedes Aegypti* and *Aedes Albopictus* infected mosquitoes widely spread across Brazil.[1–7] This virus has shown an amazing ability to

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spread and infect a large proportion of the population, as it has been observed in recurrent Chikungunya fever outbreaks. This arbovirus has an epidemic character with high attack rates ranging from 38% to 63%.[1] In 2016, 146,914 cases of CHIKF were confirmed in Brazil, with a higher incidence rate in the Northeast. The state of Pernambuco, located on the east coast of Brazil, was responsible for 25,692 cases of CHIKF[8] and for the highest number of deaths in 2016.[1,8]

CHIKF is typically an acute disease accompanied by arthralgia, intense asthenia, myalgia, headache and skin rash. The disease has an abrupt start after a 3-day incubation period and can evolve into 3 phases: acute - after the incubation period until the 10th day; subacute - if the joint pain persists after the acute phase, lasting up to 3 months; and chronic – the persistence of symptoms after 3 months and lasting up to 3 years.[1,2,4,5,9]

The diagnosis of CHIKF is typically clinical, since the association between acute fever and arthralgia is highly predictive in areas where the disease is endemic or epidemics have occurred,[9] such as in Pernambuco. The main laboratory finding is leukopenia with lymphopenia.[1,9] The definitive diagnosis can be made by detecting the virus by polymerase chain reaction (PCR) in the first week of illness. Serological diagnosis is made through detecting immunoglobulin M (IgM) from the 2nd to the 3rd day of illness, remaining present for 1 up to 3 months, and immunoglobulin G (IgG) starting from the 5th to the 10th day of illness, and remaining active for years.[1,2,9]

Atypical manifestations involving different systems, including the arterial vascular system, have been described in the literature involving CHIKF.[1,10,11] The most common vascular manifestations are peripheral arterial disorders and the Raynaud phenomenon.

Lymphedema in the lower limbs (LL) was observed in several patients during an outbreak of CHIKF in the state of Pernambuco (Northeastern Brazil) in 2016. As far as lymphatic manifestations are concerned, based on our knowledge there is only one study in the literature describing isolated cases of lymphedema in patients with CHIKF, however without lymphoscintigraphic confirmation.[12]

Thus, the aim of the present study was to evaluate lymphatic changes in the LL of patients with CHIKF and LL edema through lymphoscintigraphy.

2. Methods

The study population consisted of patients attended at the Ambulatory Clinic of Angiology and Vascular Surgery - Vascular Surgery Clinic of the *Hospital das Clínicas* of the Federal University of Pernambuco (*HC - UFPE*), sent for evaluation of lower limb edema with clinical epidemiological and serological diagnosis of CHIKF. Sixteen patients with positive serological diagnosis (detection of IgM antibodies for CHIKF) were included. All research stages were developed from March 2016 to November 2016, and a prospective observational study model was employed. In the same period, at the *Hospital das Clínicas* of the Federal University of Pernambuco (*HC – UFPE*), 248 cases of CHIKF were notified.[8]

The selected patients were subjected, LL doppler ultrasonography to exclude deep vein thrombosis, and 2 lymphoscintigraphies; one during the study admission period and another 90 days after.

Children, pregnant women, patients with a body mass index above 40, patients with LL ulcers of any etiology or advanced chronic venous disease of the LL (C3–C6 according to the Clinical-Etiology-Anatomy-Pathophysiology Classification of the Chronic Venous Disorders – CEAP classification), patients with renal, hepatic and/or cardiac failure, patients with a previous history of LL edema of any etiology, deep venous thrombosis, LL lymphangitis, orthopedic, vascular and/or oncologic LL surgery, patients under radiotherapy and patients with clinically evident lymphedema prior to CHIKF were all excluded from the study.

LL lymphoscintigraphy was performed at the *HC – UFPE* Nuclear Medicine Service following subcutaneous administration in the first inter-toe space of each foot of about 37 MBq (1 mCi) with a volume of 0.1 mL of dextran-70 solution marked with ^{99m}Tc. The ^{99m}Tc radionuclide was obtained from a molybdenum-technetium generator, manufactured by IPEN-CNEN-São Paulo.

Each patient walked for 10 minutes and then radiopharmaceutical bolus injection was performed with a 13x3 29G 1/2" needle after previous aspiration with the patient in the decubitus position. On-site massage was performed and patients performed flexion and ankle extension during the examination.

Dynamic images of the anterior pelvic region were acquired for 30 minutes (1 frame/minute) immediately after radiopharmaceutical injection, and whole-body images in the anterior and posterior projections (capture rate 7.5 cm/minute) about 1 hour after. Whole body images were also acquired 4 hours after the injection in case radiopharmaceutical ascension was not observed up to the height of the thoracic duct in the 1-hour images. The images were acquired by an all-purpose STARCAM 3200 gamma camera (General Electric, CA, USA) of one detector, with a collimator for low energy, 140 kiloelectronvolt (keV) photopic and 20% window. The following lymphoscintigraphic changes were analyzed for interpreting the images: asymmetry and early or delayed in radiotracer arrival in topography of inguinal and pelvic lymph nodes in the 30-minute dynamic images, poor visualization of the lymphatic vessels (medial superficial trunk of the lower limb), dermal reflux of the radiopharmaceutical, presence of collateral circulation, dilatation and tortuosity of lymphatic vessels and visualization of deep lymph nodes.

The obtained data was organized into spreadsheets (Microsoft Office Excel 2003). Descriptive statistics were used for analyzing the social, clinical and epidemiological profile and the results obtained from the lymphoscintigraphies, and the data were presented as *n* (absolute number) and % (relative number).

All patients were informed as to the nature of the research and the benefits of the diagnostic investigation, and those who agreed to participate in the study signed the Free and Informed Consent Terms. The study project was approved by the Ethics and Research Committee on Human Beings of the Federal University of Pernambuco (Opinion number: 1.710.092).

3. Results

In the periodo of the study a 248 cases of CHIKF was notified at the Hospital das Clínicas of the Federal University of Pernambuco (HC – UFPE), from them 31 were refer to the clinic as suspect Lymphedema and finally 16 was diagnosis by Lymphoscintigraphy of LL. (prevalence of confined LL: 6.4%).

Those 16 patients with soft and pitting LL edema and positive IgM serology for CHIKF in the acute or subacute phases of CHIKF selected, all from Pernambuco, have an average age of 59 years, and 13 (81%) patients were female (Fig. 1).

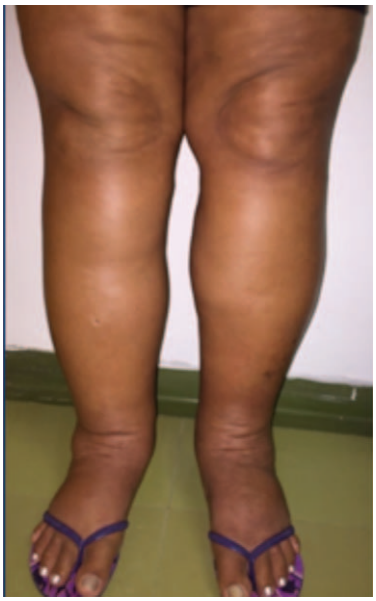

Figure 1. Patient from this study with LL lymphedema (cylindrical shape) and an edema in the dorsum of the foot included in the study.

Concerning LL affected by lymphedema, 15 (93.7%) patients presented bilateral edema and 1 (6.2%) unilateral edema.

Among the 31 LL of the 16 patients evaluated in the first lymphoscintigraphy, the presence of lymphopathy was observed in 24 (77.4%) LL. The delay in pelvic lymph nodes visualization was the most frequent alteration present in 16 (51.6%) LL, accelerated visualization of the pelvic lymph nodes in 5 (16%) LL, presence of dermal reflux in 2 (6.4%) LL, visualization of deep lymph nodes (popliteal, femoral and/or tibial) in 1 (3.2%) LL (Table 1, Figs. 2 and 3).

After the first evaluation, compression stockings (20–30 mmHg) were prescribed for all patients and the patients were advised to raise the legs when possible.

Out of the 16 study patients, 9 (56.2%) returned for a clinical reevaluation 90 days later and all of these patients continued to present bilateral LL edema.

All those 9 patients underwent a second lymphoscintigraphy. The delay in the visualization time of the pelvic lymph nodes was the most frequent abnormality, present in 13 (72.2%) LL, followed by the presence of dermal reflux in one (5.5%) LL and visualization of deep lymph nodes (popliteal, femoral and/or tibial) in one (5.5%) LL. Among the 18 LL underwent a second lymphoscintigraphy, 13(72,2%) presented lymphoscintigraphic abnormalities (Fig. 4 and Table 2).

Table 1

Lymphoscintigraphic results of the initial lymphoscintigraphy (Lympho. 1).

Lymphoscintigraphic parameters	
Lymphoscitigraphic results	**LL study sample**
N	31
Normal lymphoscintigraphy	7 (22.6%)
Abnormal lymphoscintigraphy	24 (77.4%)
Visualization of pelvic lymph nodes in less than 5 min	5 (16.1%)
Visualization of pelvic lymph nodes after 30 min (<60 min)	12 (38.7%)
Visualization of pelvic lymph nodes after 60 min	4 (12.9%)
Dermal reflux	2 (6.4%)
Deep lymph nodes	1 (3.2%)

4. Discussion

This was the first study to investigate lymphatic damage in patients with CHIKF and LL edema. Our results showed that CHIKF can cause acute lymphedema, and that lymphatic damage can progress or persist for more than 3 months causing chronic lymphedema.

The pathophysiology of lymphedema is complex[11–14] and the mechanism of lymphatic edema formation in CHIKF remains unknown. Recent studies in animal and human models suggest that inflammation is the key factor, if not the most important in the progression of lymphedema, particularly regarding interstitial changes and lymph transport.[12,15] Inflammatory joint involvement characterized by periarticular edema, tenosynovitis and arthritis is typical of CHIKF, and characterizes the main manifestation in the acute and chronic phases,[1,9] and may be associated with the development of lymphedema in these patients.

Another possibility is that CHIKF induces a mixed type II, II-III or III cryoglobulinemia. In 2009, Oliver et al evaluated the presence and persistence of cryoglobulinemia in 51 patients with CHIKF. About 20% of the patients presented vascular manifestations, and 94% of these presented cryoglobulinemia.[16] The presence of cryoglobulins may be related to vascular manifestations, but this statement still lacks studies for confirmation.

The innate and adaptive immune response to acute chikungunya virus infection has received considerable attention. Nevertheless, the pathophysiology of the manifestations of various CHIKF afflictions remains poorly characterized. Research should be performed to understand the pathophysiology of the atypical and chronic manifestations of this disease,[10] particularly of the manifestations in the lymphatic vessels. This problem will probably require new studies in order to discover biomarkers related to the severity and chronicity of the disease and genomic analysis involving these patients.[9]

This study considered normal lymphoscintigraphic findings: gradual and symmetrical rise of the radiotracer in the anterior and medial LL regions through a maximum of 5 lymphatic vessels in the calf, and up to 2 lymphatic vessels in the thigh; bilateral visualization of 2 and up to 10 pelvic lymph nodes between 10 and 15 minutes of injection; and absence of radiotracer accumulation in the subcutaneous cellular tissue in the late images of 1 and/or 4 hours. Similar criteria have been adopted by other authors.[17–21]

The delay in radiotracer ascent was the main lymphoscintigraphic alteration found in majority of the assessed LL. This lymphocytic pathological pattern is described in patients with lymphedema secondary to infectious lymphangitis of the LL,[20,22] and was the main alteration found by Soo et al in 2007 when investigating the presence of lymphopathy in patients with infectious lymphangitis.[22]

About 60% of the patients in the present study returned three months later for reassessment, and the persistence of lymphedema was observed in the majority of them. The delay in visualization time of the pelvic lymph nodes was also the most frequent alteration in the second lymphoscintigraphy. Moreover, some patients reassessed presented worsening in the lymphoscintigraphic pattern. These findings seem to indicate progression

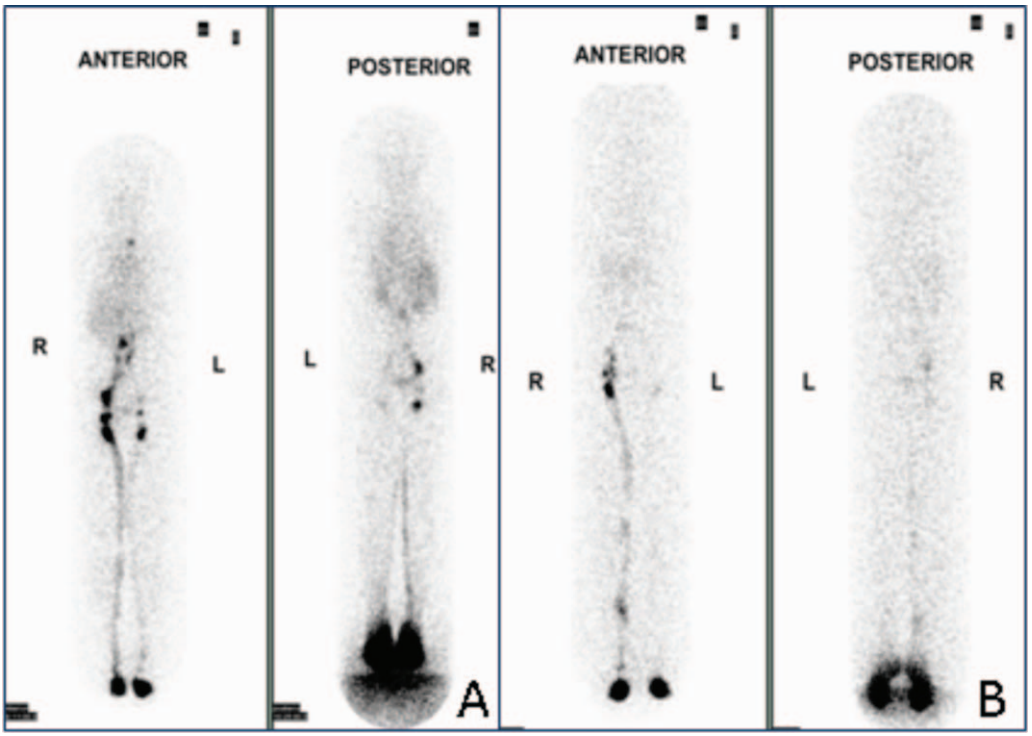

Figure 2. A: Whole body images from lymphoscintigraphy demonstrating poorly defined lymphatic vessels on the left and pelvic lymph nodes visualized just on 1-hour imaging post- 99m Tc- Dextran-70 injection. B: Whole body images from lymphoscintigraphy demonstrating poorly defined lymphatic vessels and poorly visualized pelvic lymph nodes on the left.

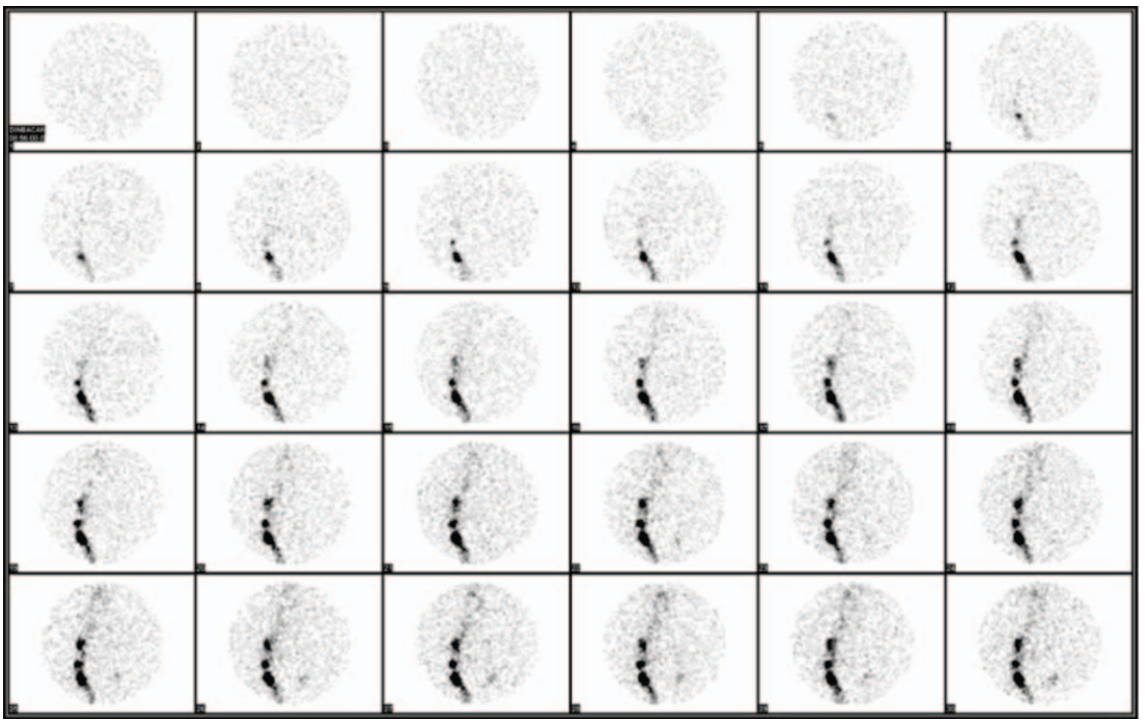

Figure 3. Lymphoscintigraphy images of the anterior pelvis acquired at 1-minute intervals during 30 minutes with absence of pelvic lymph node visualization on the left after 30 minutes of radiotracer injection.

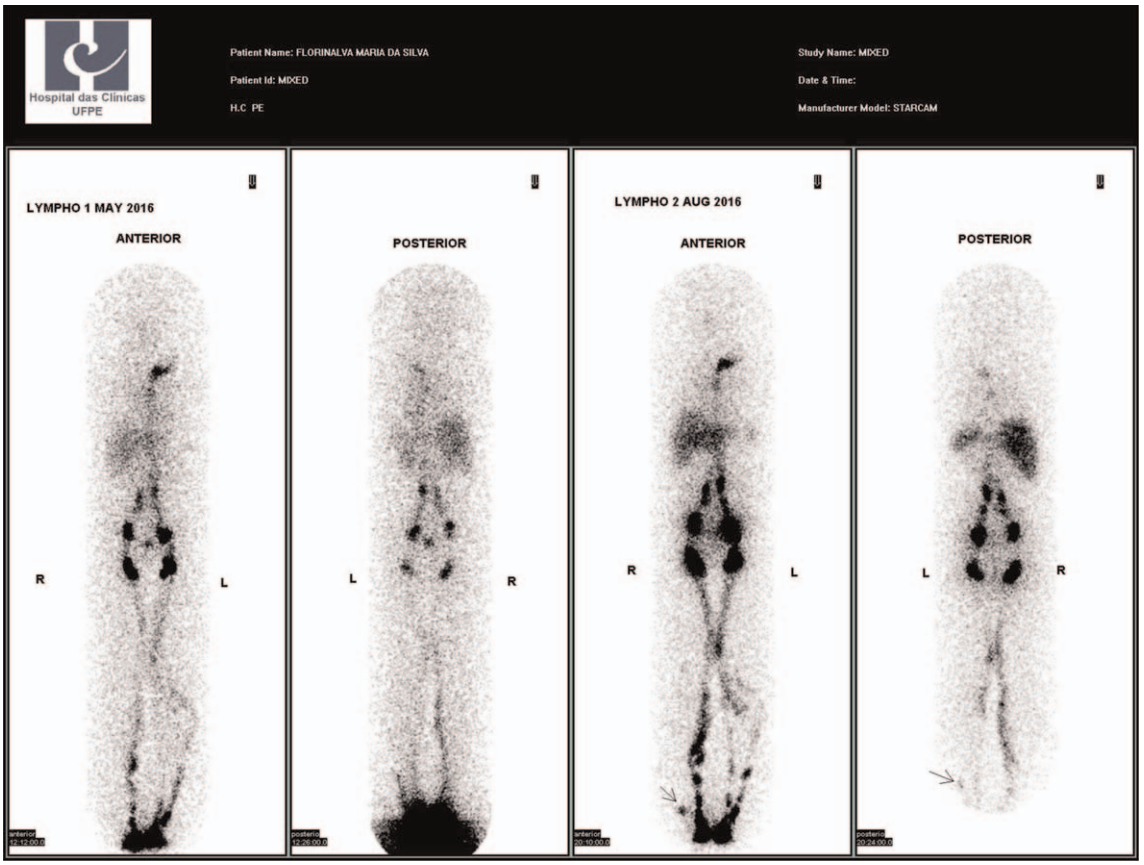

Figure 4. – LL lymphoscintigraphy showing increase of the collateral lymphatic vessels and increase in the number of deep lymph nodes in the second exam.

of the injury in the lymphatic vessels, thus demonstrating the possibility of chronic lymphatic vascular manifestations caused by CHIKF for the first time.

Unlike the present study, most studies in the literature describe lymphoscintigraphic changes in the LL of patients with chronic and/or secondary lymphedema. Advanced lymphedema phases are more likely to exhibit prominent lymphatic vessels or lymphangiectasia, the presence of collaterals, more pronounced delay in radiotracer transport, and dermal reflux in delayed images.[23] Otherwise, the present study evaluated lymphatic abnormalities in patients with the duration of edema for less than 6 months. Studies on acute lymphedema are scarce, and there is insufficient data in the literature describing expected lymphoscintigraphic changes.

Another alteration evidenced in this study was the accelerated radiotracer ascension time, with visualization of the pelvic lymph nodes varying from 1 to 9 minutes after the injection of ^{99m}Tc-Dextran 70 in about 16% of the assessed LL. Lymph flow may be normal, increased or decreased. Increased flow is characterized by pelvic lymph node visualization up to 10 minutes after radiopharmaceutical injection, and has already been associated with the presence of venous insufficiency and lymphedema.[24,25] It is possible that this accelerated pelvic lymph node visualization time may be related to lymphatic hyperflow as an attempt to compensate or increase lymphangiogenesis, and pre-existing lymphatic vessel expansion (lymphatic hyperplasia) in acute inflammatory processes.[26] More studies involving the Chikungunya virus are necessary to confirm its action over the lymphatic vessels.

Table 2

Lymphoscintigraphic results of the second lymphoscintigraphy 90 days after the first exam (Lymphoscintigraphy 2).

Lymphoscitigraphic results	Lymphoscintigraphy 2 lower limbs study sample
N	18 (100%)
Normal lymphoscintigraphy	5 (27.8%)
Abnormal lymphoscintigraphy	13 (72.2%)
Visualization of pelvic lymph nodes after 30 min (<60 min)	13 (72.2%)
Dermal reflux	1 (5.5%)
Deep lymph nodes	1 (5.5%)
Deep lymph nodes	1 (3.2%)

5. Study limitations

Although it is considered the gold standard, lymphoscintigraphy lacks a single protocol and all the technical aspects of the exam are variable.[27–30] For this study, we adopted the protocol developed by our institution, which has already been used in the elaboration of other studies.[31] The lack of current knowledge on the incidence of lymphoscintigraphic abnormalities in asymptomatic individuals and the absence of a control group of patients with CHIKF and no LL edema (denied for ethical reasons) also

limits our findings. However, all patients had a clinical diagnosis of lymphedema and a second lymphoscintigraphy was performed after 90 days, thus proving the persistence or progression of lymphoscintigraphic changes in more than half of them, and serving as a control for the first examination.

6. Conclusion

This study shows the lymphoscintigraphic abnormalities in patients with CHIKF are recurrent. CHIKF can lead to lymphedema, and lymphedema may persist or progress after 3 months of the acute or subacute phases of the disease.

Author contributions

Conceptualization: Esdras Marques Lins.
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