Rapid Virological Diagnosis of Central Nervous System Infections
Using a Multiplex RT-PCR DNA Microarray

Neurotropic Viruses Detection by RT-PCR DNA Microarray

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Abstract (264 words):

Viruses are the main etiological cause of the central nervous system (CNS) infections. A rapid molecular diagnosis is recommended to improve the therapeutic management of patients. The aim of this study was to evaluate the performances of a DNA microarray, the CLART® Entherpex kit (Genomica®, Coslada, Spain), allowing the rapid and simultaneous detection of 9 DNA and RNA neurotropic viruses: HSV-1, HSV-2, VZV, CMV, EBV, HHV-6, HHV-7, HHV-8 and the human enteroviruses (HEVs). This evaluation was performed with 28 samples from the European proficiency panels (QCMD, Glasgow, Scotland) and then with 78 cerebrospinal fluid (CSF) specimens. The majority of the QCMD results obtained by the DNA microarray were similar to those recorded by the overall QCMD participants. The main discrepant results were observed for low concentrations of HSV-2 and HEVs. From the clinical samples, the kit detected 27 of the 28 herpesviruses CNS infections and all the 30 HEVs positive CSF samples. No false positive result was observed among the 20 virus negative CSF samples. The clinical sensitivity, specificity, negative and positive predictive values of the assay were 98.3, 100, 95.2, and 100%, respectively, when the results were compared to those of commercially available PCR assays. Interestingly, HHV-7 was detected in 11 (37%) of the 30 HEVs positive CSF samples from children suffering from aseptic meningitis causing significant longer length of stay at the hospital than infection with HEVs alone (2.4 vs 1.4 days; \( P=0.038 \)). In conclusion, this preliminary study showed that this DNA microarray could be a valuable molecular diagnostic tool for single and mixed DNA and RNA virus infections of the CNS.

Keywords: Multiplex RT-PCR DNA microarray, neurotropic viruses, virological diagnosis, central nervous system infection.
Viruses are the main etiological cause of the central nervous system (CNS) infections ahead of bacterial and fungal causes (14, 34). They are responsible for encephalitis and aseptic meningitis (3, 5, 35). Encephalitis is a rare but one of the most devastating neurological disorders. Encephalitis in humans is mainly due to herpesviruses in particular herpes simplex (HSV) and varicella-zoster (VZV) viruses but also to Cytomegalovirus (CMV), Epstein-Barr virus (EBV), or Human Herpes virus type 6 (HHV-6) in the immunocompromised host (21, 35, 40). Human enteroviruses (HEVs) are the most common cause of aseptic meningitis outbreaks in children during the summer season (1, 23). In both these types of CNS infections, a rapid virological diagnosis is required to improve the therapeutic management by acyclovir therapy in cases of Herpes simplex encephalitis or to increase cost savings in hospitalized cases of HEVs-related aseptic meningitis during the epidemic season (20, 26, 30, 36). Polymerase chain reaction has been recognized as the reference method for the diagnosis of viral central nervous system infections in the cerebrospinal fluid (CSF) specimens (10, 30, 36, 38). However, the wide range of viruses potentially responsible for CNS infections as well as their genetic characteristics, both DNA or RNA viruses, rendered rapid and large virological diagnosis difficult using monoplex RT-PCR and PCR assays (4, 27).

The aim of this preliminary study was to evaluate the analytical and clinical performances of a commercially available multiplex RT-PCR DNA microarray, the CLART® Entherpex kit (Genomicia®, Coslada, Spain), allowing a rapid and simultaneous detection of 9 DNA and RNA neurotropic viruses: HSV-1, HSV-2, VZV, CMV, EBV, HHV-6, HHV-7, HHV-8 and HEVs in a single CSF sample. In a first phase, this multiplex DNA microarray assay was evaluated by testing proficiency samples of the 2008 and 2009 European proficiency panels (Quality Control for Molecular Diagnostics, Glasgow, Scotland). In a second, 78 CSF specimens from patients hospitalized for CNS infections that had been previously tested by
standardized commercially available PCR and RT-PCR assays for neurotropic virus detection, were retrospectively analyzed assessing the application of this commercially available multiplex RT-PCR DNA microarray on a routine basis.
MATERIALS AND METHODS

European proficiency panels and clinical specimens. The evaluation of the kit was first carried out with samples of the 2008 and 2009 European proficiency panels (Quality Control for Molecular Diagnostics (QCMD), Glasgow, Scotland) stored to -80°C until processing with the multiplex RT-PCR DNA microarray. Four proficiency samples for each of HSV-1, HSV-2, VZV, CMV, EBV, HHV-6 and HEVs containing concentrations ranging from $10^3$ to $10^5$ copies/mL were selected and independently tested 4 times each in order to assess the specificity, the sensitivity and the reproducibility of the DNA microarray.

In a second phase, 78 CSF samples obtained from children or adults hospitalized at the Reims University Medical Centre (France) for suspected neurological virus infections from March 2002 to May 2009 were retrospectively analyzed. These samples had been routinely submitted to the virology laboratory for neurotropic viruses detection and initially tested by both the multiplex end point PCR kit Herpes Consensus Generic® (Argène®, Verniolle, France) screening for HSV-1, HSV-2, VZV, CMV, EBV and HHV-6, and one of two HEVs RT-PCR assays (either the end point PCR kit Enterovirus Consensus® (Argène®, Verniolle, France) or the one step real-time RT-PCR kit Enterovirus TaqMan RT-PCR® (Andiatec®, Kornwestheim, Germany)) depending of the year of CSF collection (6). They were then divided in aliquots and stored to -80°C until processing with the multiplex RT-PCR DNA microarray. Among the 78 clinical samples investigated, 28 had been previously tested positive for herpesviruses (7 HSV-1, 2 HSV-2, 8 VZV, 1 CMV, 4 EBV and 6 HHV-6) and negative by HEVs RT-PCR assay; thirty samples obtained from children (sex ratio M/F: 5 ; mean age: 7 years) suffering from aseptic meningitis had been previously tested positive for HEVs and negative by Herpes Consensus Generic® kit; and twenty samples obtained from children (sex ratio M/F: 1.5 ; mean age: 6 years) suffering from aseptic meningitis had been tested negative by both Herpes Consensus Generic® kit and HEVs RT-PCR assay. Since
neither QCMD panels nor known clinical samples containing HHV-7 or HHV-8 were available, the performances of the DNA microarray regarding the detection of both these viruses have not been evaluated.

**DNA/RNA extraction.** Total nucleic acid extraction was performed using a NucliSens easyMAG® instrument (bioMerieux®, Marcy l’Etoile, France) according to the manufacturer’s instructions. Briefly, 200 µL of the QCMD specimen reconstituted according to the manufacturer’s instructions or cerebrospinal fluid were added to 2 mL of lysis buffer in a plastic vessel and incubated for 10 min at room temperature. Fifty microliters (µL) of silica were then added to the mixture. This was followed by an automatic magnetic separation phase. Nucleic acids were recovered in 50 µL elution buffer and stored at -80°C until use.

**Simultaneous detection of 9 viruses using a multiplex RT-PCR DNA microarray.** Clart® Entherpex kit (Genomica®, Coslada, Spain) is based on viral genome-specific fragments amplification by multiplex PCR and its subsequent detection via hybridization with microorganism-specific binding probe on low-density microarrays allowing simultaneous detection and identification of the eight human herpesviruses (HSV-1, HSV-2, VZV, CMV, EBV, HHV-6, HHV-7, HHV-8) and HEVs (Echovirus, poliovirus and coxsackievirus) in clinical samples. For each sample, amplification reaction is performed in two tubes containing each 5 µL of DNA/RNA extract and one of the two types of reaction mixtures allowing the detection of either HSV-1, HSV-2 and VZV or CMV, EBV, HHV-6, HHV-7, HHV-8 and HEVs. The analyses were performed in 8-wells strips according to the manufacturer’s instructions. For each sample analyzed, the accuracy of the DNA microarray detection was controlled during extraction and amplification through an internal control and during hybridization with at least 3 probes per amplified target detected. Following the phases of amplification, hybridization of the probes and colorimetric detection of the hybridized probes, each microarray system was analyzed using a microarray reader piloted by specific software.
provided by the manufacturer, which allowed an automatic detection and interpretation of the results (33).

**Viral load assessment in the CSF samples.** The viral load in the positive CSF samples selected was determined either by commercially available (EBV R-Gene®, HSV1 HSV2 VZV R-Gene®, Argene®, Verniolle, France) or by previously described in-house real-time RT-PCR and PCR assays (12, 13, 17, 25). Viral load levels were then expressed as the number of genomic DNA or RNA copies by milliliters (mL) of CSF.

**Statistical analyses.** Statistical comparison of demographic characteristics, laboratory findings and clinical features between children demonstrating single infection by HEVs or mixed infection by HHV-7 and HEVs were conducted by utilizing the two-tailed Fisher’s exact test to compare qualitative variables, and Student’s t-test for quantitative variables. These analyses were carried out with SPSS 11.0 program (SPSS, Paris, France). A *P*-value <0.05 was considered statistically significant.
RESULTS

Analytical performances of the multiplex RT-PCR DNA microarray. The European proficiency panels were processed as described in the Materials and Methods section resulting in 4 replicates for each proficiency sample. The Figure 1 depicted the results obtained from the analysis of the QCMD external quality assessment panels for HSV-1, HSV-2, VZV, CMV, EBV, HHV-6 and HEVs with the multiplex RT-PCR DNA microarray and comparison to the overall datasets submitted by the participants to the same QCMD programs. In summary, the lowest viral load detected at least once for the 6 herpesviruses tested ranged from 166 copies for HHV-6 to 266 copies/mL for EBV. Moreover, the reproducibility tests indicated that viral loads of 166, 182, 211, 171 and 524 copies/mL were detected in 3 of the 4 analyses performed (75%) for HHV-6, VZV, CMV, HSV-1 and EBV, respectively. Concerning HSV-2, 861 copies were detected only once whereas 1099 copies were constantly detected during this evaluation. Overall, QCs >250 copies/mL for the CMV, >500 copies/mL for HHV-6, and >2000 copies/mL for HSV-1, HSV-2, VZV and EBV were systematically detected reflecting the minimal concentrations at which 4 of the 4 replicates performed were positive (Fig. 1). For HEVs, the kit failed to detect the QCs corresponding to 280 and 390 genome copies/mL. The viral loads at 480 and 1480 copies/mL were detected in 50% and 75% of the analyses, respectively (Fig. 1). Neither cross-reactivity nor misidentification between herpesviruses was observed during the proficiency panels analyses with the multiplex RT-PCR DNA microarray.

Finally, the rates of detection obtained for each virus with the DNA microarray were compared to the overall datasets for the corresponding EQA programme submitted to QCMD by the participants whatever the molecular technique used. Discrepant results were mainly observed for HSV-2 and HEVs and concerned samples containing low viral concentrations: 224 and 861 copies/mL of HSV-2, and 280 and 390 copies/mL of HEVs; these samples were detected by the microarray in 25% of the analyses for HSV-2 but never for HEVs whereas the
same specimens were respectively detected at 67 and 89%, and at 27 and 41% by the overall participants to the QCMD. Besides, discrepancies were also observed for the lowest concentrations of the EBV (266 and 541 copies/mL) and HHV-6 (209 copies/mL) panel samples. These samples were detected by the DNA microarray both at 50% for EBV and at 25% for HHV-6 in comparison to the respective detection rates of 75, 88 and 51% obtained by the overall participants to the QCMD (Fig. 1).

**Clinical evaluation from CSF samples.** In order to perform a clinical evaluation of the multiplex RT-PCR DNA microarray, 78 CSF samples that had been previously analyzed by standardized commercially available RT-PCR and PCR assays were retrospectively tested. For each of the 58 CSF samples that had been previously tested positive for herpesviruses or HEVs, the viral load was determined. The Figure 2 shows for each virus the values of the viral load assessed per mL of CSF samples. Interestingly, HSV-1 and HHV-6 demonstrated the highest levels of viral load in the CSF samples selected for this study whereas the lowest viral concentrations were observed in CMV and HSV-2 infections (Fig.2).

Of the 58 virus positive clinical samples investigated, Clart® Entherpex kit detected 27 of the 28 (96%) CNS infections due to herpesviruses and all (100%) the 30 HEVs related aseptic meningitis. No misidentification among herpesviruses was observed. Only one EBV infection was missed demonstrating a 98.3% agreement (57/58) with the results initially obtained with the Argène® PCR and RT-PCR assays (Table 1). Concerning this specific case, the EBV load assessed by quantitative real-time PCR assay was 135 copies/mL corresponding to the lowest viral load identified in CSF samples whatever the virus considered (Fig. 2). Of the 20 virus negative clinical samples investigated, no discrepant result was observed between the DNA microarray and the Argène® PCR and RT-PCR assays. In summary, the DNA microarray demonstrated, by comparison to the routinely used PCR and RT-PCR assays...
considered as the “gold standard”, a sensitivity of 98.3%, a specificity of 100%, a positive predictive value of 100% and a negative predictive value of 95.2%.

Detection of HHV-7 and HEVs mixed infections. Among the 78 CSF samples tested, 12 (15%) HHV-7 infections were detected by the DNA microarray. HHV-7 was detected in 11 (37%) of the 30 HEVs infected CSF samples and as the sole virus in one of the 48 remaining CSF samples (2%) leading HHV-7 infection to be significantly associated with the HEVs infection in children suffering from aseptic meningitis (37% vs 2%; P=0.008). Detection of HHV-7 in the 12 positive CSF samples was confirmed by quantitative real-time PCR assay demonstrating viral loads ranging from 60 to 300 genome copies per mL of CSF (mean value = 163 ± 96 copies/mL) (12).

Table 2 summarizes the demographic, clinical and therapeutic characteristics of the 11 patients co-infected by HHV-7 and HEVs by comparison to the 19 children presented with single HEVs infection. Univariate statistical analyses revealed that HHV-7 and HEVs mixed infection caused significant longer length of stay at the hospital than infection with HEVs alone (2.4 vs 1.4 days; P=0.038) (Table 2). No other parameters such as demographic characteristics, biological findings, clinical data or treatments appeared to be statistically associated with HHV-7 and HEVs mixed infections (Table 2). Furthermore, no difference between the mean values of HEVs load levels in CSF samples of children presenting with mixed HHV-7 and HEVs (1.8 x 10^4 ± 2.0 x 10^4 copies/mL) or single HEVs infection (2.0 x 10^4 ± 4.2 x 10^4 copies/mL) was observed (P=0.212) (Table 2).
DISCUSSION

Molecular techniques are now considered as the “gold standard” for the detection in CSF samples of the viruses responsible for CNS infections (11, 19). The molecular tests used in routine diagnosis have to be specific and highly sensitive allowing rapid and valuable detection of RNA and DNA viruses. At the present time, the diagnosis of viral CNS infections is usually obtained through the combination of multiple PCR and RT-PCR assays resulting in laboratory confirmation of approximately 45% of physician-diagnosed cases (4, 27). This failure can be explained by the inconsistency between the small volume of CSF available and the large number of virus potentially responsible for meningitis or encephalitis (15). In a first step, the use of a multiplex PCR approach allowed to broaden the detection of neurotropic viruses (2, 6, 7, 8, 22, 29, 31, 32, 41, 44). Nevertheless, some multiplex PCR requires confirmation by hybridization of the amplicons to specific probes fixed onto microwell plates, which have limited capacities of probe-binding sites (6). The hybridization of hundreds of probes on a DNA microarray has risen above this constraint increasing the diagnostic output of the multiplex PCR assay. The previously published multiplex PCR followed by microarray-based detection showed concordant results with single end-point PCR tests assessing the reliability of the method (4, 16). The present study aimed at the preliminary evaluation of the analytical and clinical performances of the first CE and IVD marked commercially available multiplex RT-PCR DNA microarray allowing the simultaneous detection of nine neurotropic DNA and RNA viruses in CSF samples.

The first phase of the evaluation was carried out using 28 samples of the 2008 and 2009 QCMD panels to assess the sensitivity and the reproducibility of the DNA microarray for herpesviruses’ and HEVs’ detection. Through the 4 independent analyses performed, the kit demonstrated a limit of detection less than 500 copies/mL for all the 6 herpesviruses tested (Fig. 1). However, the analytical sensitivity of the test corresponding to the copy number of each one of the viruses that can be detected in 100% of the analyses performed were between
500 to 1000 for HHV-6 and greater than 2000 copies/mL for HSV-1, HSV-2, VZV and EBV. Regarding HEVs detection, the low viral loads corresponding to 280 and 390 genome copies/mL were not detected whereas 1480 copies/mL was tested positive in 3 of the 4 analyses performed, therefore suggesting a lower sensitivity of the microarray regarding these viruses. By comparison, the manufacturer’s claims regarding the analytical sensitivity of the assay are 10 copies for VZV, HHV-7 and HSV-1, 100 copies for HSV-2, CMV, EBV, HHV-6, HHV-8 and the coxsackieviruses, and 1000 copies for the echoviruses and the polioviruses per PCR reaction containing 5 µL of DNA/RNA extract. Finally, the comparison of these data to those recorded by the overall QCMD participants, whatever the molecular technique used, showed discrepant results for QCMD samples containing low concentration of HSV-2 and HEVs (Fig. 1).

The second phase of the evaluation consisted of the retrospective analysis of 78 CSF samples initially tested negative or positive for either herpesviruses or HEVs by standardized commercially available RT-PCR and PCR assays. This clinical evaluation confirmed the reliable diagnosis of the herpesviruses’ CNS infections by the DNA microarray since 27 of the 28 herpesvirus positive CSF samples were detected demonstrating high concordance with the results initially obtained using the multiplex end point PCR kit Herpes Consensus Generic® (Argène®, Verniolle, France) (Table 1). Only one EBV infection characterized by a low viral load was not detected by the DNA microarray. Moreover, Clart® Entherpex kit did not reveal any sensitivity defect regarding HEVs detection as previously suggested by the QCMD samples analysis since all (100%) the 30 HEVs positive CSF samples were detected. This could be explained by the viral load levels assessed in CSF samples, which ranged from 2.9 to 5.2 log_{10} copies/mL. The viral loads thus appeared to be high in case of HEVs-related meningitis and most often higher than the virus quantities contained in the lowest QCMD samples not detected by the microarray (Fig. 2). This finding was confirmed by previously
published studies demonstrating that the viral loads ranged from 3.1 to 4.6 log\(_{10}\) copies/mL of CSF in HEVs CNS infections (9, 18, 24).

Interestingly, the multiplex RT-PCR DNA microarray detected 11 (37%) HHV-7 and HEVs mixed infections among the 30 paediatric aseptic meningitis cases initially related to HEVs. While HEVs are well known neurotropic viruses, HHV-7 infection remains a neglected topic (42, 45). However, this CD4+ T-lymphotropic herpesvirus has been shown to contribute significantly to the burden of disease in young children with suspected encephalitis or severe convulsions with fever (37, 39, 46). In the present study, the presence of HHV-7 was confirmed by qPCR (12). Moreover, the lack of correlation between HHV-7 detection and CSF leukocyte counts suggested that the HHV-7 DNA was from actively replicating virus and not just latent HHV-7 DNA carried in inflammatory cells (\(P=0.29\)) (Table 2). Statistical analyses of the demographic, clinical and therapeutic characteristics revealed that HHV-7 and HEVs mixed infection resulted in significant longer length of hospitalization for children suffering from aseptic meningitis than infection with HEVs alone (\(P=0.038\)) (Table 2). Risk factors and consequences of this co-infection should be assessed in larger prospective studies.

However, these preliminary data let open the question of the role of HHV-7 as HEV meningitis co-factor associated with increased severity of the disease. Finally, this first description of combined HHV-7 and HEVs infection highlighted the advantage of the DNA microarray technology for the detection of mixed viral CNS infections as well as for the investigation of the pathogenicity of neglected viruses (33).

Concerning the practical aspects of the kit, the CSF sample analysis with the DNA microarray was performed from 10µL of total nucleic acids extract. The total time to complete the assay was approximately 8 hours from specimen extraction to microarray detection, thereby allowing laboratory to provide the answer to the clinician in a single working day. However, several limitations of the DNA microarray were observed during this
study. Numerous handlings were required during the analysis and opportunities of automation remained limited. Moreover, the kit cannot be used without a microarray reader piloted by specific software provided by the manufacturers thus limiting the implementation of the kit in virology laboratories. By comparison to the one-step real-time PCR assays frequently used in current molecular virological diagnosis, the major drawback of the technique was the handling of amplicons. Although no contamination event was observed during this evaluation, a potential risk of contamination could not be ruled out. Finally, DNA microarray technology, which is a multiplex end point PCR system, did not allow the quantitation of the viral load in the clinical samples. However, it has been assumed that quantitation of viral nucleic acid may be useful in monitoring the effectiveness of antiviral therapy, and for establishing the prognosis of the disease (9, 28, 43).

In conclusion, the evaluated multiplex RT-PCR DNA microarray appeared to be a sensitive and a specific test allowing rapid (in one working day) and simultaneous detection from a small volume of CSF sample of the main neurotropic DNA (herpesviruses) and RNA (enteroviruses) viruses. Moreover, our findings suggest that this molecular diagnostic tool could improve the routine virological diagnosis and the therapeutic management of CNS infections by detecting multiple viral infections potentially responsible for meningitis or encephalitis with increased severity. However, due to the small number of CSF specimens tested, further studies are needed to better characterize the performances of this test before its use in routine patient care.
Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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References

   Infect. Dis. 26:403-412.


Figures captions:

Fig.1. Analysis of the QCMD external quality assessment panels with the multiplex RT-PCR DNA microarray and comparison to the overall datasets submitted by the participants to the QCMD. The quality controls were independently tested 4 times each. Rates of detection obtained with the microarray ranging from 0 to 100% are depicted with the shade of grey or the pattern used for the histograms while the numbers at the top of the bars indicate the results obtained by the participants to the QCMD. The performances of the DNA microarray regarding the detection of HHV-7 and HHV-8 have been not evaluated since no QCMD panels containing these viruses are available.

Fig.2. Viral load levels determined in CSF samples, initially positive for either herpesvirus (HSV-1 to HHV-6) or human enteroviruses, retrospectively tested with the DNA microarray. Copy numbers were plotted on a logarithmic scale. The mean value of the viral loads is indicated for each virus within the graph and depicted by a black line. The numbers indicated within the square brackets on the X-axis correspond to the range values of the viral loads determined for each virus in CSF samples. The number of CSF specimens analysed for each virus is indicated below. The sample initially tested positive for EBV with EBV R-Gene® and missed by CLART Entherpex® is depicted by an empty square. No known CSF sample positive for HHV-7 or HHV-8 has been tested.
**Table 1.** Summary of the CSF specimens testing results obtained with the multiplex RT-PCR DNA microarray by comparison to the standardized commercially available PCR and RT-PCR assays for neurotropic virus detection.

<table>
<thead>
<tr>
<th>Herpes Consensus Generic®</th>
<th>HEVs RT-PCR assays</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Multiplex RT-PCR DNA microarray</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>50</td>
</tr>
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</table>
Table 2. Comparison of demographic, clinical and biological parameters and of the patient’s management between children co-infected by HHV-7 and HEVs and children presented with single HEVs infection. Mean values are indicated ± the standard deviation.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Enterovirus/HHV-7 mixed infection N=11</th>
<th>Enterovirus single infection N=19</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (years)</td>
<td>5.73 ± 3.79</td>
<td>8.44 ± 6.66</td>
<td>0.19</td>
</tr>
<tr>
<td>Male</td>
<td>9 (81.8%)</td>
<td>13 (81.3%)</td>
<td>0.97</td>
</tr>
<tr>
<td>Fever (°C)</td>
<td>38.3 ± 0.87</td>
<td>38.1 ± 0.39</td>
<td>0.64</td>
</tr>
<tr>
<td>CSF analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-alpha (UI/mL)</td>
<td>7.60 ± 7.36</td>
<td>3.36 ± 3.32</td>
<td>0.12</td>
</tr>
<tr>
<td>Protein (mg/dL)</td>
<td>0.76 ± 0.88</td>
<td>0.39 ± 0.12</td>
<td>0.20</td>
</tr>
<tr>
<td>Leukocytes (/µL)</td>
<td>49 ± 34</td>
<td>91 ± 148</td>
<td>0.29</td>
</tr>
<tr>
<td>Enterovirus load (copies/mL)</td>
<td>$1.8 \times 10^4 \pm 2.0 \times 10^4$</td>
<td>$2.0 \times 10^4 \pm 4.2 \times 10^4$</td>
<td>0.21</td>
</tr>
<tr>
<td>Blood analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes ($10^9$ cells/L)</td>
<td>12.0 ± 2.6</td>
<td>12.5 ± 4.5</td>
<td>0.74</td>
</tr>
<tr>
<td>AST (UI/L)</td>
<td>37.0 ± 7.6</td>
<td>32.5 ± 17.2</td>
<td>0.56</td>
</tr>
<tr>
<td>ALT (UI/L)</td>
<td>42.0 ± 32.4</td>
<td>43.9 ± 50.4</td>
<td>0.94</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>10.55 ± 14.6</td>
<td>25.1 ± 30.3</td>
<td>0.12</td>
</tr>
<tr>
<td>Procalcitonin (ng/mL)</td>
<td>0.82 ± 1.11</td>
<td>0.26 ± 0.18</td>
<td>0.28</td>
</tr>
<tr>
<td>Patient’s management</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>antibiotic use</td>
<td>(27.3%)</td>
<td>(14.3%)</td>
<td>0.42</td>
</tr>
<tr>
<td>antiviral use</td>
<td>(0%)</td>
<td>(14.3%)</td>
<td>0.19</td>
</tr>
<tr>
<td>length of stay (day)</td>
<td>2.40 ± 1.26</td>
<td>1.38 ± 0.65</td>
<td><strong>0.038</strong></td>
</tr>
<tr>
<td>Death</td>
<td>(10%)</td>
<td>(7.1%)</td>
<td>0.80</td>
</tr>
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</table>