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Short Communication

# Marseillevirus prevalence in multitransfused patients suggests blood transmission

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ABSTRACT

*Background:* Emerging viral infections in humans are appearing at an increasing rate. Recently, we identified a new Marseillevirus, named Giant Blood Marseillevirus (GBM), by performing viral metagenomics on asymptomatic blood donors.
*Objectives:* To study and compare the prevalence of Marseillevirus between asymptomatic blood donors and thalassemia patients.
*Design:* Here, we present a combined molecular and serological study on 174 asymptomatic blood donors and 22 patients with thalassemia who receive repeated blood transfusions to estimate the prevalence of Marseillevirus in these two populations.
*Results:* We identified Marseillevirus genomic DNA in 4% of donors, whereas 9.1% of the thalassemia patients were positive for this virus. Moreover, IgG seropositivity was detected in 22.7% of patients in the thalassemia group, whereas this seropositivity was observed in 12.6% of the blood donor population.
*Conclusion:* These results suggest that Marseillevirus infection is not rare in healthy persons and may be transmitted by transfusion, thus raising speculation regarding the long-term consequences of this viral infection, particularly in patients requiring repeated blood transfusions.
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## 1. Background

New viral infections represent constant threats to public health [1–3]. Currently, blood safety depends on improved donor selection procedures, pathogen reduction, and nucleic acid testing and selective antigen/antibody screening for known viruses [4]. However, methodological limitations, such as a low viral DNA load, the abundance of host and mitochondrial DNA, and the absence of genes that are conserved among all viruses, make detecting emerging blood-borne viruses elusive and may represent a threat in the case of frequent blood transfusions [5].

The thalassemia group of genetic diseases are characterized by globin chain production disorders [6]. Patients with thalassemia require iterative blood transfusions, approximately once per month, leading to higher risks of blood-borne viral infections, representing a secondary cause of death [7–10].

Marseilleviruses are amoeba-infecting pathogens that were initially isolated from environmental samples [11–13]. Marseillevirus possesses a 368 kilobase-pair-long double-stranded DNA genome, enclosed in a 250 nm icosahedral capsid. Accumulating data emphasize the presence of Marseilleviruses in humans. Sequences homologous to Marseilleviruses were found in various human metagenomics samples [14]. Moreover, Senegalvirus, a member of the *Marseilleviridae* family, was isolated from a human stool sample in an asymptomatic patient [14,15]. Finally, we recently identified a new giant virus known as Giant Blood Marseillevirus (GBM) [16] in the blood of asymptomatic blood donors. GBM was detected by performing viral metagenomics on 0.45 μm filtered sera and was found to infect human T-cells *in vitro*.

## 2. Objectives

In this study, we investigated the prevalence of Marseillevirus and related viruses using ELISA and PCR to test the sera of two

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cohorts composed of asymptomatic blood donors and thalassemia patients.

## 3. Study design

### 3.1. Samples

Sera collected from asymptomatic blood donors ($n = 174$; male/female: 91/83; median age = 46 years; range = 21–70 years) and thalassemia patients ($n = 22$; male/female: 12/10; median age = 23.5 years; range = 8–49 years) were obtained from Blood supply center (Montpellier, France) and the Timone Hospital (Marseille, France) respectively. The blood donors tested negative for HIV-1/2, human T leukemia virus, hepatitis B virus surface antigen and anti-hepatitis B core antibody and hepatitis C virus (HCV), whereas serology to cytomegalovirus indicated negativity or past infection. Serological data for the thalassemia patients is summarized in Table S1. Control sera ($n = 9$) were also collected from 6- to 12-month-old children (Timone Hospital). This study was a non-interventional and did not require more than the routine procedures. Biological material and clinical data were only obtained for standard diagnosis in clinical virology, as prescribed by physicians, and did not require any specific collection of clinical samples, change in sampling protocol or supplementary questioning of the patient. Data were collected and then analyzed from an anonymized database. According to the French Law of Public Health (CSP Art L 1121-1.1), this type of study is exempt from written informed consent. This study was approved by the local ethics committee "Comité d'éthique de l'IFR 48, Service de Médecine Légale," under the accession numbers N°13-016 and N°13-025.

### 3.2. Viral DNA detection

DNA from serum samples was extracted using the High Pure Viral Nucleic Acid Kit (Roche Applied Science, Mannheim, Germany). PCR was performed using the following primers: ATPase_T19F (5′AGACCCAAACTCGCAGCTTA-3′) and ATPase_T19R (5′-CCGGAAGATTCCAAGTTTCA-3′) to target the Marseillevirus *orf* 152. Amplification using *Phusion* DNA Polymerase (Thermo Fisher Scientific, Illkirch, France) started with an initial denaturation step at 95 °C for 30 s, followed by 35 cycles at 98 °C for 10 s, 53 °C for 30 s and 72 °C for 20 s. Sequencing reactions were carried out with the reagents of the BigDye® Terminator v1.1 Cycle Sequencing Kit (Life Technologies, Saint Aubin, France) according to the manufacturer's instructions. Sequences were analyzed using ChromasPro software and aligned using the ClustalW tool of BioEdit software.

### 3.3. Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed as previously described [16]. Average results for the IgG levels were obtained from two independent experiments. Negative ($OD_{490\,nm} = 0.182$; $OD_{490\,nm} = 0.139$) and positive controls ($OD_{490\,nm} = 0.474$ and 0.487, respectively) were included in each plate. Thresholds for the blood donors and thalassemic patients ($OD_{490\,nm} = 0.260$ and 0.233, respectively) were calculated using the relative percentage of positivity (RPP%) formula $RPP\% = \frac{OD_{threshold} - OD_{neg.\ control}}{OD_{pos.\ control} - OD_{neg.\ control}}$. RPP = 27% was used, for which a 97.1% relative specificity and 97.2% relative sensibility of the ELISA results were predicted.

### 3.4. Statistical analysis

Statistical analysis was performed using PASW Statistics software version 17.0 (SPSS Inc., Chicago, IL, USA) to carry out Chi$^2$ tests. $P$-values less than or equal to 0.05 were considered to be significant.

**Table 1**
Serological and molecular survey of Marseillevirus infection in 174 blood donors and 22 thalassemia patients.

| | Blood donors | Thalassemia patients | $p$-Value[a] |
|---|---|---|---|
| Sample number | 174 | 22 | |
| Male/female (male %) | 91/83 (52.3%) | 12/10 (54.5%) | |
| Median age (range) | 46 (21–70) | 23.5 (8–49) | |
| IgG positive | | | |
| Number | 22/174 (12.6%) | 5/22 (22.7%) | 0.165 |
| Male/female (male %) | 10/12 (45.5%) | 3/2 (60%) | |
| Median age (range) | 47.5 (22–70) | 30 (17–49) | <0.0001 |
| PCR positive | | | |
| Number | 7/174 (4%) | 2/22 (9.1%) | 0.267 |
| Male/female (male %) | 3/4 (42.9%) | 1/1 (50%) | |
| Median age (range) | 37 (21–61) | 31 (21–41) | |
| IgG positive/PCR positive | | | |
| Number | 4/174 (2.3%) | 1/22 (4.5%) | 0.452 |
| Male/female (male %) | 2/2 (50%) | 1 (0%) | |
| Median age (range) | 31 (22–53) | 41 | |

Summary results from serological and molecular testing for the presence of Marseillevirus IgGs and DNA in serum, performed on 174 blood donors and 22 thalassemic patients. PCR-positive sera were systematically verified using DNA sequencing. See Study design section for further information.
[a] Chi$^2$ test.

## 4. Results

By analyzing 196 serum samples, we detected IgG levels above the threshold values in 22/174 (12.6%) of the blood donors and 5/22 (22.7%) of the thalassemic patients (Table 1, Table S1). Notably, in one patient with thalassemia (#21), we detected seropositivity 22 days after the last transfusion ($OD_{490\,nm}$ increase = 1.6-fold).

We further performed viral DNA detection by amplifying a conserved 200 base pair region in the Marseillevirus ORF152. We found Marseillevirus DNA in the sera from 7 (4%) blood donors and 2 (9.1%) thalassemia patients. Two of the seven blood donors, #45 (Acc. Number # KF223992) and #127 (Acc. Number # KF223993) presented single nucleotide polymorphisms at positions 136 (A/G) and 55 (T/C), respectively (Fig. 1). It is worth noting that 4 of the blood donors and 1 of the thalassemia patients who were PCR positive also presented elevated IgG levels (Table 1, Table S2).

## 5. Discussion

In the present study, we showed that the presence of Marseillevirus among blood donors is not uncommon. In fact, we found that 12.6% of the analyzed serum samples had anti-Marseillevirus IgG levels above the cut-off values, whereas 4% presented circulating Marseillevirus DNA. In comparison, the thalassemia patient cohort presented a higher prevalence of IgG- and PCR-positive subjects. However, the observed difference was not statistically significant ($p$-value > 0.05), indicating that additional samples may be required to estimate the significance of the observed phenomenon. Interestingly, we observed that thalassemia patients acquired IgG seropositivity toward Marseillevirus at a younger age compared to those in the blood donors cohort ($p$-value < 0.0001), suggesting that frequent blood transfusions may represent a risk factor for Marseillevirus transmission. However, the IgG to Marseillevirus detected in thalassemia patients (who received transfusions of concentrated erythrocytes approximately once per month) may have been transferred passively from the seropositive blood donors. Therefore, additional pre and post transfusion monitoring of blood donors and thalassemic blood recipients should shed light on Marseillevirus transmission in humans.

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