Circulating virome and inflammatory proteome in patients with ST-elevation myocardial infarction and primary ventricular fibrillation

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Primary ventricular fibrillation (PVF) is a life-threatening complication of ST-segment elevation myocardial infarction (STEMI). It is unclear what roles viral infection and/or systemic inflammation may play as underlying triggers of PVF, as a second hit in the context of acute ischaemia. Here we aimed to evaluate whether the circulating virome and inflammatory proteome were associated with PVF development in patients with STEMI. Blood samples were obtained from non-PVF and PVF STEMI patients at the time of primary PCI, and from non-STEMI healthy controls. The virome profile was analysed using VirCapSeq-VERT (Virome Capture Sequencing Platform for Vertebrate Viruses), a sequencing platform targeting viral taxa of 342,438 representative sequences, spanning all virus sequence records. The inflammatory proteome was explored with the Olink inflammation panel, using the Proximity Extension Assay technology. After analysing all viral taxa known to infect vertebrates, including humans, we found that non-PVF and PVF patients only significantly differed in the frequencies of viruses in the *Gamma-herpesvirinae* and *Anelloviridae* families. In particular, most showed a significantly higher relative frequency in non-PVF STEMI controls. Analysis of systemic inflammation revealed no significant differences between the inflammatory profiles of non-PVF and PVF STEMI patients. Inflammatory proteins associated with cell adhesion, chemotaxis, cellular response to cytokine stimulus, and cell activation proteins involved in immune response (IL6, IL8, CXCL-11, CCL-11, MCP3, MCP4, and ENRAGE) were significantly higher in STEMI patients than non-STEMI controls. CDCP1 and IL18-R1 were significantly higher in PVF patients compared to healthy subjects, but not compared to non-PVF patients. The circulating virome and systemic inflammation were not associated with increased risk of PVF development in acute STEMI. Accordingly, novel strategies are needed to elucidate putative triggers of PVF in the setting of acute ischaemia, in order to reduce STEMI-driven sudden death burden.

In cases of acute myocardial infarction with ST-segment elevation (STEMI), morbidity and mortality have substantially decreased following the establishment of regional and national reperfusion networks, and the use of newer evidence-based drugs32,33. However, ventricular fibrillation (VF) during the acute phase of myocardial infarction and related complications continue to be the leading causes of death in patients with STEMI. Efforts to identify novel pharmacological and non-pharmacological strategies to prevent VF and improve patient outcomes are ongoing.

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Infarction—also known as primary ventricular fibrillation (PVF)—is still the leading cause of sudden prehospital cardiac death, and is a factor that predicts poor short-term prognosis. In this context, numerous studies have attempted to identify predictors of PVF, without significant progresses. It is likely that susceptibility to VF during acute ischemia might be modulated by several factors, including hemodynamic dysfunction, electrolyte alterations, autonomic dysregulation, genetic factors, and certain environmental influences.

Particularly, an association between viral infections and acute myocardial infarction (AMI) has been proposed. Furthermore other authors have found seasonal variations in sudden cardiac death (SCD), typically with a peak in winter, suggesting that viral exposure is a trigger of VF in patients suffering from acute ischemia. However, only influenza virus and some enteroviruses have been investigated for roles in SCD, with contradictory results. To date, evidence is also scarce and unclear regarding an association between inflammatory biomarkers and SCD in an asymptomatic population. For instance, interleukin 6 (IL-6) is reported as a predictor of sudden death in healthy men. Additionally, growth differentiation factor 15 (GDF-15) has been described as a risk factor for SCD during the acute phase of myocardial infarction, and as a predictor of short-term mortality in patients with PVF. Nevertheless, the association between systemic inflammation at the time of STEMI and PVF remains unknown.

In the present study, we aimed to conduct a pilot study with two objectives: (1) to examine the circulating virome sequence, and (2) to explore the systemic inflammatory proteome in patients with STEMI, with and without PVF.

**Methods**

**Patient population.** The RUTI-STEMI-PVF cohort is a prospective single-centre registry of consecutive STEMI patients treated with primary percutaneous coronary intervention (PCI) and within the Codi IAM reperfusion network. STEMI was defined according to the Third Universal Definition of Myocardial Infarction. Patient management was decided by the physicians, following recommended guidelines. Upon admission, blood samples were obtained by venipuncture and centrifuged, and then heparin-plasma was stored at −80 °C until assay.

Patients were divided into two groups: those who had suffered PVF, and those who had not (non-PVF). PVF was defined as ventricular fibrillation occurring ≤ 24 h after diagnosis of myocardial infarction, and not preceded by heart failure or shock.

Blood samples for virome analyses were obtained from non-PVF (n = 9) and PVF (n = 11) STEMI patients at the time of primary PCI. The patients had a mean age of 60 ± 10 years and were 85% men. Patients with a first STEMI were selected, matched by sex, age, diabetes, and anterior myocardial infarction. Table 1 shows clinical and demographic characteristics of the studied groups. The inflammatory proteome was analysed among the same patients with available samples, PVF and non-PVF, as well as in non-STEMI healthy controls (without history of cardiovascular disease or cancer; mean age, 58.6 ± 1.2 years, 60% men). Written informed consent was obtained from all patients. The study was approved by the local ethics committee (The Ethics Committee of the Clinical Investigation of Germans Trias i Pujol Hospital) and was conducted in accordance with the Declaration of Helsinki.

**Nucleic acid extraction.** DNA was purified from total blood collected in BD Vacutainer EDTA tubes, using the FlexiGene DNA Kit (QIAGEN GmbH, Germany). DNA concentration was evaluated using the Qubit BR Assay Kit (Thermo Scientific, Wilmington, DE, USA). Integrity was checked by gel electrophoresis.

**Molecular assays.** We analysed a total of 20 human blood samples, from 9 patients with STEMI and non-PVF, and from 11 patients with STEMI and PVF; using VirCapSeq-VERT (Virome Capture Sequencing Platform for Vertebrate Viruses). VirCapSeq-VERT is a virome capture sequencing platform targeting viral taxa that infect vertebrates, using a database of 342,438 representative sequences spanning all virus sequence records. When compared with other enrichment procedures, the utilized procedure allows for reduction of background viral genomes, mapping of filtered reads and generation of counts, and analysis of viral communities. Kraken tools were used to remove sequenced human and bacterial reads from among the total sequencing reads generated for each sample. A quality check and adapter trimming were performed using the quality control tool FASTQC. Assembly of the host depleted trimmed reads was performed using SPAdes software version 3.15.2. Generation of the index and the mapping was done using BWA software version 0.7.17. Amplification duplicates that might confound the count were remove using SAMtools software version 1.12. Finally, mapping statistics were generated using the MultiQC tool.

**Epstein–Barr real time PCR and immunoassay.** Real time PCR was performed in non-PVF (n = 6) and PVF (n = 9) DNA samples by EBV Amplification Reagent Kit (Abbott Molecular, 08N54-085).

IgG-class antibodies to Epstein–Barr virus nuclear antigen (EBV-EBNA-1) were determined in plasma. Non-PVF (n = 94) and PVF (n = 82) plasma samples were by Epstein–Barr Virus (EBNA-1) IgG ELISA (Demeditec, DE4246, lot 109G/K041).
Inflammation proteomic analysis. The inflammatory proteomic profiles of non-PVF and PVF patients were analysed using the Olink Inflammation panel, based on Proximity Extension Assay technology. This multiplex immunoassay enables analysis of 92 inflammation-related proteins. Non-PVF patients (n = 7), PVF patients (n = 7), and healthy subjects (n = 5) were analysed using the Olink Inflammation panel.

Statistical analysis. Summary data were represented by mean and standard error of the mean (SEM), or by median and interquartile range (IQR) depending on the data normality. The D’Agostino and Pearson test was used to evaluate the normality of data. Two-groups comparisons were performed using the unpaired t-test or Mann Whitney test, and three-groups comparisons were performed using Kruskal–Wallis test or ANOVA, depending on the data normality. Fisher’s exact test was used when required. Statistical significance was assumed.

| Variable                                      | PVF STEMI (n = 11) | non-PVF STEMI (n = 9) | P value |
|-----------------------------------------------|--------------------|-----------------------|---------|
| Age, years, mean (standard deviation)         | 59.5 (11.7)        | 60.2 (9)              | 0.889   |
| Male sex, n (%)                               | 9 (81.8%)          | 8 (88.9%)             | 0.579   |
| Medical history, n (%)                        |                    |                       |         |
| Hypertension                                  | 5 (45.5%)          | 6 (66.7%)             | 0.311   |
| Hyperlipidaemia                               | 6 (54.5%)          | 7 (77.8%)             | 0.272   |
| Diabetes mellitus                             | 2 (18.2%)          | 2 (22.2%)             | 0.625   |
| Current smoker                                | 7 (63.6%)          | 6 (66.7%)             | 0.630   |
| Persistent or permanent atrial fibrillation   | 1 (9.1%)           | 0                     | 0.550   |
| Previous treatment, n (%)                    |                    |                       |         |
| Aspirin                                       | 0                  | 1 (11.1%)             | 0.450   |
| Beta-blocker                                  | 0                  | 1 (11.1%)             | 0.450   |
| Statin                                        | 2 (18.2%)          | 1 (11.1%)             | 0.579   |
| Angiotensin-converting enzyme inhibitor        | 1 (9.1%)           | 2 (22.2%)             | 0.421   |
| Angiotensin II receptor blocker               |                    |                       |         |
| Clinical characteristics                      |                    |                       |         |
| At least 2 angina episodes in the last 24 h, n (%) | 3 (27.3%)         | 1 (11.2%)             | 0.375   |
| Killip–Kimball class, n (%)                   |                    |                       |         |
| I                                             | 6 (54.4%)          | 8 (88.9%)             |         |
| II                                            | 1 (9.1%)           | 1 (11.1%)             |         |
| III                                           | 0                  | 0                     |         |
| IV                                            | 4 (36.4%)          | 0                     |         |
| Killip–Kimball > 1                            | 5 (45.5%)          | 1 (11.1%)             | 0.119   |
| ECG characteristics                            |                    |                       |         |
| Anterior STEMI, n (%)                         | 7 (63.6%)          | 4 (44.4%)             | 0.342   |
| Atrial fibrillation on first ECG, n (%)       | 3 (27.3%)          | 0                     | 0.145   |
| Echocardiography                               |                    |                       |         |
| LVEF after PCI, %, median (IQR)               | 42 (40–55)         | 56 (52–61)            | 0.003   |
| Culprit lesion, n (%)                         |                    |                       |         |
| Left anterior descending artery               | 5 (45.5%)          | 3 (33.3%)             | 0.465   |
| Circumflex artery                             | 0                  | 2 (22.2%)             | 0.189   |
| Right coronary artery                         | 5 (45.5%)          | 4 (44.4%)             | 0.658   |
| Left main coronary artery                     | 1 (9.1%)           | 0                     | 0.550   |
| Multivessel disease, n (%)                    | 7 (63.6%)          | 3 (33.3%)             | 0.185   |
| Primary percutaneous coronary intervention, n (%) | 10 (90.9%)        | 9 (100%)              | 0.550   |
| Complete revascularization, n (%)             | 5 (45.5%)          | 5 (55.6%)             | 0.500   |
| Timing of procedure                            |                    |                       |         |
| Symptoms onset to first medical contact, minutes, median (IQR) | 18 (10–25) | 45 (27–170)           | 0.053   |
| Symptoms onset to PPCI, minutes, median (IQR) | 135 (107–208)      | 144 (113–261)         | 0.543   |
| Symptoms onset to PPCI < 120 min, n (%)       | 7 (63.6%)          | 7 (77.8%)             | 0.426   |
| Clinical events during hospitalization, n (%) |                    |                       |         |
| Recurrent ischemic event                      | 1 (9.1%)           | 0                     | 0.550   |
| Atrial fibrillation or flutter                | 2 (18.2%)          | 1 (11.1%)             | 0.579   |
| Sustained ventricular tachycardia              | 1 (9.1%)           | 0                     | 0.550   |
| Post-anoxic encephalopathy                    | 7 (63.6%)          | 0                     | 0.004   |
| Intrahospital mortality                       | 5 (45.5%)          | 0                     | 0.030   |

Table 1. Clinical characteristics of PVF and non-PVF STEMI patients.
when \( P \) was < 0.05. Statistical analyses were performed using Prism 9 for macOS version 9.0.2 (134) and 9.3.1 (350).

**Results**

**Circulating virome.** The virome capture sequencing platform VirCapSeq-VERT was used to target viral taxa in human blood samples from non-PVF patients \((n = 9)\) and PVF patients \((n = 11)\). The capture results were sequenced using Illumina HiSeq/NovaSeq. Human and bacterial reads were removed from the sequencing files, and the remaining reads ranged from 148–543 k pairs of reads per sample (Supplementary Table 1). Along all reads and samples, we found good quality per base position. However, we detected a high amount of PCR duplicates, due to the amplification and enrichment protocol (Supplementary Table 2). We also identified and removed common sequencing adapters. Details in the statistics regarding the trimming process for each sample are shown in Supplementary Table 3. The host depleted trimmed reads were assembled using SPAdes software to generate longer sequences, and for an additional and improved homology search (Supplementary Table 4). All viral reference genomes available in GenBank NCBI database were used to create a BLAST database, which was used for the homology search, with the generated assembled host depleted trimmed reads as input. We observed a total of 51 different genome entries (Table 2).

The identified genome entries were then used to create an alignment index, to map the reads corresponding to their exact position in the reference genomes. Nearly half of the reads did not map to retrieved viral sequences, likely because the host depleted reads may have contained archaea, yeast, or unclassified taxon reads. These results correspond with the low number of reads identified as viral (Supplementary Table 1). Supplementary Tables 5 and 6 summarize the length of each reference sequence, and the percent of base pairs covered (%), in results correspond with the low number of reads identified as viral (Supplementary Table 1). Supplementary Tables 5 and 6 summarize the length of each reference sequence, and the percent of base pairs covered (%), in non-PVF and PVF patients. Six genome entries found in the homology search did not generate reads mapping, since the better sensitivity of the mapping enabled more confident placing of a read compared to with BLAST.

**Alpha diversity.** We further assessed alpha diversity to determine the diversity and to enable comparisons of the type and quantity of virus species between non-PVF and PVF patients. Alpha diversity is a statistic used in this kind of sample, in which reads reflect the abundance of each of the identified operational taxonomical units (OTUs). Richness and diversity are alpha diversity metrics.

As a result, species richness did not significantly differ between non-PVF patients and PVF patients \((26.44 ± 2.69 \text{ vs. } 20.45 ± 2.39; \text{ } P = 0.112)\) (Fig. 1A). PVF and non-PVF patients also did not significantly differ in other richness indexes, such as the Chao1 Richness Estimate \((28.23 ± 2.63 \text{ vs. } 21.93 ± 2.54; \text{ } P = 0.105)\) (Fig. 1B) and Abundance Coverage Estimator (ACE) index \((29.02 ± 2.69 \text{ vs. } 22.00 ± 2.44; \text{ } P = 0.069)\) (Fig. 1C). We also used a simple linear regression model to explore whether the species richness correlated with the number of raw read pairs sequenced. We identified a slight correlation between the number of raw read pairs sequenced and the observed richness \((R^2 = 0.14; \text{ } P = 0.099)\) (Fig. 1D), Chao1 \((R^2 = 0.13; \text{ } P = 0.113)\) (Fig. 1E), and ACE index \((R^2 = 0.20; \text{ } P = 0.049)\) (Fig. 1F). Concerning the species diversity, we found no significant differences between non-PVF and PVF patients using Shannon's Diversity Index \((0.614 ± 0.044 \text{ vs. } 0.552 ± 0.018; \text{ } P = 0.252)\) (Fig. 2A), the Simpson Index \((0.239 ± 0.019 \text{ vs. } 0.212 ± 0.006; \text{ } P = 0.456)\) (Fig. 2B), or the Inverse Simpson Index \((1.322 ± 0.036 \text{ vs. } 1.271 ± 0.011; \text{ } P = 0.423)\) (Fig. 2C).

**Frequent sequences.** In addition, due to species richness of viruses did not significantly differ between non-PVF and PVF patients, we explored whether specific virus families were differentially expressed. Supplementary Table 7 summarizes the top 10 OTUs. The predominant OTU was Human endogenous retrovirus K113 \((NC_022518.1)\) (Fig. 3A), and its frequency did not significantly differ between non-PVF and PVF patients \((0.869 ± 0.012 \text{ vs. } 0.885 ± 0.004; \text{ } P = 0.381)\).

Among the most frequent OTUs, the genus most commonly found was Lymphocryptovirus \((Fig. 3A)\), belonging to the Herpesviridae family. In particular, we detected the complete genomes of three viruses of this family—Human gammaherpesvirus 4 \((NC_007605.1)\), human herpesvirus 4 type 2 (Epstein–Barr virus), Human herpesvirus 4 type 2 (Epstein–Barr virus type 2), and Macacine gammaherpesvirus 4 (Rhesus lymphocryptovirus), respectively (Table 2).

Non-PVF and PVF patients only significantly differed in the frequencies of Human herpesvirus 4 type 2 and Macacine gammaherpesvirus 4. As shown in Fig. 4A, Human herpesvirus 4 type 2 \((NC_009334.1)\) significantly more common in non-PVF patients than in PVF patients \((0.0011 ± 0.000 \text{ vs. } 0.0004 ± 0.000; \text{ } P = 0.042)\), while Macacine gammaherpesvirus 4 \((NC_006146.1)\) was significantly less frequent in non-PVF patients than in PVF patients \((0.009 ± 0.000 \text{ vs. } 0.011 ± 0.000; \text{ } P = 0.024)\) (Fig. 4B). Non-PVF and PVF patients did not significantly differ in the frequency of Human gammaherpesvirus 4 \((NC_007605.1)\) \((0.0045 ± 0.000 \text{ vs. } 0.0042 ± 0.000; \text{ } P = 0.939)\) (Fig. 4C). Human gammaherpesvirus 4 \((NC_007605.1)\) results were also validated by RT-PCR and ELISA. Human gammaherpesvirus 4 \((NC_007605.1)\) viral load of 15 patients was evaluated by RT-PCR. Viral load was detected in 13 patients and no significant differences were observed between non-PVF and PVF patients \((2147 ± 887.2 \text{ vs. } 1113 ± 683.6 \text{ UI/mL}; \text{ } P = 0.731)\) (Fig. 5A).

IgG-class antibodies to Epstein–Barr nuclear antigen were detected in 94.8% of the analyzed population. Similar to the non-significant differences found in Human gammaherpesvirus 4 \((NC_007605.1)\) frequencies between non-PVF and PVF patients by sequencing, IgG-class antibody detection levels did not significantly differ between non-PVF and PVF patients \((31.42 ± 1.121 \text{ vs. } 33.40 ± 1.125 \text{ Units}; \text{ } P = 0.196)\) (Fig. 5B).

Supplementary Table 8 details the relative frequencies of the rest of the identified OTUs. Among the less common OTUs, the Alphatorquevirus genus \((Fig. 3B)\), belonging to the Anelloviridae family, was most prominently represented, as we identified the complete genome or specific genes of up to 27 Torque teno viruses, also referred to as transversion transmitted viruses or TTVs. PVF and non-PVF patients showed significantly different
Table 2. Taxonomic information of the sequence identified.
Figure 1. Species richness alpha diversity. (A–C) Species richness represented by the following metrics: (A) Observed richness values, (B) Chao1 Richness Estimate (Chao1), and (C) Abundance Coverage Estimator (ACE). (D–F) Simple linear regression model between the number of sequenced raw read pairs and (D) observed richness, (E) Chao 1, and (F) ACE.
frequencies of *Torque teno virus* 18 (NC_043414.1) and *Torque teno virus* 8 (NC_014084.1). The relative frequency of *Torque teno virus* 18 was significantly higher in non-PVF patients than in PVF patients (0.00058 ± 0.0002 vs. 0.00013 ± 0.00006; \( P = 0.0297 \)) (Fig. 4D). Similarly, the relative frequency of *Torque teno virus* 8 was significantly increased in non-PVF patients compared to PVF patients (0.00038 ± 0.00011 vs. 0.00004 ± 0.00003; \( P = 0.0097 \)) (Fig. 4E).

**Systemic inflammation.** The inflammatory proteomic profile was analysed using the Olink Inflammation panel (Supplementary Table 9). Our results did not show that PVF and non-PVF patients significantly differed in any of the analysed inflammatory-related proteins (Table 3). Indeed, 53 of the 92 analysed proteins showed no differences between any of the studied groups (Supplementary Figs. 1, 2, 3 and 4). On the other hand, 16 of the 92 proteins significantly differed between healthy subjects versus both non-PVF and PVF patients (Supplementary Fig. 5). No differences were found between non-PVF and PVF patients; however, some proteins significantly differed between the healthy control group and one of the AMI groups. Compared to healthy subjects, PVF patients showed significantly higher circulating levels of CUB domain containing protein 1 (CDCP1) (\( P = 0.0364 \)) (Fig. 6A) and Interleukin-18 receptor 1 (IL18-R1) (\( P = 0.0488 \)) (Fig. 6B).

Monocyte chemotactic protein 1 (MCP-1), C–C motif chemokine 4 (CCL4), Tumor necrosis factor receptor superfamily member 9 (TNFRSF-9), Interleukin-10 (IL-10), Chemokine (C–C motif) ligand 19 (CCL19),
Figure 4. Relative frequency of (A) Human herpesvirus 4 type 2 (NC_009334.1), (B) Macacine gammaherpesvirus 4 (NC006146.1), (C) Human gammaherpesvirus 4 (NC_007605.1), (D) Torque teno virus 18 and (NC_043414.1), and (E) Torque Teno virus 8 (NC_014084.1).

Figure 5. (A) Epstein–Barr viral load (UI/mL) and (B) IgG-class antibodies to Epstein–Barr nuclear antigen levels (units) in non-PVF patients and PVF patients.
eraldS) study showed that PVF during first STEMI was most significantly associated with SNP rs2824292 at patients, but did not exhibit significant differences when compared with PVF patients. MCP1 (\( P = 0.0221 \)) (Fig. 6C), CCL4 (\( P = 0.0281 \)) (Fig. 6D), IL-10 (\( P = 0.0279 \)) (Fig. 6E), and TNFRSF-9 (\( P = 0.0499 \)) (Fig. 6F) levels were significantly promoted in non-PVF patients in comparison with the control groups. Conversely, NT-3 (\( P = 0.0092 \)) (Fig. 6G) and CCL19 (\( P = 0.0093 \)) (Fig. 6H) levels were significantly lower in non-PVF patients compared with in healthy subjects.

### Discussion

Primary ventricular fibrillation (PVF) is among the leading causes of prehospital sudden cardiac death. It is presently unknown what factors increase the probability of PVF development during acute ischemia, complicating the identification of PVF predictors. We thus aimed to evaluate possible PVF predictors or triggers, including the complete DNA virome and the inflammatory proteome in PPCI-treated STEMI patients.

A growing number of viruses have been determined to be associated with inflammatory cardiomyopathy. Previous data suggest that viral exposure could increase PVF susceptibility, although this has not been conclusively proven. In this context, Andréoletti et al. identified coxsackievirus B infection in post-mortem endomyocardial tissue of patients who died suddenly due to AMI\(^1\). Additionally, the AGNES (Arrhythmia Genetics in the Netherlands) study showed that PVF during first STEMI was most significantly associated with SNP rs2824292 at
chromosome 21q21, where the CXADR gene is found. CXADR encodes the coxsackie and adenovirus receptor protein, which has been implicated in myocarditis, dilated cardiomyopathy, and ventricular conduction and arrhythmia vulnerability. However, this association was not replicated in at least two additional studies. Extreme influenza epidemics are also reportedly associated with out-of-hospital cardiac arrest. However, no other relationships have been found between PVF occurrence and enterovirus or influenza exposure.

The present pilot study is the first to include a circulating virome analysis of all DNA viruses that infect vertebrates. Our findings indicate that non-PVF and PVF patients significantly differed only in the levels of Macacine gammaherpesvirus 4 (Rhesus lymphocryptovirus), Human herpesvirus 4 type 2 (Epstein–Barr virus type 2), and Torque teno viruses 8 and 18 (transfusion transmitted viruses).

Gamma-herpesvirinae family viruses are lymphotropic viruses that infect lymphoid cells. Epstein–Barr virus (EBV) is a highly ubiquitous herpesvirus which asymptomatically infect over 90% of the population. Once infected, EBV persists in B-cells for life and could be reactivated in immunosuppression cases. In terms of the heart, EBV reportedly induces severe infection of T-cells in the myocardium of patients with ongoing myopericarditis, as well as in abdominal or coronary aneurysms. EBV infection may also influence the development of atherosclerosis. Here we identified EBV (Human gammaherpesvirus 4) and EBV type 2 (Human herpesvirus 4 type 2). The relative frequency of EBV did not significantly differ between non-PVF and PVF patients. Along this line, we did not find significant differences in the viral load or in the IgG-class antibodies to EBV between non-PVF and PVF patients measured by RT-PCR and ELISA, respectively. On the other hand, the EBV type 2 frequency was significantly higher in non-PVF patients than in PVF patients, and is thus not a risk factor for second-hit ischaemia-driven cardiac arrest. Any of the patients analysed took immunosuppressive treatment or had any malignancy.

Furthermore, Torque teno viruses (TTVs) are small DNA viruses that have been detected in many mammalian hosts, and whose prevalence in humans is > 90% . It is not clear that TTVs act as primary pathogens, and it appears that TTVs usually establish chronic infections without causing pathology. It has been suggested that TTVs could be used as markers of viral environmental contamination, since TTVs are potential contaminants.
in water sources and hospitals, including in the blood supply. This may explain why we detected 27 species of TTVs in the presented study. Remarkably, among 20 human samples, only 1 tested negative for all detected TTV species. Although they are not among the 10 most frequent relative entries, TTV-8 and TTV-18 were the most frequently detected TTVs, and their frequencies significantly differed between non-PVF and PVF patients. However, the relative frequencies of TTV-8 and TTV-18 were significantly higher in non-PVF patients than in PVF patients, and thus do not provide information to predict sudden cardiac arrest. Takeuchi et al. detected one TTV sequence read in a patient with acute myocarditis, but could not establish it as a potential pathogen of myocarditis. Both our results and Takeuchi’s findings support the widespread idea that TTVs are unlikely to act as primary pathogens.

The second objective of this study was to examine the systemic inflammatory response, which is known to play important roles in the pathophysiology of acute coronary syndrome and atherosclerosis. Notably, in recent years, its involvement in SCD has also been studied, although attempts to find predictive biomarkers have yielded inconclusive results. The Physicians’ Health Study showed that C-reactive protein (CRP) levels are an independent risk factor for SCD (OR, 2.78; 95% CI, 1.35–5.72). In contrast, the Nurses’ Health Study did not confirm any significant correlation between SCD and highly sensitive CRP. Among healthy European middle-aged men who participated in the PRIME Study, higher IL-6 was a strong predictor of sudden death, with an OR of 3.06 (95% CI, 1.20–7.81), but CRP was not shown to predict SCD, as in the Nurses’ Health Study. Furthermore, our group identified growth differentiation factor 15 (GDF-15) as a predictor of mortality and CV morbidity, and Anderson et al. detected GDF-15 as a risk factor for sudden cardiac death in the acute phase of MI, with an OR of 1.47 (95% CI, 1.11–1.95).

Our analyses revealed no significant differences between non-PVF and PVF patients for any of the analysed inflammatory-related proteins. We did identify differential protein expression between healthy subjects and STEMI patients (including both non-PVF and PVF patients) (Supplementary Fig. 5). Compared to healthy subjects, STEMI patients showed significantly higher levels of inflammatory proteins related to cell adhesion, chemotaxis, and cellular response to cytokine stimulus, and cell activation proteins involved in immune response, such as IL-6, IL-8 CXCL11, CCL11, MCP3, MCP4, and ENRAGE. The roles of IL-6 and IL-8 in AMI have been previously described. CCL11 has potent eosinophil chemotaxant activity, and is expressed by cardiac macrophages. Here we found that CCL11 levels were increased in STEMI patients compared to healthy subjects, thus confirming the previously observed association between CCL11 and myocardial infarction. MCP-3 plays an important role in cell recruitment to inflammatory sites, specifically, it has been described that MCP-3 recruits mesenchymal stem cells and improved cardiac remodeling. Mao et al., found that MCP-3 levels were decreased in patients with cardiac remodeling after AMI compared to MI and control groups; in addition, MCP-3 values were not differential between MI and healthy subjects. These results do not agree with what was found in our pilot study, so delving into the role that MCP-3 plays in STEMI patients would be interesting.

Although no differences were found between non-PVF and PVF patients, some proteins significantly differed between the healthy control group and one of the STEMI groups. For example, CDCP1 and IL18-R1 were significantly higher in PVF patients than in healthy subjects. Shia et al. conducted genome-wide association analyses, and identified variations in the DNA sequence that affect the expression of 3p21.31 (CDCP1), which were associated with myocardial infarction. Those authors did not specify whether the patients had PVF. In the other hand, Fonsekeno et al. also found that a polymorphic variant of IL18R1 was associated with an increased risk of MI in CAD patients with coronary artery disease. Based on our results, it would be interesting to further examine into the studies related to CDCP1 or IL18-R1 and PVF. In contrast, MCP-1, CCL4, TNFRSF-9, and NT-3 showed different expression profiles in healthy subjects compared to non-FVP patients, but not compared with FVP patients. The association of some of them with cardiovascular disorders has already been previously described by other authors. MCP-1, which recruit circulating monocytes, plays a major role in the immunologic profile of ischemia/reperfusion injury in the heart; CCL4 is directly involved in the atheroma plaque stabilization; and elevated NT-3 plasma levels are associated with an increased risk atrial fibrillation recurrence. However, there remains a need to elucidate potential key roles of these proteins in inflammatory process development in AMI; and they do not seem to be involved in PVF.

This study has several limitations. It was a pilot study with a limited sample size. Despite comprehensive examination of both the virome and the proteome, we did not identify any clear trend. The VirCapSeq-VERT panel can capture both DNA and RNA viruses; however, due to the storage conditions and available blood material, we cannot fully exclude the presence of undetected RNA viruses. In addition, we have not been able to make the correlation between the OTUs and the inflammatory protein levels because, although the population is the same, some samples were used to the virome screening study and others to the inflammation analyses. Lastly, to confirm the presence of a viral genome within the myocardium during the acute phase of STEMI, we would need to perform endomyocardial biopsies, which is ethically unacceptable.

In conclusion, our observations revealed no clear trend in associations between the circulating virome or inflammatory proteome and PVF in STEMI. Hence, there remains a critical need for new strategies to better elucidate the possible triggers of PVF; and to identify individuals at high risk of SCD.

Data availability
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions
T.O., E.R.-L., C.G.-G., C.G.-M. and A.B.-G. designed the work. T.O., C.G.-G., F.R., C.L., M.F., S.M., N.E.-O. and M.J.M. acquired data for the work by completing the RUTI-STEMI-PVF registry. T.O., E.R.-L., C.G.-G., F.R., C.L., M.F., S.M., N.E.-O. and A.B.-G. performed the virome and inflammatory proteome analysis. T.O., E.R.-L., C.G.-G., F.R., C.L., M.F., S.M., N.E.-O. and A.B.-G. interpretated the results. T.O. and E.R.-L. drafted the article. A.B.-G. revised it critically for important intellectual content. All the authors have reviewed the manuscript and approved the final version to be published.

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