https://doi.org/10.29001/2073-8552-2021-36-3-111-118
УДК 616.12-005.4-036.11:616.124.2-073.27]:[616-022.6:578.825.11]

Features of the course of acute decompensated ischemic heart failure and/or ongoing adverse left ventricular remodeling in patients with identified human herpes virus type 6

Ekaterina V. Kruchinkina, Oksana N. Ogurkova, Anna M. Gusakova, Tatyana E. Suslova, Vyacheslav V. Ryabov

Cardiology Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences,

111a, Kievskaya str., Tomsk, 634012, Russian Federation

Abstract

Objective. To determine serum levels of immunoglobulin M (IgM) and G (IgG) antibodies to human herpes virus type 6 (HHV-6) (anti-HHV-6) and features of clinical and morphological portrait in patients with acute decompensated heart failure (ADHF) of ischemic genesis and/or adverse left ventricular (LV) remodeling.

Material and Methods. This open-label, nonrandomized, single-center, prospective trial was registered at clinicaltrials.gov (#NCT02649517) and comprised 25 patients (84% men) with ADHF and LV ejection fraction (EF) ≤ 40%. All patients underwent endomyocardial biopsy (EMB) with immunohistochemistry (IHC) analysis for the presence of HHV-6, compliment C1q, major histocompatibility complex of class II (MHC II), and B-lymphocyte antigen (CD19) as the markers of autoimmune reaction as well as the serum levels of anti-HHV-6 IgM and IgG. Serum levels of IgM and IgG were measured using enzyme-linked immunosorbent assay (ELISA) with the calculation of positivity coefficient (PC) according to manufacturer instructions. The test results were interpreted as positive when PC value was greater than 0.8.

Results. The endomyocardial biopsy study detected HHV-6 antigen expression in 15 (60%) out of 25 enrolled patients including 10 cases with diagnosed HHV-6-positive myocarditis and five patients with carriage of viruses. According to IHC, the autoimmune HHV-6 myocarditis was confirmed in three cases (30%). The data of ELISA (n = 18) detected anti-HHV-6 IgM in 5 patients (28%) and anti-HHV-6 IgG in 11 cases (61%). The simultaneous presence of both anti-HHV-6 IgM and IgG was detected in two patients (11%). In addition, anti-HHV-6 IgM and IgG were absent in two (11%) cases. Eight patients (44%) with HHV-6-positive myocarditis included three patients (17%) tested positive for serum anti-HHV-6 IgM, three patients (17%) tested positive for serum anti-HHV-6 IgG, and two patients (11%) who had nether anti-HHV-6 IgM nor anti-HHV-6 IgG in blood serum. Among virus carriers, one patient (20%) was tested positive for anti-HHV-6 IgM and four patients (80%) were tested positive for anti-HHV-6 IgG. The patients without HHV-6 antigen expression (n = 5, 28%) included one patient (5.6%) tested positive for anti-HHV-6 IgM and two patients (11%) tested positive for anti-HHV-6 IgG. The entire sample of patients was divided into two groups depending on the serum level of anti-HHV-6 IgM: group 1 comprised patients tested positive for anti-HHV-6 IgM (n = 5); group 2 comprised patients (n = 13) tested negative for anti-HHV-6 IgM. Clinical and instrumental parameters differed only in the duration of CHF history, which was greater in group 1 than in group 2 (11.0 [8.0; 12.0] vs. 22.5 [14.5; 75.5] months, respectively (p = 0.045). The groups did not significantly differ in the studied markers in myocardial tissue according to the results of IHC analysis. No associations were found between the severity of HHV-6 antigen expression and serum levels of anti-HHV-6 IgM and IgG.

Conclusion. Patients with ADHF and/or adverse LV remodeling after complete myocardial revascularization had higher percentage of HHV-6 antigen expression whose severity was not associated with the serum levels of anti-HHV-6 IgM and IgG.

Keywords:	acute decompensated heart failure, adverse left ventricular remodeling, human herpesvirus type 6, ischemic heart disease, endomyocardial biopsy, anti-HHV-6 IgM and IgG.
Conflict of interest:	the authors do not declare a conflict of interest.
Financial disclosure:	no author has a financial or property interest in any material or method mentioned.

Кручинкина Екатерина Владимировна, e-mail: katy990@mail.ru.

Adherence to ethical standards:	informed consent was obtained from all patients. The study was approved by the Ethics Committee of Cardiology Research Institute of Tomsk NRMC (protocol No. 114 from 16.12.2015).
For citation:	Kruchinkina E.V., Ogurkova O.N., Gusakova A.M., Suslova T.E., Ryabov V.V. Features of the course of acute decompensated ischemic heart failure and/or ongoing adverse left ventricular remodeling in patients with identified human herpes virus type 6. *The Siberian Journal of Clinical and Experimental Medicine.* 2021;36(3):111–118. https://doi.org/10.29001/2073-8552-2021-36-3-111-118.

Особенности течения острой декомпенсации ишемической сердечной недостаточности и/или продолжающегося неблагоприятного ремоделирования левого желудочка у пациентов с выявленным вирусом герпеса человека 6-го типа

Е.В. Кручинкина, О.Н. Огуркова, А.М. Гусакова, Т.Е. Суслова, В.В. Рябов

Научно-исследовательский институт кардиологии, Томский национальный исследовательский медицинский центр Российской академии наук,
634012, Российская Федерация, Томск, ул. Киевская, 111а

Аннотация

Цель: определить сывороточные уровни иммуноглобулинов класса M и G к вирусу герпеса человека 6-го типа (анти-HHV-6 IgM и IgG) и особенности клинического и морфологического портрета больных острой декомпенсацией ишемической хронической сердечной недостаточностью (ОДХСН) и/или неблагоприятным ремоделированием левого желудочка (ЛЖ).

Материал и методы. Выполнено открытое нерандомизированное, одноцентровое, проспективное исследование, зарегистрированное на сайте Clinicaltrials.gov (# NCT02649517). В исследование включены 25 пациентов (84% составляют мужчины, фракция выброса (ФВ) ЛЖ ≤ 40%). Всем пациентам выполнена инвазивная коронарная ангиография и эндомиокардиальная биопсия (ЭМБ). Проведен иммуногистохимический (ИГХ) анализ ткани миокарда на экспрессию антигенов HHV-6, определение комплимента C1q, главного комплекса гистосовместимости класса II (MHC II), B-лимфоцитарного антигена (CD-19). Установлен сывороточный уровень анти-HHV-6 IgM и G с помощью иммуноферментного анализа (ИФА) с расчетом коэффициента позитивности (КП). За положительный результат принимали величину КП ≥ 0,8.

Результаты. По результатам ЭМБ, у 15 (60%) из 25 включенных в исследование пациентов выявлена экспрессия антигенов HHV-6, из них в 10 случаях диагностирован HHV-6 – позитивный миокардит, у 5 больных – вирусоносительство. Вирусно-аутоиммунный характер миокардита, по данным морфологии, подтвержден в 3 (30%) случаях. По данным ИФА (18 больных), анти-HHV-6 IgM выявлены у 5 пациентов (28%), анти-HHV-6 IgG определялись чаще у 11 (61%). Одновременное наличие анти-HHV-6 IgM и анти-HHV-6 IgG было зарегистрировано у 2 (11%) пациентов. Кроме того, в 2 (11%) случаях анти-HHV-6 отсутствовали. У 8 (44%) пациентов с HHV-6 – позитивным миокардитом анти-HHV-6 IgM – в 3 (17%), анти-HHV-6 IgG – в 3 (17%) случаях определялись в сыворотке крови и отсутствовали анти-HHV-6 IgM, IgG – в 2 (11%) случаях. Вместе с тем при вирусоносительстве обнаруживался анти-HHV-6 IgM в 1 (20%) и анти-HHV-6 IgG в 4 (80%) случаев. У 5 (28%) пациентов при отсутствии экспрессии антигенов HHV-6 в 1 (5,6%) случае выявляли анти-HHV-6 IgM, в 2 (11%) случаях – анти-HHV-6 IgG. В зависимости от уровня анти-HHV-6 IgM в сыворотке крови пациенты разделены на 2 группы: 1-я группа – пациенты с положительным анти-HHV-6 IgM (n = 5), 2-я группа - пациенты без положительного анти-HHV-6 IgM (n = 13). Среди клинико-инструментальных показателей различия установлены только в продолжительности анамнеза ХСН, она была большей в 1-й группе 22,5 [14,5; 75,5] против 11,0 [8,0; 12,0] (p = 0,045). Согласно результатам имммуногистохимического исследования, среди изученных маркеров в ткани миокарда значимых различий между группами не выявлено. Не установлена взаимосвязь между выраженностью экспрессии антигенов HHV-6 в ткани миокарда и сывороточными уровнями анти-HHV-6 IgM и IgG.

Выводы. У пациентов с ОДХСН и/или неблагоприятным ремоделированием ЛЖ после полной реваскуляризации миокарда диагностирована высокая частота встречаемости экспрессии антигенов HHV-6 в ткани миокарда, степень выраженности которой не ассоциировалась с сывороточными уровнями анти-HHV-6 IgM и IgG.

Ключевые слова:	острая декомпенсация хронической сердечной недостаточности, неблагоприятное ремоделирование левого желудочка, вирус герпеса человека 6-го типа, ишемическая болезнь сердца, эндомиокардиальная биопсия, антитела к HHV-6 класса M и G.
Конфликт интересов:	авторы заявляют об отсутствии конфликта интересов.
Прозрачность финансовой деятельности:	никто из авторов не имеет финансовой заинтересованности в представленных материалах или методах.
Соответствие принципам этики:	информированное согласие получено от каждого пациента. Исследование одобрено этическим комитетом НИИ кардиологии Томского НИМЦ (протокол № 114 от 16.12.2015 г.).
Для цитирования:	Кручинкина Е.В., Огуркова О.Н., Гусакова А.М, Суслова Т.Е., Рябов В.В. Особенности течения острой декомпенсации ишемической сердечной недостаточности и/или продолжающегося неблагоприятного ремоделирования левого желудочка у пациентов с выявленным вирусом герпеса человека 6-го типа. *Сибирский журнал клинической и экспериментальной медицины.* 2021;36(3):111–118. https://doi.org/10.29001/2073-8552-2021-36-3-111-118.

Introduction

Heart failure (HF) is a long-term chronic condition that progressively worsens with time and leads to high hospitalization rate and over 50% mortality within five years. The prevalence of HF is increasing over the years reaching up to 15 million in Europe and over six million in the USA [1]. Acute decompensated heart failure (ADHF) accounts for more than one million hospitalizations in the USA [2]. Previous myocardial infarction can trigger left ventricular (LV) remodeling, which is the major contributor to the development of HF in most of patients. The rate of ADHF reaches 50% [3] despite the advances in coronary revascularization and optimal medical therapy [4]. Moreover, in case of the most common ischemic HF, there is a loss of LV function as a result of ischemic damage to the myocardial tissue and LV remodeling [5]. Chronic myocardial ischemia causes a vicious circle, activating compensatory neurohormonal and inflammatory mechanisms [6, 7], leading to the progression of HF, and resulting in ischemic cardiomyopathy. The use of optimal pharmacological and surgical treatment improved the survival rate of patients with HF during the period from 1979 to 2000 [8]. However, in recent years, numerous attempts aiming to improve the outcomes in patients with HF decompensation using therapeutic approaches were unsuccessful [9] suggesting the lack of complete understanding of HF pathophysiology.

Therefore, studying the inflammatory response as a fundamental link in the pathogenesis of HF and as the main component of HF decompensation is currently of a particular interest. The elimination of myocardial ischemia is not associated with a decrease in the incidence of HF whereas the proportion of patients with adverse cardiac remodeling remains high [7, 8]. Over time, there was a transition of views in regard to chronic and physiological inflammation occurring in response to any tissue damage (myocardial ischemia, hypertension, etc.), which is necessary for recovery. In the presence of ongoing inflammation, the inflammatory response can become pathologic, potentially leading to the development and progression of HF [1, 10]. Any damage to the myocardium triggers sterile inflammation, whereas the infectious pathogens contribute to the development of non-sterile inflammation in the myocardium [10]. There are viral infections that cause latent diseases and can also manifest as viral carriage [1]. Most of these infections are caused by the viruses with an inherent high prevalence in the population [11]. Evidence suggests that the human herpesvirus type 6 (HHV-6) is the most prevalent cardiac herpesvirus detected by endomyocardial biopsy (EMB) in patients with HF [12] and it is also lymphotropic virus [13]. The HHV-6 has been well studied in cases of myocarditis [14]. There is also evidence on the association between HHV-6 and HF progression. However, there is a lack of knowledge regarding the role of acute and chronic HHV-6 infection in the clinical course of HF [14, 15]. In this article, we wanted to test the hypothesis that chronic HHV-6 infection is the cornerstone of acute decompensated heart failure (ADHF) of ischemic genesis and/or adverse LV remodeling. The HHV-6 antigen expression in the myocardium and the presence of immunoglobulin M and G antibodies to human herpes virus type 6 (anti-HHV-6 IgM and IgG) in blood serum were taken as markers of herpes infection activity.

The aim of this study was to determine the serum levels of anti-HHV-6 IgM and IgG and the features of clinical and morphological portrait in patients with ADHF and/or adverse LV remodeling.

Study design

Patients

This open-label, nonrandomized, single-center, prospective trial was registered at clinicaltrials.gov (#NCT02649517). A total of 25 patients (84% men, LV ejection fraction (EF) of 29.17 ± 9.4%) with ADHF and/or adverse LV remodeling were included.

Inclusion criteria: ADHF; LVEF ≤ 40%; medical history of ischemic heart disease (IHD); not earlier than six months after optimal surgery (PCI or/and CABG) and optimal drug treatment for HF according to ESC guidelines. Exclusion criteria: hemodynamically significant valvular heart disease; acute coronary syndrome; thrombosis of the right atrium and right ventricle; condition after the operation impeding access to the right ventricle (vena cava filters, vena cava plication, and mechanical prosthetic tricuspid valve); and severe comorbidities.

All patients were divided into 2 groups depending on the presence of the ratio of anti-HHV-6 IgM in blood serum: group 1 comprised patients tested positive for anti-HHV-6 IgM ($n = 5$) and group 2 comprised patients ($n = 13$) tested negative for anti-HHV-6 IgM. IgM and IgG test results were interpreted as positive when positivity ratio was greater than 0.8.

Clinical, instrumental, and laboratory parameters are presented in Table 1. Patients did not have any clinical manifestations of herpes infection.

Table 1. Baseline characteristics of patients included in the study

Parameter	All patients (*n* = 25)	All patients with ELISA* (*n* = 18)	Group 1 (*n* = 5)	Group 2 (*n* = 13)	*p*-value
Men, *n* (%)	21 (82)	14 (78)	5 (100)	9 (69)	0.160
Age, years	61.0 [56.0; 67.0]	59.0 [56.0; 65.0]	56.0 [46.0; 57.0]	61.0 [57.5; 66.0]	0.095
BMI, kg/m^2	29.7 [25.7; 31.9]	30.4 [28.0; 31.9]	30.3 [28.0; 31.0]	30.6 [28.1; 34.7]	0.502
Duration of HF, months	17.0 [8.0; 48.0]	20.0 [11.0; 48.0]	11.0 [8.0; 12.0]	22.5 [14.5; 75.5]	0.045
Duration of IHD to the development of HF, months	72.0 [13.0; 114.0]	60.0 [12.0; 98.0]	14.0 [5.0; 72.0]	70.0 [12.5; 117.0]	0.143
NYHA FC 4	8 (32)	5 (28)	2 (40)	3 (23)	–
NYHA FC 3 by	15 (60)	13 (72)	3 (60)	10 (77)	–
Type 2 diabetes mellitus	12 (48)	10 (56)	3 (60)	7 (54)	–
Ventricular tachycardia	13 (52)	10 (56)	2 (40)	8 (62)	–
Atrial fibrillation/atrial flutter	44 (11)/28 (7)	10 (56)/5 (28)	3 (40)/1 (20)	7 (54)/4 (31)	–
2nd-3rd degree atrioventricular block	1 (4)	1 (6)	–	1 (8)	–
Left bundle branch block	8 (32)	6 (33)	2 (40)	4 (31)	–
Right bundle branch block	6 (24)	5 (28)	2 (40)	3 (23)	–
QRS > 120 ms	10 (40)	9 (50)	3 (60)	6 (46)	–
Prior PCI	19 (76)	15 (83)	5 (100)	10 (77)	0.242
Prior CABG	15 (48)	10 (56)	3 (60)	7 (54)	–
Prior PCI and CABG	12 (48)	7 (39)	2 (40)	5 (39)	–
ICD (before/during the study)	6 (24)/4 (16)	4 (22)/1 (6)	2 (40)	4 (31)	–
CRT (before/during the study)	1 (4)/–	–/–	–/–	–/–	–
CRT–D (before / during the study)	2 (8)/3 (12)	–/1 (6)	–/–	2 (15)/2 (15)	–
Symptoms of ADHF					
Cyanosis	11 (44)	6 (33)	1 (20)	4 (31)	–
Shortness of breath: physical activity/ at rest	13 (52)/8 (36)	11 (61)/5 (28)	3 (60)/1 (20)	3 (23)/2 (15)	–
Wheezing	11 (44)	8 (44)	2 (40)	6 (46)	–
Edema/welts	7 (28)/4 (16)		2 (40)/1(20)	4 (31)/1 (8)	–
Increase in body weight in the last week	6 (24)	6 (33)	2 (40)	4 (31)	–
Jugular venous distention	8 (32)	4 (22)	–	4 (31)	–
Abnormal heart rhythm	12 (48)	10 (56)	4 (80)	7 (54)	–
Liver (up to 5 cm/more than 5 cm)	14 (56)/2 (8)	10 (56)/–	3 (60)/-	8 (62)/–	–
Classification of ADHF					
Warm and dry	14 (56)	11 (61)	3 (60)	8 (54)	–
Warm and wet	1 (4)	1 (6)	–	1 (8)	–
Cold and dry	2 (8)	1 (6)	1 (20)	1 (8)	–
Cold and wet	5 (20)	5 (28)	1 (20)	3 (23)	–
Vital signs at admission:					
Systolic blood pressure, mm Hg	128.0 [104.0;138.0]	130.0 [100.0;130.0]	120.0 [102.0;130.0]	130 [100.0;134.0]	0.924
Diastolic blood pressure, mm Hg	80.0 [70.0; 80.0]	70.0 [60.0; 80.0]	70.0 [70.0; 80.0]	75.0 [60.0; 80.0]	0.703
Heart rate, beats/min	72.0 [65.0; 87.0]	68.0 [64.0; 82.0]	64.0 [64.0; 72.0]	70.0 [68.0: 84.0]	0.289
Respiratory rate, inhalation/min	19.0 [17.0; 20.0]	18.0 [17.0; 20.0]	18.0 [18.0; 20.0]	18.5 [16.5; 20.0]	0.920
Medications at discharge					
Diuretics (parenteral/oral)	15 (60)/17 (68)	14 (78) /12 (67)	2 (40) / 4 (80)	12 (92) / 8 (62)	–
Inotropic drugs	2 (8)	–	–	–	–
ACE/ARA	16 (64)	13	3 (60)	10 (77)	–
Beta blockers	16 (64)	12	3 (60)	9 (69)	–
MCRA	17 (68)	14	4 (80)	10 (77)	–

Note: data are presented as the median, interquartile range [Q1; Q3] for continuous non-normally distributed variables or by frequency *n* (%). * – group of patients with ELISA performed; IHD – ischemic heart disease; HF – heart failure, BMI – body mass index, PCI – percutaneous coronary intervention, CABG – coronary artery bypass grafting, ICD – implanted cardioverter-defibrillator, CRT – resynchronization therapy, CRT-D – resynchronization therapy with defibrillator function, ACE – inhibitors of angiotensin-converting enzyme, ARA – angiotensin II receptor antagonists, MCRA – mineralocorticoid receptor antagonists.

Echocardiography was performed at least one day before the EMB. The values of LVEF, end-diastolic volume index (EDVI), and end-systolic volume index (ESVI) were determined in 2- and 4-chamber views according to the recommendations of European Association of Echocardiography [16]. Invasive coronary angiography was performed in all patients to rule out the progression of coronary atherosclerosis. All patients underwent EMB with immunohistochemistry (IHC) analysis for the presence of HHV-6. Patients with diagnosed HHV-6 by EMB received antiviral therapy.

Seven patients who did not have blood samples for ELISA due to any reasons were excluded from the analysis.

Endomyocardial biopsy by immunohistochemical examination

EMB was performed by femoral access. Three biopsy samples were taken from the interventricular septum, apical region, and right ventricular outflow tract. IHC analysis was performed using mouse monoclonal antibodies to monocytes/macrophages (CD68 +) and T-lymphocytes (CD3+, CD45+) as well as monoclonal antibodies against HHV-6. We used the compliment C1q, MHC II, and CD19 as the markers of autoimmune reaction; quantitative parameters of cells in the myocardial tissue were not determined at that time [13, 17]. IHC criteria of myocarditis were at least 14 leukocytes per sq. mm in the myocardium including up to four monocytes and seven or more CD3+ T lymphocytes per square millimeter [17]. The activity of the inflammatory process in the myocardium and the semi-quantitative degree of fibrosis severity were evaluated using a 5-point system [18].

ELISA for determination of serum HHV-6-specific IgM and IgG antibodies

Serum levels of anti-HHV-6 IgM and IgG were assessed at admission. Qualitative/semi-quantitative analysis of anti-HHV-6 IgM and IgG levels in blood serum was performed by ELISA using the test systems “Vector-Best” and “VIDITEST” with the calculation of positivity coefficient (PC) according to manufacturer instructions. Results were interpreted as positive, negative, or equivocal depending on PC value, namely: the results were considered positive when PC was greater than 1, negative when PC was below 0.8, and equivocal when PC value was between 0.8 and 1. Optical density assessment, calibration graph preparation, and evaluation of quantitative and semi-quantitative parameters were done using the Infinite F50 microplate reader and Magellan Tracker software (Austria).

ELISA for assessment of inflammatory biomarkers in blood serum

Blood samples were obtained by venipuncture between 08.00 and 09.00 a.m., incubated at room temperature and centrifuged at 3000 rev/min for 15 minutes; serum samples were stored at −40 °C with a single freeze-thaw cycle. Serum levels of biomarkers were analyzed from the same blood samples using ELISA before myocardial biopsies.

Serum levels of highly sensitive C-reactive protein (CRP (hs)) were determined by the CRP (hs) test system (Biomedica, Austria). Serum levels of N-terminal fragment of brain natriuretic peptide precursor (NT–proBNP) were assessed using the NT–proBNP kit (Biomedica, Slovakia). The levels of Troponin I were assessed using the test system Troponin I (Human cardiac-specific) (BIOMERICA, Austria). Serum levels of IL-1beta, IL-10, TNF-α, INF-γ, and IL-6 were determined by the kit (Vector–Best, Russia). The measurements were expressed in ng/mL.

Statistical analysis

Statistical data analyzes were performed with STATISTICA 10.0 software. Nonparametric statistical methods were used. Continuous variables are described as median and interquartile range. Statistical differences between groups were detected using the χ^2-test for categorial variables. Univariate comparisons of two independent groups were performed with the Mann – Whitney *U*-test. A value of $p \leq 0.05$ was considered statistically significant.

Results

Among a total of 25 patients with ADHF and/or adverse LV remodeling, we identified patients (n = 15, 63%) tested positive for HHV-6 according to EMB results (Table 2). HHV-6-positive myocarditis was confirmed in 10 (40%) cases and viral carriage was found in five (20%) cases by EMB. The autoimmunity in HHV-6 myocarditis was confirmed in three (30%) cases according to IHC. The data of ELISA (n = 18) showed the presence of anti-HHV-6 IgM in five patients (28%) and anti-HHV-6 IgG in 11 cases (61%). The simultaneous presence of anti-HHV-6 IgM and anti-HHV-6 IgG was detected in two (11%) patients. In addition, anti-HHV-6 IgM and IgG were absent in two (11%) cases (Table 2).

Table 2. The presence of HHV-6 antigen expression by EMB and serum anti-HHV-6 IgM and G in patients with ADHF and/or adverse LV remodeling on admission

Patient's number	Myocarditis	HHV-6 antigen expression in EMB	Anti-HHV-6 IgM in serum	Anti-HHV-6 IgG in serum
1	+	++	+	–
2	–	+	–	+
3	+	+	+	+
4	–	+++	–	+
5	–	+	–	+
6	–	+	–	+
7	–	–	–	+
8	+	+++	–	+
9	+	+++	+	–
10	–	+	+	–
11	–	–	+	+
12	+	+	–	–
13	+	+++	–	–
14	–	–	–	+
15	–	–	–	–
16	+	+	–	+
17	+	++	–	+
18	–	–	–	–

Note: + – low HHV-6 antigen expression, ++ – moderate HHV-6 antigen expression, +++ – high HHV-6 antigen expression.

Among eight (44%) patients with HHV-6-positive myocarditis, the anti-HHV-6 IgM was detected in three (17%) cases; the anti-HHV-6 IgG was found in three (17%) cases; and blood serum anti-HHV-6 IgM and IgG were absent in two (11%) cases. At the same time, among virus carriers, anti-HHV-6 IgM was detected in one (20%) case and anti-HHV-6 IgG was found in four (80%) cases. In five (28%) patients without HHV-6 antigens expression, anti-HHV-6 IgM was found in one (5.6%) case and anti-HHV-6 IgG was detected in two (11%) cases (Table 2).

In all cases, anti-HHV-6 IgM value of 0.82 [0.78; 0.83] corresponded to a dubious result.

The groups had the clinical course of ADHF and/or adverse LV remodeling as follows: duration of HF was higher in group 1 (p = 0.045) than in group 2 (11.0 [8.0; 12.0] vs. 22.5 [14.5; 75.5] months, respectively). Furthermore, the period from the diagnosis of IHD to the development of HF was shorter by 80% in group 1 (p = 0.143) compared with group 2 (14.0 [5.0; 72.0] vs. 70.0 [12.5; 117.0] months, respectively). Patients of group 1 had higher rates of heart rhythm disturbances and absent jugular venous

distention. Patients of group 1 did not require inotropic drugs at admission.

In group 1, serum content of anti-HHV-6 IgM was higher by 52% (p = 0.0002) compared with the corresponding value in group 2 (0.82 [0.78; 0.83] vs. 0.39 [0.22; 0.55], respectively). The anti-HHV-6 IgG level was lower by 70% in group 1 (p = 0.849) compared with the corresponding value in group 2 (0.69 [0.59; 6.94] vs. 2.35 [1.03; 4.15], respectively).

The presence of HHV-6 antigen expression in group 1 was confirmed by EMB in four (22%) cases. In these cases, 25% (n = 1) of patients had high expression of HHV-6 antigen, 25% (n = 1) of patients had moderate expression of HHV-6 antigen; and 50% (n = 2) of patients had low expression level of HHV-6 antigen. In group 2, the presence of HHV-6 antigen expression was confirmed by EMB in nine (69%) cases: 23% (n = 3) of patients had high level of HHV-6 antigen expression; 15% (n = 2) of patients had moderate level of HHV-6 antigen expression; and 31% (n = 4) of patients had low level of HHV-6 antigen expression. No relationships were found between HHV-6 expression in the myocardium and serum levels of the antibodies. Positive tests for anti-HHV-6 IgG were more often detected in patients with the low expression level of herpes antigens according to EMB.

No differences in echocardiographic parameters and serum biomarkers were found between the groups (Table 3).

Table 3. Serum biomarkers and echocardiography parameters in patients with ADHF and/or adverse LV remodeling on admission

Parameter	Group 1 (n = 5)	Group 2 (n = 13)	p-value
NT-proBNP, ng/mL	398.41 [386.21; 2424.70]	297.90 [81.62; 665.20]	0.208
Troponin I, ng/mL	0.18 [0.01; 0.19]	0.22 [0.16; 0.53]	0.503
CRP (hs), mg/L	4.03 [0.66; 10.19]	7.80 [1.40; 9.50]	0.775
IL-1β, ng/mL	1.16 [1.15; 1.34]	1.15 [1.04; 1.65]	0.633
IL-10, ng/mL	4.63 [4.51; 4.73]	1.16 [3.74; 4.85]	0.503
IL-6, ng/mL	3.34 [1.51; 10.51]	4.09 [3.15; 4.86]	0.849
TNF-α, ng/mL	2.76 [1.64; 3.5]	3.07 [2.37; 3.32]	0.775
INF-γ, ng/mL	5.50 [5.28; 6.11]	4.70 [3.85; 6.57]	0.633
LVEF, %	22.5 [19.2; 28.8]	26.6 [18,6; 29,5]	0.924
EDVI, mL/m^2	99.7 [99.2; 118.5]	96.3 [69.7; 108.5]	0.566
ESVI, mL/m^2	71.0 [70.3; 80.2]	76.2 [40.2; 91.2]	0.775

Note: Data are presented as the median, interquartile range [*Q1;Q3*] for continuous non-normally distributed variables. TNF-α – tumor necrosis factor alpha, INF-γ – interferon gamma, IL – interleukin, C-RP (hs) – C-reactive protein (highly specific), NT-proBNP – N-terminal prohormone of brain natriuretic peptide, LVEF – left ventricular ejection fraction, EDVI – left ventricular end-diastolic volume index, ESVI – left ventricular end-systolic volume index.

There were no significant differences between the groups, according to the results of IHC and EMB (Table 4). Increased numbers of CD68+, CD3+, and MHC II cells were observed in all patients. Group 1 had higher CD3+ cell count and higher complement C1q expression than the corresponding parameters in group 2. In group 1, areas of fibrosis and sclerosis in the myocardium and cases of HHV-6-positive myocarditis were observed more often. CD 19+ cells were not detected in either group. The activity of inflammation in myocardial tissue corresponded to G2; S3 in both groups (Table 4).

Table 4. Data of endomyocardial biopsy and immunohistochemical analysis in patients with ADHF and/or adverse LV remodeling on admission

Parameter	Total group (n = 25)	Group 1 (n = 5)	Group 2 (n = 13)	p-value
CD68+	15.0 [9.0; 16.0]	10.0 [8.0; 16.0]	15.0 14.0; 16.0]	0.289
CD3+	8.0 [3.0; 13.0]	8.0 [1.0; 17.0]	6.0 [1.0; 17.0]	1.000
CD45+	12.0 [8.0; 20.0]	12.0 [8.0; 20.0]	12.0 [8.0; 18.0]	0.849
HHV-6 - positive myocarditis	8 (32)	3 (60)	5 (39)	0.410
Virus carrier	5 (20)	1 (20)	4 (31)	0.648
CD 19	–	–	–	–
C1q	2.0 [1.0; 5.0]	4.0 [2.0; 4.5]	1.5 [1.0; 3.0]	0.142
MHC II	4.0 [2.0; 16.0]	5.5 [4.5; 6.0]	4.0 [1.0; 5.0]	0.188
Myocardial necrosis	12 (48)	4 (80)	7 (54)	0.414
Myocardial fibrosis (interstitial/ endocardial)	20 (80)/16 (64)	5 (100)/ 5 (100)	12 (92)/ 10 (77)	0.257 0.137
Myocardial sclerosis	5 (20)	3 (60)	2 (15)	0.649
The activity of inflammation in myocardial tissue according to the classification of Basso, C. and et. al. [25]:				
Grading (burden of myocyte damage and inflammation) (G)	2.0 [1.0; 3.0]	2.0 [1.0; 2.0]	2.5 [1.0; 3.0]	0.600
Staging (fibrosis) (S)	3.0 [3.0; 4.0]	3.0 [3.0; 3.0]	3.0 [3.0; 4.0]	0.299

Note: Data are presented as the median, interquartile range [*Q1;Q3*] for continuous non-normally distributed variables, or by frequency n (%), C1q – compliment component, MHC II – class II major histocompatibility complex, CD 19 – B-lymphocyte antigen, CD45 + – T-lymphocytes; CD3 + – T-killers; CD68 + – monocytes/macrophages.

Discussion

In the present study, we show a high detection rate of HHV-6-positive myocarditis (42%) and HHV-6 carriage (21%) according to EMB study in patients with ADHF and/or adverse LV remodeling after complete myocardial revascularization. The obtained data on the prevalence of HHV-6 in the myocardial tissue correspond to the world literature. Over the past 30 years, there has been extensive discussion of whether viruses are involved in HF [18–20]. It has been shown that HHV-6 is more frequently detected in EMB samples from patients with myocarditis [21]. However, the question on the effect of chronic HHV-6 infection in ADHF and/or adverse LV remodeling remains controversial. Numerous works were devoted to studying the role of herpes type 6 in pediatrics and neurology [22] due to the vivid clinical picture [23]. This became another reason for studying the HHV-6 prevalence and the clinical, instrumental, and laboratory features in HHV-6-positive patients with ADHF and/or adverse LV remodeling.

According to the results of ELISA, anti-HHV-6 IgM in blood serum was detected in 28% (n = 5) of patients and serum anti-HHV-6 IgG was found in 61% (n = 11) of patients. However, we did not find any correlation between HHV-6 antigen expression in the myocardial tissue and serum levels of anti-HHV-6 IgM and IgG. Our findings agree with the study by F. Mahfou

et al. who were the first to show that detection of acute viral infections by serological antibody tests does not correlate with the detection of viral genome in EMB in patients with suspected myocarditis [24]. The low detectability of serum anti-HHV-6 IgM and the absence of relationships between HF decompensation and previous infection indicate the latency and chronic course of HHV-6 infection. Therefore, HHV-6 seropositivity is not always associated with active inflammation in the myocardial tissue whereas the negative tests for anti-HHV-6 IgM do not rule out HHV-6 antigen expression and the presence of inflammation in the myocardial tissue.

The clinical course of ADHF and adverse LV remodeling were studied depending on the presence of anti-HHV-6 IgM in blood serum, but we did not find any differences between the groups. However, the following characteristics were found in patients with high serum level of anti-HHV-6 IgM: these patients had rapid HF development, predominance of heart rhythm disturbances over other symptoms of decompensated HF, and no need for inotropic support.

It is known that HHV-6 is a T-lymphotropic virus and the presence of activated T cells is observed as a sign of cardiac damage in virus-induced myocarditis as shown by animal models [18]. Our IHC data showed high numbers of CD3+ lymphocytes and CD68+ macrophages, signs of autoimmune process (high complement C1q and MHC II expression), and foci of myocardial fibrosis and sclerosis according to the classification (C. Basso et. al), which all confirmed the presence of chronic myocardial inflammation.

It is important to note, that the cytokine levels did not go beyond the reference values. This may suggest the manifestation of a minimal latent inflammatory response or immune incompetence.

F. Eschera et al. showed that persistence of cardiac HHV-6B genomes is significantly associated with cardiac dysfunction whereas hemodynamic parameters improve in association with HHV-6B clearance. Our data differ from reports of other authors as we did not find any differences in echocardiographic parameters depending on the presence of anti-HHV-6 IgM in blood serum and HHV-6 antigen expression in the myocardial tissue.

Conclusion

Patients with ADHF and/or adverse LV remodeling after complete myocardial revascularization had a higher percentage of HHV-6 antigen expression in the myocardium and its severity was not associated with the serum levels of anti-HHV-6 IgM and IgG.

Limitations

This study is a single-center study with the enrollment of a small number of patients. Further studies are needed to prove the role of HHV-6 in HF onset. PCR tests were not performed in this study. The further trials are needed to determine the serologic types of HHV-6 and to elucidate its role in ischemic HF decompensation.

Литература / References

1. Virani S.S., Alonso A., Benjamin E.J., Bittencourt M.S., Callaway C.W., Carson A.P. et al. Heart Disease and Stroke Statistics – 2020 Update: A Report From the American Heart Association. *Circulation.* 2020;141(9):e139–e596. DOI: 10.1161/CIR.0000000000000757.
2. Degoricija V., Trbušić M., Potočnjak I., Radulović B., Terešak D.S., Pregartner G. et al. Acute Heart Failure developed as worsening of Chronic Heart Failure is associated with increased mortality compared to *de novo* cases. *Sci. Rep.* 2019;9(1):8403. DOI: 10.1038/s41598-019-44069-7.
3. Farmakis D., Parissis J., Lekakis J., Philippathos G. Acute Heart Failure: Epidemiology, risk factors, and prevention. *Rev. Esp. Cardiol. (Engl. Ed.).* 2015;68(3):245–248. DOI: 10.1016 / j.rec.2014.11.004.
4. Rich J.D., Burns J., Freed B.H., Maurer M.S., Burkhoff D., Shah S.J. et al. Meta-analysis Global Group in Chronic Heart Failure (MAGGIC): Validation of a Simple Tool for the Prediction of Morbidity and Mortality in Heart Failure With Preserved Ejection Fraction. *J. Am. Heart Assoc.* 2018;7(20):e009594. DOI: 10.1161/JAHA.118.009594.
5. Pryds K., Schmidt M.R., Bjerre M., Thiel S., Refsgaard J., Bøtker H.E. et al. Effect of long-term remote ischemic conditioning on inflammation and cardiac remodeling. *Scand. Cardiovasc J.* 2019;53(4):183–191. DOI: 10.1080/14017431.2019.1622770.
6. Bacmeister L., Schwarzl M., Warnke S., Stoffers B., Blankenberg S., Westermann D. et al. Inflammation and fibrosis in murine models of heart failure. *Basic Res. Cardiol*. 2019;114(3):19. DOI: 10.1007/s00395-019-0722-5.
7. Kercheva M., Gusakova A.M., Ryabova T.R., Suslova T.E., Kzhyshkowska J., Ryabov V.V. Serum levels of bone morphogenetic proteins 2 and 4 in patients with acute myocardial infarction. *Cells.* 2020;9(10):2179. DOI: 10.3390/cells9102179.
8. Mozaffarian D., Benjamin E.J., Go A.S., Arnett D.K., Blaha M.J., Cushman M. et al. Heart Disease and Stroke Statistics – 2016 Update. A Report from the American Heart Association. *Circulation.* 2016;133(4):e38–e360. DOI: 10.1161/CIR.0000000000000350.
9. Bertero E., Störk S., Maack C. REPORT-HF reveals global inequalities in health care provision and prognosis of patients with acute heart failure. *Cardiovasc. Res.* 2020;116(10):e112–e114. DOI: 10.1093/cvr/cvaa194.
10. Zhang Y., Bauersachs J., Langer H.F. Immune mechanisms in heart failure. *Eur. J. Heart Fail.* 2017;19(11):1379–1389. DOI: 10.1002/ejhf.942.
11. Caforio A.L., Pankuweit S., Arbustini E., Basso C., Gimeno-Blanes J., Felix S.B. et al. Current state of knowledge on aetiology, diagnosis, management, and therapy of myocarditis: A position statement of the European Society of Cardiology Working Groupon Myocardial and Pericardial Diseases. *Eur. Heart J.* 2013;34(33):2636–2648, 2648a–2648d. DOI: 10.1093/eurheartj/eht210.
12. Maisch B., Alter P. Treatment options in myocarditis and inflammatory cardiomyopathy. *Herz*. 2018;43(5):423–430. DOI: 10.1007/s00059-018-4719-x.
13. Tschöpe C., Ammirati E., Bozkurt B., Caforio A.L., Cooper L.T., Felix S.B. et al. Myocarditis and inflammatory cardiomyopathy: Current evidence and future directions. *Nat. Rev. Cardiol.* 2021;18(3):169–193. DOI: 10.1038/s41569-020-00435-x.
14. Agut H., Bonnafous P., Gautheret-Dejean A. Laboratory and clinical aspects of human herpesvirus 6 infections. *Clin. Microbiol. Rev.* 2015;28(2):313–335. DOI: 10.1128/CMR.00122-14.
15. De Bolle L., Naesens L., De Clercq E. Update on human herpesvirus 6 biology, clinical features, and therapy. *Clin. Microbiol. Rev*. 2005;18(1):217–245. DOI: 10.1128/CMR.18.1.217-245.2005.
16. Lang R.M., Badano L.P., Mor-Avi V., Afilalo J., Armstrong A., Ernande L. et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J. Am. Soc. Echocardiogr.* 2015;28(1):1–39.e14. DOI: 10.1016/j.echo.2014.10.003.
17. Caforio A.L., Marcolongo R., Jahns R., Fu M., Felix S.B., Iliceto S. Immune-mediated and autoimmune myocarditis: Clinical presentation, diagnosis and management. *Heart Fail. Rev*. 2013;18(6):715–732. DOI: 10.1007/s10741-012-9364-5.
18. Basso C., Calabrese F., Angelini A., Carturan E., Thiene G. Classification and histological, immunohistochemical, and molecular diagnosis of inflammatory myocardial disease. *Heart Fail. Rev*. 2013;18(6):673–681. DOI: 10.1007/s10741-012-9355-6.
19. Maisch B., Pankuweit S. Inflammatory dilated cardiomyopathy: Etiology and clinical management. *Herz.* 2020;45(3):221–229. DOI: 10.1007/s00059-020-04900-8.
20. Escher F., Kühl U., Gross U., Westermann D., Poller W., Tschöpe C. et al. Aggravation of left ventricular dysfunction in patients with biopsy-proven cardiac human herpesvirus A and B infection. *J. Clin. Virol*. 2015;63:1–5. DOI: 10.1016/j.jcv.2014.11.026.
21. Шарипова Е.В., Бабаченко И.В., Левина А.С. Поражение сердечно-сосудистой системы при вирусных инфекциях. *Журнал инфектологии.* 2017;9(4):14–23. DOI: 10.22625/2072-6732-2017-9-4-14-23.

Sharipova E.V., Babachenko I.V., Levina A.S. Defeat of the cardiovascular system in viral infections. *Journal Infectology*. 2017;9(4):14–23 (In Russ.). DOI: 10.22625/2072-6732-2017-9-4-14-23.

22. Никитская Е.А., Марюхнич Е.В., Саввинова П.П., Пинегина Н.В., Шпектор А.В., Васильева Е.Ю. и др. Вирусы герпеса человека и атеросклероз. Современный взгляд. *Креативная кардиология*. 2015;9(2):54–62. DOI: 10.15275/kreatkard.2015.02.05.
Nikitskaya E.A., Maryukhnich E.V., Savvinova P.P., Pinegina N.V., Shpektor A.V., Vasilieva E.Yu. et al. Human herpesviruses and atherosclerosis. Modern point of view. *Creative Cardiology*. 2015;9(2):54–62 (In Russ.). DOI: 10.15275/kreatkard.2015.02.05.
23. King O., Al Khalili Y. Herpes virus type 6. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021.
24. Mahfou F., Gartner B., Kindermann M., Ukena C., Gadomski K., Klingel K. et al. Virus serology in patients with suspected myocarditis: Utility or futility? *Eur. Heart J.* 2011;32(7):897–903. DOI: 10.1093/eurheartj/ehq493.

Information on author contributions

Kruchinkina E.V. – management of patients, data obtaining and interpretation, statistical processing of data, and writing the manuscript.

Ogurkova O.N. – enzyme immunoassay tests, writing the section of materials and methods, and database provisioning.

Gusakova A.M. – enzyme-linked immunosorbent assay tests, writing the section of materials and methods, and revision of essential intellectual content.

Suslova T.E. – revision of essential intellectual content and final approval of the manuscript for publication.

Ryabov V.V. – development of concept and design of the study, coordination of study implementation, and final approval of the manuscript for publication.

Информация о вкладе авторов

Кручинкина Е.В. – ведение пациентов, получение и интерпретация данных, статистическая обработка, написание текста статьи.

Огуркова О.Н. – проведение иммуноферментного анализа сыворотки крови, написание раздела материалов и методов, составление базы данных.

Гусакова А.М. – проведение иммуноферментного анализа сыворотки крови, написание раздела материалов и методов, проверка критически важного интеллектуального содержания.

Суслова Т.Е. – проверка критически важного интеллектуального содержания, окончательное утверждение содержания для публикации рукописи.

Рябов В.В. – разработка концепции и дизайна исследования, координация выполнения исследования, окончательное утверждение содержания для публикации рукописи.

Information about the authors

Ekaterina V. Kruchinkina, Junior Research Scientist, Cardiologist, Department of Emergency Cardiology, Cardiology Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences. ORCID 0000-0002-9409-9085.
E-mail: katy990@mail.ru.

Oksana N. Ogurkova, Cand. Sci. (Med.), Research Scientist, Clinical Diagnostic Laboratory, Cardiology Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences. ORCID 0000-0001-8397-0296.
E-mail: oon@cardio-tomsk.ru.

Anna M. Gusakova, Cand. Sci. (Pharm.), Research Scientist, Clinical Diagnostic Laboratory, Cardiology Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences. ORCID 0000-0002-3147-3025.
E-mail: mag_a@mail.ru.

Tatiana E. Suslova, Cand. Sci. (Med.), Head of Clinical Diagnostic Laboratory, Cardiology Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences. ORCID 0000-0001-9645-6720.
E-mail: tes@cardio-tomsk.ru.

Vyacheslav V. Ryabov, Dr. Sci. (Med.), Leading Research Scientist, Head of Department of Emergency Cardiology, Cardiology Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences. ORCID 0000-0002-4358-7329.
E-mail: rvvt@cardio-tomsk.ru.

Ekaterina V. Kruchinkina, e-mail: katy990@mail.ru.

Сведения об авторах

Кручинкина Екатерина Владимировна, младший научный сотрудник, отделение неотложной кардиологии, Научно-исследовательский институт кардиологии, Томский национальный исследовательский медицинский центр Российской академии наук. ORCID 0000-0002-9409-9085.
E-mail: katy990@mail.ru.

Огуркова Оксана Николаевна, канд. мед. наук, научный сотрудник, отделение клинической лабораторной диагностики, Научно-исследовательский институт кардиологии, Томский национальный исследовательский медицинский центр Российской академии наук. ORCID: 0000-0001-8397-0296.
E-mail: oon@cardio-tomsk.ru.

Гусакова Анна Михайловна, канд. фарм. наук, научный сотрудник, отделение клинической лабораторной диагностики, Научно-исследовательский институт кардиологии, Томский национальный исследовательский медицинский центр Российской академии наук. ORCID 0000-0002-3147-3025.
E-mail: mag_a@mail.ru.

Суслова Татьяна Евгеньевна, канд. мед. наук, заведующий отделением клинической лабораторной диагностики, Научно-исследовательский институт кардиологии, Томский национальный исследовательский медицинский центр Российской академии наук. ORCID 0000-0001-9645-6720.
E-mail: tes@cardio-tomsk.ru.

Рябов Вячеслав Валерьевич, д-р мед. наук, заместитель директора по научной и лечебной работе, заведующий отделением неотложной кардиологии, Научно-исследовательский институт кардиологии, Томский национальный исследовательский медицинский центр Российской академии наук. ORCID 0000-0002-4358-7329.
E-mail: rvvt@cardio-tomsk.ru.

Кручинкина Екатерина Владимировна, e-mail: katy990@mail.ru.