Article

Discovery Medicine 2025; 37(194): 583–590
https://doi.org/10.24976/Discov.Med.202537194.50

Analysis of *hsa_circ_0006459* and *hsa_circ_0015962* Expression in Peripheral Blood Mononuclear Cells of Dengue-Infected Patients and Healthy Donors

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Published: 20 March 2025

Background: Although evidence exists on the potential involvement of circular RNAs (circRNAs) in the pathogenesis of several viral infections, the expression levels, and the exact role that *hsa_circ_0006459* and *hsa_circ_0015962* could play during the Dengue virus (DENV) infection are still unclear. These two circRNAs were identified as possible biomarkers for diagnosis and prognosis of DENV disease in peripheral blood mononuclear cells (PBMC) of Dengue-positive patients. This study aimed to evaluate the expression levels of *hsa_circ_0006459* and *hsa_circ_0015962* in DENV-infected patients and compare them with healthy donors (HD) to provide new insights into the biological significance of these two circRNAs' expression.
Methods: We examined the presence and expression levels of *hsa_circ_0006459* and *hsa_circ_0015962* in PBMC of DENV-patients throughout a period of 28 days after the DENV diagnosis. HD was used as a control group.
Results: Our results show different expression levels and patterns between *hsa_circ_0006459* and *hsa_circ_0015962*, both in DENV patients and HD.
Conclusion: Possible change in the *hsa_circ_0006459* expression during DENV infection was observed, mainly at the time of diagnosis, but without a consistent pattern among patients during follow-up. Further studies are needed to clarify their expression levels and function both in Dengue-positive patients and HD.

Keywords: Dengue virus; Dengue fever; circRNA; biomarkers; *hsa_circ_0006459*; *hsa_circ_0015962*

Introduction

Circular RNAs (circRNAs) are a class of endogenous non-coding RNAs characterized by a special single-stranded, covalently closed circular structure that exerts many biological functions by acting as transcriptional regulators, microRNA sponges, and protein templates, also playing a significant role in cell proliferation, angiogenesis, and apoptosis [1–5]. Since 1976, when circRNAs were discovered [6,7], they have been increasingly studied, as more molecules related to various human diseases, such as neurodegenerative disorders (e.g., Alzheimer's disease), cancer (e.g., breast cancer and leukaemia), and cardiovascular diseases have been identified [1,3,8–12]. Some biochemical features of circRNAs, such as their stability and tissue specificity, make them potential biomarkers for different clinical settings [3,8–12].

During viral infections, circRNAs often show abnormal expression and can be detected in the host's cells, body fluids, or tissues [1]. Moreover, circRNAs play a key role in regulating the host's immune response and virus replication [1]. For instance, circRNAs are involved in proline-glutathione metabolisms and possibly in the mechanistic target of rapamycin (mTOR) signaling pathway after human papillomavirus 16 infection [13], enhance influenza A virus replication by suppressing autophagy [14], and exert an antiviral effect promoting oncogenesis during the herpesvirus type 8 infection [15,16]. These findings make circRNAs potentially useful as a novel biomarker for diagnosis and clinical progression of viral infections. Nevertheless, the role of circRNAs in Dengue virus (DENV) infection is not well understood [17]. In 2019, He *et al.* [18] identified two circRNAs (*hsa_circ_0006459* and *hsa_circ_0015962*) as potential biomarkers of disease progression in peripheral blood mononuclear cells (PBMC) from patients with Dengue fever (DENF), showing the

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down-regulation of the *hsa_circ_0006459* and the up-regulation of the *hsa_circ_0015962*, probably influencing the host response to DENV treatment.

DENV infection affects more than 100 countries in tropical and subtropical regions, including the Americas, Asia, Australia, and Africa, and causes about 400 million cases and 22,000 deaths globally every year [19]. Although DENV is endemic in most countries, a recent expansion of DENV cases beyond historical areas of presence has been observed, and higher transmission rates are expected in the future in other geographic areas due to favorable climate conditions for the spread of mosquitoes [20]. In 2023 a total of 377 cases of DENV infection were reported in Italy; among these, 82 had no travel history and were considered autochthonous [21], with 50% (41 out of 82) cases occurring in the Lazio region [21].

Given the lack of biomarkers for DENV infection progression, which could be very useful in predicting disease evolution in patients, the potential diagnostic use of these two circRNAs, previously analyzed from other groups, was investigated in the follow-up of infected patients. To this aim, we retrospectively analysed the prevalence and expression levels of the *hsa_circ_0006459* and *hsa_circ_0015962* circRNAs at different time points [i.e., on the day of the first DENV positive result (T0), and then on days 7 (T7), 15 (T15), and 28 (T28) from the diagnosis] in patients admitted to the National Institute for Infectious Diseases "Lazzaro Spallanzani" in Rome with a history of fever during the 2023 epidemic. We also analyzed a control group of healthy donors (HD) at two different time points to evaluate the basal expression level of these two circRNAs without DENV infection. The further purpose of this study was to compare the expression levels of the *hsa_circ_0006459* and *hsa_circ_0015962* in HD versus DENV-infected patients to give new insight into the biological significance of the expression of these two circRNAs.

Materials and Methods

Study Population and Samples

The control group consisted of fourteen HD (9 females and 5 males; mean age, 39 years, Interquartile Range (IQR): 20, range, 24 to 61 years) selected only on the basis of their good health state and absence of comorbidities. The group with DENV infection included 10 patients who came to the National Institute for Infectious Diseases "Lazzaro Spallanzani" with a suspected diagnosis of DENV infection from September to December 2023. The only criterion for inclusion was not having an epidemiological link of travel to endemic areas, therefore excluding imported cases. The mean age was 51 years (IQR: 35), three patients were males, and seven were females. At admission, patients were tested for DENV non-structural protein 1 (NS1) and IgM and IgG by Standard F Dengue NS1 Ag FIA and Standard F Dengue IgM/IgG FIA (SD Biosensor, Suwon, Republic of Korea [22]; Lot: 6475523AC and 6485181AC), respectively. Additionally, specific real-time reverse transcription polymerase chain reaction (RT-PCR) tests on blood or plasma matrix for confirming the diagnosis of DENV infection, for the DENV serotype discrimination, and during follow-up were performed according to the Centers for Disease Control and Prevention [23]. Briefly, RNA was reverse transcribed and amplified using Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR System (Thermo Fisher Scientific, Waltham, MA, USA; Lot: 2151250) for the detection and typing of DENV. Primers used for PCR amplification were previously described [23,24] (**Supplementary Table 1**).

After giving consent, patients had blood drawn with ethylenediaminetetraacetic acid (EDTA) tubes on T0, T7, T15, and T28. Total PBMC were separated right after blood collection by standard Ficoll-Hypaque (Cedarlane, Burlington, Ontario, Canada; Lot: 25-65) density-gradient centrifugation, rinsed with plenty of phosphate-buffered salines, and then treated with Red Blood Cell Lysis Solution (Miltenyi Biotec, Bologna, Italy; Lot: 5240508454) to achieve maximum removal of the remaining red blood cells. Plasma and PBMC samples were immediately stored at –80 °C and kept frozen until use. Various biological parameters (i.e., red blood cell count, haemoglobin, haematocrit, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red blood cell distribution, platelet, mean platelet volume, white blood cell, neutrophil, lymphocyte, monocyte, eosinophilia, basophil) were assessed in the blood of infected patients at T0.

RNA Extraction and CircRNA Detection

Nucleic acid extraction was performed from PBMC of HD and DENV patients by the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany; Lot: 178018946) on the QiaSymphony automatic extractor (Qiagen, Hilden, Germany). After extraction, samples were treated with the TURBO DNA-free™ Kit (Thermo Fisher Scientific, Waltham, MA, USA; Lot: 01340918) and RNase R (Abcam, Cambridge, UK; Lot: 1021059238) to isolate circRNAs, following the manufacturer's instructions.

KAPA SYBR One-Step qRT-PCR Master Mix Kit (Merck, Milano, Italy; Lot: 0000203714) was used to reversetranscribe, amplify, and quantify the *hsa_circ_0006459* and *hsa_circ_0015962* circRNAs. Primers used for PCR amplification were previously described by He *et al*. [18] (**Supplementary Table 1**). *β-actin* was used as an internal control (**Supplementary Fig. 1**). For statistical calculations, an arbitrary value of 450.1 cycle-threshold (Ct) was assigned to negative samples.

Statistical Analysis

GraphPad Prism version 9.3.1 (GraphPad Software, La Jolla, CA, USA) was used for data management

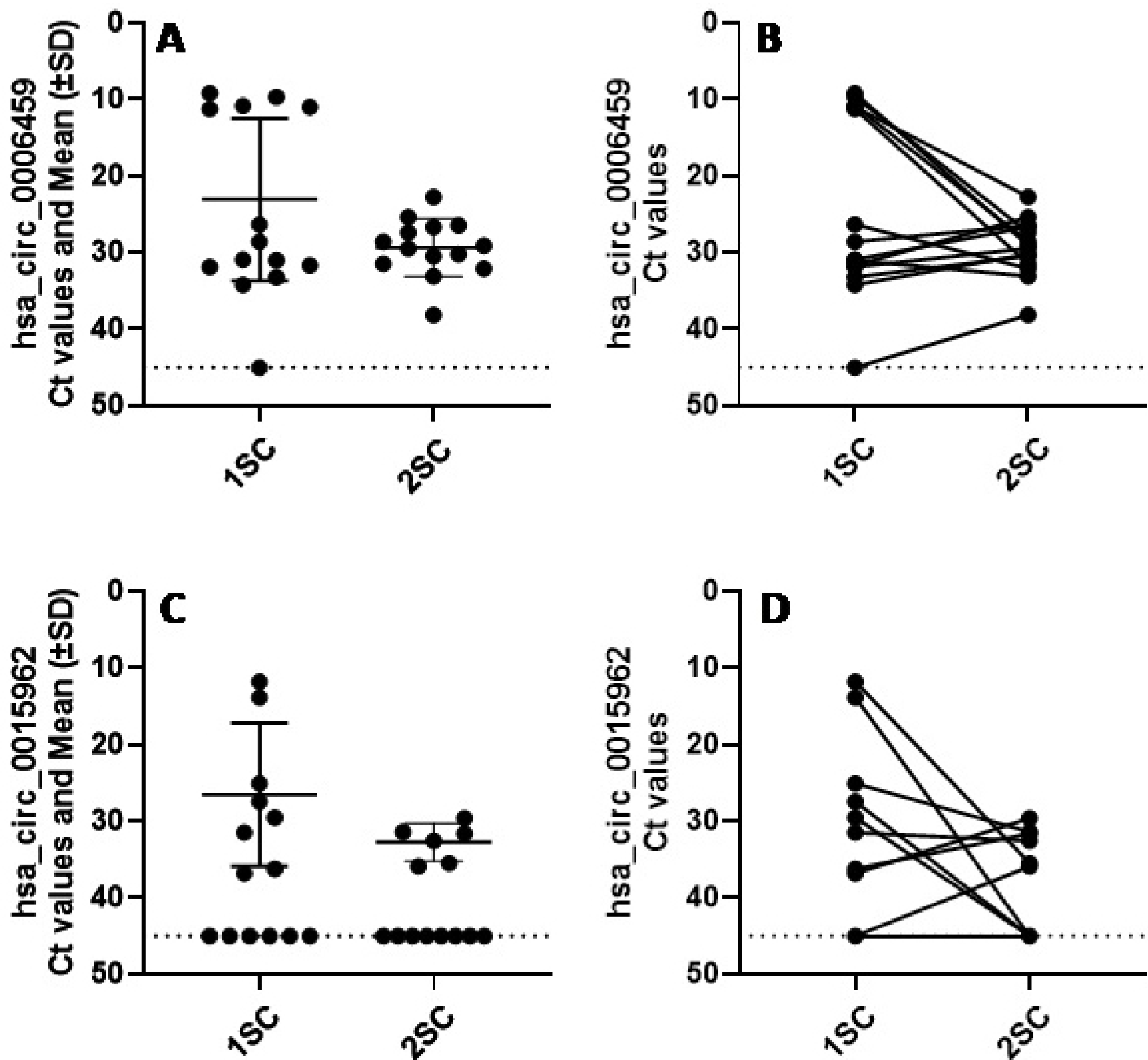

Fig. 1. Levels of the two circRNAs measured in PBMC of HD at the first and second sample collections. (A) Levels of the *hsa_circ_0006459*. (B) Graphical representation of changes in the expression of the *hsa_circ_0006459*. (C) Levels of the *hsa_circ_0015962*. (D) Graphical representation of changes in the expression of the *hsa_circ_0015962*. The dotted line indicates the low limit of detection. Negative samples were assigned a cycle-threshold (Ct) value of 45.01. The mean Ct value (± standard deviation) refers to circRNA-positive samples. 1SC, first sample collection; 2SC, second sample collection; circRNAs, circular RNAs; PBMC, peripheral blood mononuclear cells; HD, healthy donors.

and statistical analyses: Spearman correlation, median, IQR, mean, standard deviation (SD), and Mann-Whitney test. In particular, the Spearman correlation of confidence interval was used to analyze the correlation between *hsa_circ_0015962*, *hsa_circ_0006459*, and biological parameters; the Mann-Whitney test to check whether there are significant differences in the expression level of *hsa_circ_0015962* and *hsa_circ_0006459* between HD and DENV-positive patients. A p-value < 0.05 was considered significant.

Results

Clinical Characteristics of DENV-Positive Patients

DENV-positive patients had a median of 6 days (IQR: 2) between the onset of symptoms and the laboratory confirmation. They were confirmed as DENV-positive by a rapid screening test: nine out of ten patients had the DENV antigen NS1, seven had anti-DENV IgM, and three had anti-DENV IgG. At T0, the mean (± SD) Ct value of DENV was 25.5 ± 8.2, and, after 28 days, no DENV-RNA was found

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Table 1. Prevalence of the *hsa_circ_0006459* and *hsa_circ_0015962* circRNAs in healthy donors and DENV-positive patients.

		hsa_circ_0006459	*hsa_circ_0015962*
		N. positive/total examined (%)	N. positive/total examined (%)
Healthy donors	1SC[1]	13/14 (93)	8/14 (57)
	2SC[2]	14/14 (100)	6/14 (43)
DENV[3]-positive patients	T0	6/7 (86)	2/7 (29)
	T7	6/10 (60)	3/10 (30)
	T15	6/10 (60)	0/10 (0)
	T28	5/10 (50)	1/10 (10)

[1] 1SC: first sample collection; [2] 2SC: second sample collection; [3] DENV: Dengue virus; T0, T7, T15, and T28: first positive DENV result (T0), and then on days 7 (T7), 15 (T15), and 28 (T28) from the diagnosis. The small number of patients at T0 is because we ran out of samples, so we could not perform the analysis.

in any patient. Three patients had a DENV-1 serotype infection, one patient had a DENV-2 serotype infection, and six patients had a DENV-3 serotype infection. The most common symptoms of DENV-positive patients were fever (90%), arthromyalgia (90%), skin rash (60%), headache (40%), and nausea (40%). Noteworthy, all patients got the same treatment based on paracetamol following international guidelines [25].

Laboratory tests performed at the time of the hospital admission, showed a median of 3900/mm^3 for white blood cells (IQR: 230), 890/mm^3 for lymphocytes (IQR: 540), 134 mm^3 for platelets (IQR: 126), 59 U/L for aspartate aminotransferase (IQR: 44), and 48 U/L for alanine transaminase (IQR: 45). Haemoglobin and creatinine levels were normal. Spearman correlation did not show a significant correlation between any of these parameters and circRNA expression in DENV-positive patients.

None of the patients had Chikungunya virus, human immunodeficiency virus, hepatitis B virus, or hepatitis C virus infections.

Detection and Levels of the hsa_circ_0006459 and hsa_circ_0015962 CircRNAs in PBMC of HD and DENV-Positive Patients

Analysis of *hsa_circ_0006459* and *hsa_circ_0015962* was performed twice in PBMC of HD to verify both their presence and the stability of the levels over time. As shown in Table 1, on the first sample collection (1SC), 13 out of 14 (93%) HD tested positive for the expression of the *hsa_circ_0006459*, while the *hsa_circ_0015962* was detected in 8 out of 14 (57%) individuals. In the second sample collection (2SC), there was a slight difference in the occurrence of the two circRNAs: 100% and 43%, respectively. In detail, one HD who tested negative for both circRNAs in the 1SC became positive for the *hsa_circ_0006459* in the 2SC; moreover, three HDs turned negative for the *hsa_circ_0015962* in the 2SC.

In DENV-positive patients, *hsa_circ_0006459* and *hsa_circ_0015962* were monitored at selected time points: T0, T7, T15, and T28. As shown in Table 1, at baseline, the *hsa_circ_0006459* and *hsa_circ_0015962* circRNAs were detected in 6 out of 7 patients (86%) and 2 of 7 patients (29%), respectively.

During the follow-up (T7–T28), changes in occurrence for both circRNAs were observed in some patients (Table 1). The *hsa_circ_0006459* was detected in 60–50% of patients, while the *hsa_circ_0015962* was found in 30%, 0%, and 10% of patients at T7, T15, and T28, respectively.

We also measured the two circRNAs in PBMC of HD and DENV-positive patients at the indicated time points. In HD, the *hsa_circ_0006459* had a mean ($\pm$ SD) Ct of 23.1 $\pm$ 10.6 in the first sample and a mean ($\pm$ SD) Ct of 29.4 $\pm$ 3.8 in the second sample (no statistically significant difference was observed; Fig. 1A). In only one HD from a negative result in the 1SC, the *hsa_circ_0006459* becomes positive at 2SC (Fig. 1B).

For the other circRNA, the Ct values are shown in Fig. 1C,D. The mean ($\pm$ SD) Ct was 26.5 $\pm$ 9.4 in the 1SC, while in the 2SC, three donors turned negative and one became positive, and the mean ($\pm$ SD) Ct was 32.8 $\pm$ 2.5 among the six positive donors (no statistically significant difference was observed, $p > 0.05$).

The mean Ct value ($\pm$ SD) of the *hsa_circ_0006459* in DENV-positive patients at T0 was Ct 26.4 $\pm$ 9.6 (Fig. 2A). However, there was a wide variation in the individual *hsa_circ_0006459* Ct values among the patients, from 13.3 to 37.5 (95% confidence interval: 16.3–36.5), and one patient even tested negative.

This circRNA was undetected in 4/10 patients at T7 and at T15, and one patient showed this non-coding RNA above 28.0 Ct both at T7 and T15. At T28, the *hsa_circ_0006459* was absent in the PBMC of half of the patients. At T7, positive patients showed a mean Ct value $\pm$ SD of 30.8 $\pm$ 4.0, 30.0 $\pm$ 4.6 at T15, and 29.6 $\pm$ 2.5 at T28.

Regarding the *hsa_circ_0015962*, two out of ten individuals tested positive at T0, but with high Ct levels (mean Ct values ($\pm$ SD): 38.4 $\pm$ 0.4) (Fig. 2B). One patient had a high level of *hsa_circ_0015962* at T7 (Ct 24.9); all samples were negative at T15; one patient turned positive at

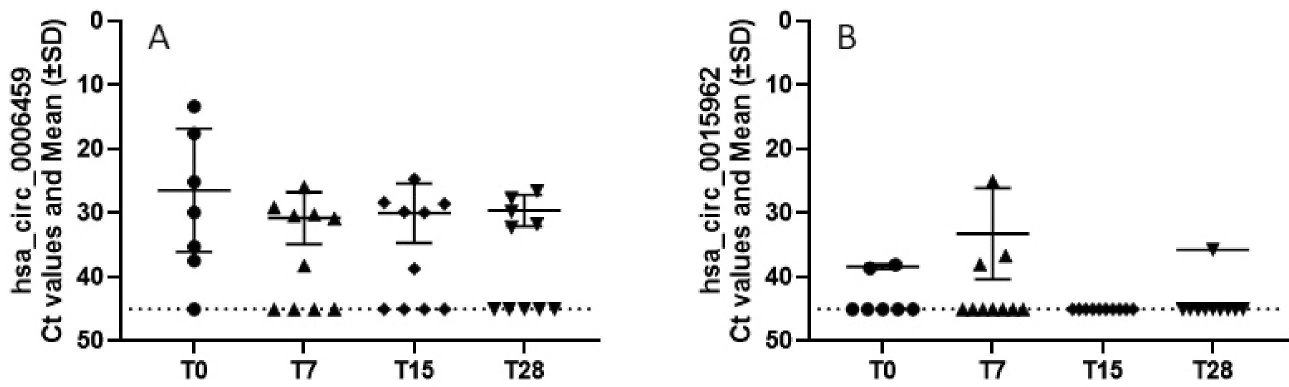

Fig. 2. Graphical representation of levels of the two circRNAs measured in PBMC of DENV patients at indicated time points. (A) Levels of the *hsa_circ_0006459* at indicated time points. (B) Levels of the *hsa_circ_0015962* at indicated time points. The dotted line indicates the low limit of detection. Negative samples were assigned a cycle-threshold (Ct) value of 45.01. The mean Ct value (± standard deviation) refers to circRNA-positive samples. circRNAs, circular RNAs; PBMC, peripheral blood mononuclear cells; HD, healthy donors.

T28, after being negative throughout the follow-up. A statistically significant difference was observed between the *hsa_circ_0015962* in DENV-positive patients at T0 and HD at 1SC (p = 0.0444).

Hence, the expression of these two circRNAs varied throughout the observation period in follow-up, showing large individual differences, and, for each donor, between different time points: the changes observed are shown in Fig. 3 along with the kinetics of DENV viral loads. For instance, some patients (e.g., patients C, E, F, and G) had mostly stable Ct values of *hsa_circ_0006459* at all selected time points; others (patients A and I) showed a similar pattern between *hsa_circ_0006459* and DENV RNA; patients D, H, and J had an unrelated trend to the viral load.

Moreover, the *hsa_circ_0015962* was often negative, with 60% of patients never expressing it (Fig. 3). In patients positive for the *hsa_circ_0015962* expression, two were PCR positive only at T7 (patient C) and T28 (patient G), both with a very low Ct value, while other two patients (patients I and J) were circRNA positive at T0 and T7, dropping to negativity at T15 (Fig. 3).

Overall, although all patients had cleared the infection by the end of follow-up (DENV viral load not detected), the viral load decline seemed to be independent of the expression levels of the two circRNAs, having expression kinetics variable from one patient to another, without a consistent or replicable pattern (Fig. 3).

Discussion

Despite the role of different signaling pathways throughout DENV infection having been explored, the complex nature of DENV pathogenesis has prevented a full understanding of the underlying molecular mechanisms [26]. In recent decades, many significant steps forward have been taken in revealing the function of host and viral non-coding RNAs in DENV infection [27]; nevertheless, there is still a large gap in examining the role of other host non-coding RNAs, such as circRNAs, in DENV pathogenesis. CircRNAs are known to participate in multiple regulatory processes and to be ubiquitous, tissue- and cell-specific [3,5,13,28,29]. More recently, these circRNAs have been shown to play an important role in innate antiviral immunity against many viruses and to have potential as therapeutic and diagnostic biomarkers of viral diseases [1,14–16,30]. Based on their features, circRNAs can be used for the development of innovative models aimed at discovering, studying, understanding, and modulating the regulatory pathways in which they are involved, with an important flap in achieving a deeper insight into the pathogenesis of several severe viral infections, including DENV infection.

In a previous study, He *et al*. [18] collected PBMC from patients with DENV before and after the treatment and analyzed the circRNA expression profiles. Two circRNAs showed the most change, with a lower expression of *hsa_circ_0006459* and a higher expression of *hsa_circ_0015962* at the end of the illness. Based on these findings, the authors suggested the role of these two circRNAs as potential biomarkers for patients with DENV [18].

To investigate the possibility of using these two circRNAs as diagnostic criteria, we analyze the prevalence of the *hsa_circ_0006459* and the *hsa_circ_0015962* in patients with DENV, and we monitored their expression levels for one month after diagnosis of infection. In addition, we analyzed these circRNAs in HD to verify their changes by collecting samples at two different times.

Unlike He *et al*. [18], no statistically significant correlation was detected between biological parameters, DENV subtypes, and circRNA expression in DENV-positive patients at diagnosis of infection, thus suggesting other factors potentially responsible for the variable behavior of these circRNAs in infected individuals.

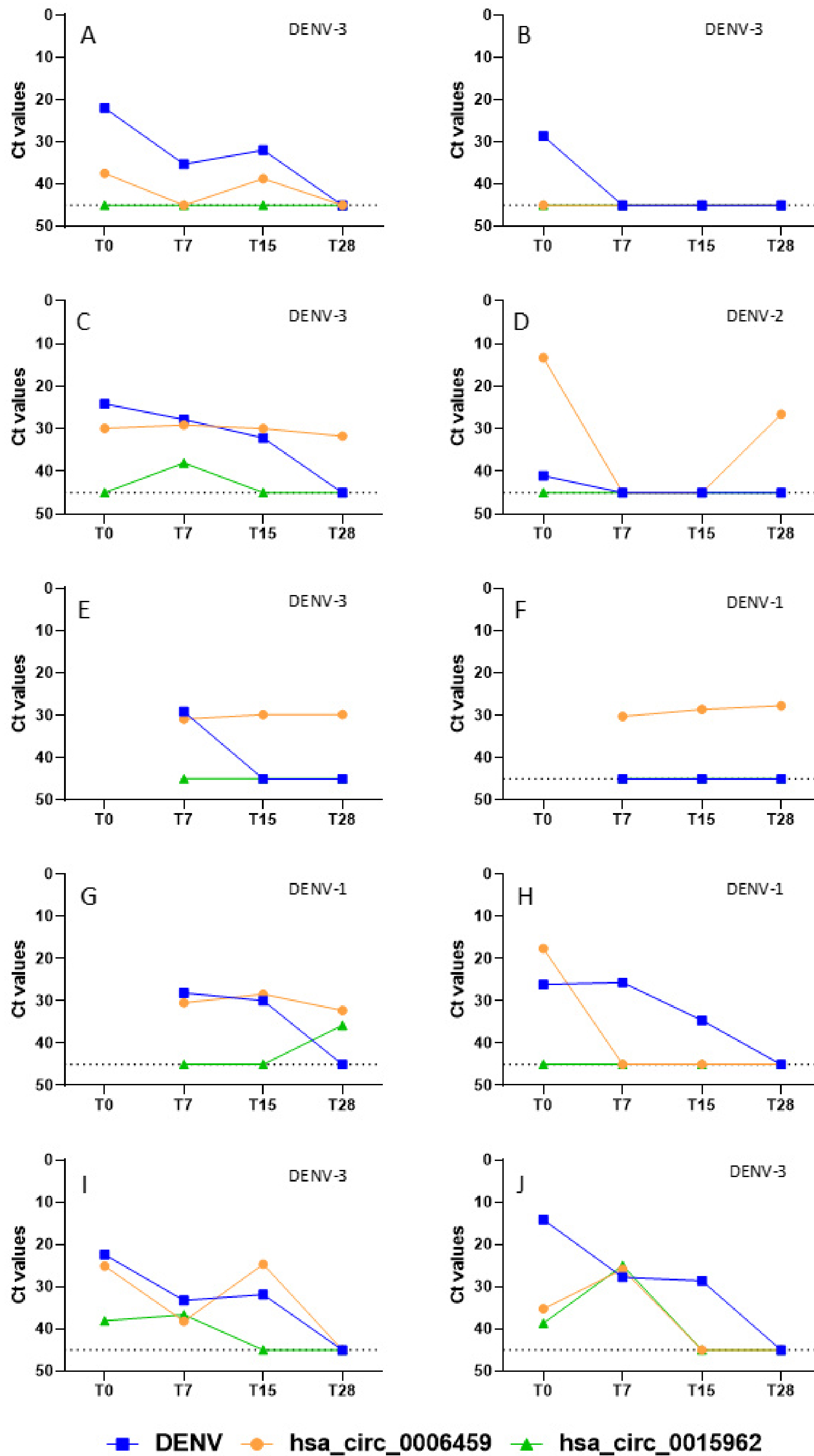

Fig. 3. Results of Ct values of DENV RNA in blood/plasma samples and of *hsa_circ_0006459* and *hsa_circ_0015962* in PBMC at different time points in each patient. Patients were indicated by capital letters (A–J). DENV serotypes of each patient are indicated in each panel. For patients (D) and (J), the T0 results were obtained from the plasma matrix, as a blood sample was not available. The dotted line indicates the low limit of detection. Negative samples were assigned a cycle-threshold value of 45. PBMC, peripheral blood mononuclear cells.

When considering the expression levels, the *hsa_circ_0006459* was detected in almost all the HD with variation among subjects both at 1SC and 2SC; differently, the *hsa_circ_0015962* was detected in about half of the HD at 1SC, some of which being negative at 2SC.

Such differences in prevalence and levels of the two circRNAs were also observed in patients with DENV infection during a 4-week follow-up. In particular, already at the time of diagnosis, some patients showed higher expression of the *hsa_circ_0006459* with respect to others. Furthermore, during the follow-up, different kinetics were observed: in four cases, this circRNA remained at a constant level throughout the entire period, in five patients a fluctuating trend was detected and only one case was always negative. Looking at the *hsa_circ_0015962*, a slight increase one week after diagnosis in three DENV-positive patients was observed, becoming undetected at T15; one patient tested positive only at T28.

Starting from the consideration that it is not possible to know the expression levels of the two circRNAs in our patients before the DENV infection, the data here described suggest that there is no unique behavior of these two circRNAs in HD and DENV infection. Although notable changes in the expression levels of the first circRNA (the *hsa_circ_0006459*) were observed during the illness, we did not recognize a trend correlated with the kinetics of the DENV load or with the progressive resolution of the symptoms, nor a common or reproducible trend among DENV-positive patients.

The small number of patients and the different ethnicities of the population examined compared to the work of He *et al*. [18] could certainly be a limitation of the study, justifying the differences between the results obtained in the two studies.

Conclusion

Although the two circRNAs were previously identified as potential biomarkers of DENV disease progression in infected patients, with their modulation supposed to reflect the host response during illness, our data clearly show a fluctuating trend, raising many questions about the real possibility of using them as biomarkers in DENV infection.

Since the expression levels of the circRNAs analyzed in this study appear individual-specific either in HD or in DENV-positive patients, we hypothesize a possible influence by different factors, probably unrelated to the DENV infection, such as host-dependent factors and population genetics.

Therefore, to better clarify the behaviour and impact of these two circRNAs, it is mandatory to perform further analyses using an expanded number of HD and patients, which could allow us to understand if these circRNAs are related to illness, treatment, genetic population, or infection-related inflammatory state and to investigate deeper if the modulation of circRNA levels can guide the prognosis of DENV infection in the clinic.

Availability of Data and Materials

The datasets generated and/or analyzed during the current study are not publicly available due to privacy policy, but are available from the corresponding author on reasonable request.

Author Contributions

Conceptualization, GS; methodology, GS, EL, AA, and LB; formal analysis, GS, and CM; resources, AM, MLG, and EN; data curation, GS, and CM; interpretation of data, GS, EL, AA, LB, CM, AM, MLG, EN, EG, and FM; writing—original draft preparation, GS; writing—review and editing, LB, AA, EL, EN, EG, and FM; supervision, EG, EN, and FM; funding acquisition, FM. All authors contributed significantly to editorial changes of important content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the institutional review board of the National Institute for Infectious Diseases, L. Spallanzani, IRCCS (protocol n. 14/2015, approved on 17 February 2015, and amendments 9/2020, 38/2023, 56/2023). Data for biologic samples collected for diagnostic purposes were used only after their complete anonymization. Informed consent was obtained from all subjects involved in the study.

Acknowledgment

Not applicable.

Funding

This study was supported by funds allocated to the National Institute for Infectious Diseases "Lazzaro Spallanzani", IRCCS, 00149, Rome (Italy), from the Italian Ministry of Health (Programma Ricerca Corrente—Linea 1 on emerging and re-emerging infections).

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202537194.50.

References

[1] Sberna G, Maggi F, Amendola A. Virus-Encoded Circular RNAs: Role and Significance in Viral Infections. International Journal of Molecular Sciences. 2023; 24: 16547.

[2] Zhang X, Chen L, Xiao B, Liu H, Su Y. Circ_0075932 in adipocyte-derived exosomes induces inflammation and apoptosis in human dermal keratinocytes by directly binding with PUM2 and promoting PUM2-mediated activation of AuroraA/NF-κB pathway. Biochemical and Biophysical Research Communications. 2019; 511: 551–558.

[3] Lukiw WJ. Circular RNA (circRNA) in Alzheimer's disease (AD). Frontiers in Genetics. 2013; 4: 307.

[4] Xu Y, Leng K, Yao Y, Kang P, Liao G, Han Y, *et al*. A Circular RNA, Cholangiocarcinoma-Associated Circular RNA 1, Contributes to Cholangiocarcinoma Progression, Induces Angiogenesis, and Disrupts Vascular Endothelial Barriers. Hepatology. 2021; 73: 1419–1435.

[5] Zhao F, Zhang X, Pei X, Yang D, Han M. Deregulated Expression of Circular RNAs Is Associated with Immune Evasion and Leukemia Relapse after Allogeneic Hematopoietic Stem Cell Transplantation. Genes. 2022; 13: 1986.

[6] Sanger HL, Klotz G, Riesner D, Gross HJ, Kleinschmidt AK. Viroids are single-stranded covalently closed circular RNA molecules existing as highly base-paired rod-like structures. Proceedings of the National Academy of Sciences of the United States of America. 1976; 73: 3852–3856.

[7] Kolakofsky D. Isolation and characterization of Sendai virus DI-RNAs. Cell. 1976; 8: 547–555.

[8] Lei K, Bai H, Wei Z, Xie C, Wang J, Li J, *et al*. The mechanism and function of circular RNAs in human diseases. Experimental Cell Research. 2018; 368: 147–158.

[9] Bao H, Li J, Zhao Q, Yang Q, Xu Y. Circular RNAs in Breast Cancer: An Update. Biomolecules. 2024; 14: 158.

[10] Ugalde AP, Roiz-Valle D, Moledo-Nodar L, Caravia XM, Freije JMP, López-Otín C. Noncoding RNA Contribution to Aging and Lifespan. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences. 2024; 79: glae058.

[11] Zhao J, Wang G, Yan G, Zheng M, Li H, Bai Y, *et al*. Hsa_circ_0006010 and hsa_circ_0002903 in peripheral blood serve as novel diagnostic, surveillance and prognostic biomarkers for disease progression in chronic myeloid leukemia. BMC Cancer. 2024; 24: 1172.

[12] Tan WLW, Lim BTS, Anene-Nzelu CGO, Ackers-Johnson M, Dashi A, See K, *et al*. A landscape of circular RNA expression in the human heart. Cardiovascular Research. 2017; 113: 298–309.

[13] Zheng SR, Zhang HR, Zhang ZF, Lai SY, Huang LJ, Liu J, *et al*. Human papillomavirus 16 E7 oncoprotein alters the expression profiles of circular RNAs in Caski cells. Journal of Cancer. 2018; 9: 3755–3764.

[14] Yu T, Ding Y, Zhang Y, Liu Y, Li Y, Lei J, *et al*. Circular RNA GATAD2A promotes H1N1 replication through inhibiting autophagy. Veterinary Microbiology. 2019; 231: 238–245.

[15] Yao S, Jia X, Wang F, Sheng L, Song P, Cao Y, *et al*. CircRNA ARFGEF1 functions as a ceRNA to promote oncogenic KSHV-encoded viral interferon regulatory factor induction of cell invasion and angiogenesis by upregulating glutaredoxin 3. PLoS Pathogens. 2021; 17: e1009294.

[16] Tagawa T, Gao S, Koparde VN, Gonzalez M, Spouge JL, Serquiña AP, *et al*. Discovery of Kaposi's sarcoma herpesvirus-encoded circular RNAs and a human antiviral circular RNA. Proceedings of the National Academy of Sciences of the United States of America. 2018; 115: 12805–12810.

[17] circAtlas 3.0. 2024. Available at: https://ngdc.cncb.ac.cn/circatlas/disease.php (Accessed: 2 July 2024).

[18] He J, Ming Y, MinLi Y, Han Z, Jiang J, Zhou J, *et al*. hsa_circ_0006459 and hsa_circ_0015962 affect prognosis of Dengue fever. Scientific Reports. 2019; 9: 19425.

[19] World Health Organization. Dengue guidelines, for diagnosis, treatment, prevention and control. 2009. Available at: https://www.who.int/publications/i/item/9789241547871 (Accessed: 2 July 2024).

[20] De Carli G, Carletti F, Spaziante M, Gruber CEM, Rueca M, Spezia PG, *et al*. Outbreaks of autochthonous Dengue in Lazio region, Italy, August to September 2023: preliminary investigation. European Communicable Disease Bulletin. 2023; 28: 2300552.

[21] Istituto Superiore di Sanità – EpiCentro. Febbre dengue. 2024. Available at: https://www.epicentro.iss.it/febbre-dengue/aggiornamenti (Accessed: 2 July 2024).

[22] SD Biosensor. 2024. Available at: https://www.sdbiosensor.com (Accessed: 2 July 2024).

[23] Centers for Disease Control and Prevention. Molecular Tests for Dengue Virus. 2025. Available at: https://www.cdc.gov/dengue/hcp/diagnosis-testing/molecular-tests-for-dengue-virus.html?CDC_AAref_Val=https://www.cdc.gov/dengue/healthcare-providers/testing/molecular-tests/index.html (Accessed: 18 February 2025).

[24] Santiago GA, Vergne E, Quiles Y, Cosme J, Vazquez J, Medina JF, *et al*. Analytical and clinical performance of the CDC real time RT-PCR assay for detection and typing of dengue virus. PLOS Neglected Tropical Diseases. 2013; 7: e2311.

[25] Long M, Wang H, Ning X, Jia F, Zhang L, Pan Y, *et al*. Functional analysis of differentially expressed long non-coding RNAs in DENV-3 infection and antibody-dependent enhancement of viral infection. Virus Research. 2022; 319: 198883.

[26] Krishnamoorthy P, Raj AS, Kumar P, Das N, Kumar H. Host and viral non-coding RNAs in dengue pathogenesis. Reviews in Medical Virology. 2022; 32: e2360.

[27] Gu A, Jaijyan DK, Yang S, Zeng M, Pei S, Zhu H. Functions of Circular RNA in Human Diseases and Illnesses. Non-Coding RNA. 2023; 9: 38.

[28] Ikeda Y, Morikawa S, Nakashima M, Yoshikawa S, Taniguchi K, Sawamura H, *et al*. CircRNAs and RNA-Binding Proteins Involved in the Pathogenesis of Cancers or Central Nervous System Disorders. Non-coding RNA. 2023; 9: 23.

[29] Wang X, Li J, Bian X, Wu C, Hua J, Chang S, *et al*. *CircURI1* interacts with hnRNPM to inhibit metastasis by modulating alternative splicing in gastric cancer. Proceedings of the National Academy of Sciences of the United States of America. 2021; 118: e2012881118.

[30] Zhen Y, Sun G, Chen C, Li J, Xiao R, Xu Z. Circular RNA hsa_circ_0064559 affects tumor cell growth and progression of colorectal cancer. World Journal of Surgical Oncology. 2023; 21: 171.