Annals of Case Reports
Beligni G, et al. Ann Case Rep: 9: 101695
www.doi.org/10.29011/2574-7754.101695
www.gavinpublishers.com

Case Report

Detection of RSV Genome in Cerebrospinal Fluid of a Patient with Guillain Barrè Syndrome: A Case Report

Giada Beligni[1], Sabina Bartalini[2], Maria Grazia Cusi[1*]

[1]Department of Medical Biotechnologies, University of Siena, Viale Bracci, 1, 53100, Siena, Italy

[2]Department of Medical, Surgical and Neuroscience, Neurology and Clinical Neurophysiology Unit, Policlinico 'S. Maria delle Scotte', 53100 Siena, Italy

***Corresponding author**: Maria Grazia Cusi, Department of Medical Biotechnologies, University of Siena, Viale Bracci, 1, 53100, Siena, Italy; Email: mariagrazia.cusi@unisi.it

Citation: Beligni G, Bartalini S, Cusi MG (2024) Detection of RSV Genome in Cerebrospinal Fluid of a Patient with Guillain Barrè Syndrome: A Case Report. Ann Case Report. 9: 1695. DOI:10.29011/2574-7754.101695

Received: 04 March 2024, **Accepted:** 09 March 2024, **Published:** 12 March 2024

Abstract

Respiratory syncytial virus is one of the major causes of serious lower respiratory tract infections in childhood leading to bronchiolitis and pneumonia. In addition to the common respiratory symptoms, non-respiratory manifestations due to RSV have also been reported. We report a case of a man, with a suspicion of a GBS-like syndrome, obscured by a compromised respiratory clinical picture. Although the routine lumbar puncture did not reveal the presence of any infectious agent, a secondary metagenomic analysis revealed the presence of RSV type B, broadening the knowledge on demyelinating disorders, which appear unrelated to this virus in adults. This is the first case of Guillain Barré syndrome with a clinical diagnosis associated with a metagenomic analysis revealing RSV genome in the cerebrospinal fluid. This fact underlines the importance of investigating respiratory viruses in subjects with neuropathies through CSF molecular testing.

Keywords: RSV; Guillain Barrè Syndrome; Metagenomic analysis

Introduction

Respiratory Syncytial Virus (RSV) is the major cause of serious lower respiratory tract infections in childhood [1], leading to bronchiolitis and pneumonia [2], as well as being the major cause of hospitalization in pediatric age [3]. RSV may infect a various spectrum of people of all ages: young, adults and elderly [4]. Besides the respiratory symptoms, extrapulmonary manifestations have also been reported; there are evidence of myocarditis [5], hepatitis [6] and encephalopathy [7]. The disease is often mild, but it can cause severe complications, especially when involving the central nervous system (CNS). Patients with an involvement of CNS showing convulsion and seizures have been identified in approximately 1.2-1.8% of patients hospitalized with RSV [8]. Specific adult cases of RSV-related meningoencephalitis have been reported in a limited number of patients in association with other viral infections, such as influenza [9]. Guillain-Barré syndrome (GBS), which is a rare, acute, acquired, monophasic autoimmune disorder of peripheral nerves, has been associated to RSV. This disease develops in susceptible individuals after infection and, in rare cases, after immunization [10,11]. It leads to acute, flaccid paralysis and can be associated with some microorganisms, such as Campylobacter jejuni, Zika virus, SARS-CoV-2 [12] and, recently, RSV [13]. Most people fully recover from GBS, but some have permanent nerve damage. About two-thirds of people with GBS are sick with diarrhea or respiratory illness, days or weeks before developing symptoms, therefore an infectious etiology seems likely. Direct CNS infection and neurological symptoms

are usually confirmed by molecular tests. The detection of viral genome in the cerebrospinal fluid (CNS) supports the clinical diagnosis. Unfortunately, since the routine diagnostic tests are not always able to identify the presence of a causative agent inside a patient sample, Metagenomic analysis can help to identify the infective causative agent with a Next Generation Sequencing (NGS) approach, as previously demonstrated [14]. Here we report a case of a man, coming to our attention for a suspected GBS-like syndrome, hidden by a compromised respiratory clinical picture. The routine lumbar puncture resulted negative for all investigated viruses, but a secondary metagenomic analysis revealed the presence of RSV, broadening the knowledge on demyelinating disorders, which appear unrelated to this virus in adults. A literature systematic review was conducted on PubMed and Web of Science. The keywords 'RSV' and 'Guillain Barrè' was used. The results are listed in Table1.

Materials and Methods

RNA and DNA were extracted from a CSF sample (200ml) by using ELITe InGenius instrument (Elitech, France), which integrates extraction, amplification for viruses and bacteria. Epstein-Barr virus, Herpes simplex virus type 1, Herpes simplex virus type 2, Herpesvirus-6, Cytomegalovirus, Varicella Zoster virus, Adenovirus, Measles virus, Picornavirus, JC Polyomavirus, West Nile Virus, Toscana Virus, Mycoplasma pneumoniae, Borrelia and Mycobacterium tuberculosis were tested. HTLV-1 DNA was extracted from CSF by use of an EZ1&2 DNA Blood kit (Qiagen GmbH, Germany) and amplified as described (Moens B et al., 2009).

Next Generation Sequencing of Cerebrospinal fluid

DNA was extracted with RNeasy plus mini kit (Qiagen GmbH, Germany) where a selective depletion of the genomic DNA and the Ribosomal RNA (rRNA) is carried out using specific filtering columns. Quantification of the obtained RNA was carried out with the Qubit RNA high sensitivity assay kit and the Qubit 3.0 fluorometer (Thermo Fisher Scientific, Rodano, (MI), Italy). The retro transcription and library preparation were assessed using the NEBNext Single Cell/Low Input RNA Library Prep kit for Illumina (New England Biolabs, Milan, Italy) according to the manufacturer's protocol. The obtained library was then quantified with Qubit DNA high sensitivity assay kit and the Qubit 3.0 fluorometer (Thermo Fisher Scientific). The median size was assessed with a 2100 biological analyzer (Agilent Technologies, Milan, Italy) using a high sensitivity DNA Chip. Libraries were then pooled and denatured according to Illumina's instructions and charged on a V2 micro MiSeq 300 cycles flow cell (Illumina S.r.l. Milan, Italy). FastQ analysis was carried with the CZ ID and Genome detective online platform (https://czid.org) (https://www.genomedetective.com)

Case Presentation

On January 5th, 2022, a 25-year-old man was admitted to the Emergency Department of the 'S. Maria delle Scotte' Hospital in Siena, Italy, with fever, cough, pneumonia, hypoasthenia in limbs (left>right), pain in the left leg and speech disorder. Laboratory results, including complete blood count, metabolic panel and blood cultures, were unremarkable; only an increase of leukocytes (11,96 x 103/mm^3) was evidenced. The oropharyngeal swab revealed the presence of RSV by RT-PCR, while influenza virus type A/B and SARS-CoV-2 were negative. Thorax RX did not show parenchymal alterations at foci, nor signs of pleural effusion. A few days later, the patient developed weakness in the lower limbs, more pronounced on the left side, along with pain and language impairment and on January 17th he was admitted to the Neurology Unit. The neurological examination revealed difficulty walking on tiptoes. Romberg test showed slight multidirectional swaying. Bilateral facial diplegia with the remaining cranial nerves was within normal limits. No strength deficits against gravity or resistance in all four limbs were present. Deep tendon reflexes were normal and symmetrical in the upper limbs, absent in the lower limbs except for the patellar reflexes. Cerebellar tests were correctly performed. The patient reported numbness in the lower limbs, especially on the left side. Laboratory tests (complete blood count, negative procalcitonin, C-reactive protein, lactate dehydrogenase, creatine kinase, serum electrolytes, liver and kidney function) were normal. Chest X-ray did not show any abnormalities. A CT scan and later an MRI scan of the cranial and lumbar spine were normal. Cerebrospinal fluid (CSF) analysis was normal; In particular, there was no albuminocytologic dissociation. Isoelectric focusing showed the presence of some oligoclonal IgG at the same isoelectric point in serum and cerebrospinal fluid. CSF polymerase chain reaction assay (PCR) for Epstein-Barr virus, Herpes simplex virus type 1, Herpes simplex virus type 2, Herpesvirus 6, Cytomegalovirus, Varicella Zoster virus, Adenovirus, HTLV-1, Measles virus, Picornavirus, JC Polyomavirus, West Nile Virus, Toscana Virus, Mycoplasma pneumoniae, Borrelia and Mycobacterium tuberculosis was negative. Furthermore, the search for anti-ganglioside and sulfatide antibodies and anti-onconeural antigen antibodies were negative in both blood and cerebrospinal fluid. A neurophysiological study documented an acute motor axonal neuropathy (AMAN). The patient was diagnosed as a case of AMAN (a GBS subtype) based on history, clinical findings and EMG examination. He was treated with plasma-exchange (5 sessions) with good tolerability and complete clinical recovery. During the ten-day stay in hospital, the patient improved, in particular the orbicularis oculi bilateral deficit decreased, although less improvement for the orbicularis

oris muscle deficit was observed. Due to the negative results in molecular diagnosis, a second-generation sequencing technology (NGS), not limited by targeted primers, was performed on the CSF. NGS has a fast detection speed and is capable of diagnosing infectious diseases of the central nervous system with speed and efficiency.

Discussion and Conclusions

Direct information on pathogenic microorganisms from brain tissue and CSF can be obtained via NGS with high throughput, thus speeding up the detection process and eliminating the drawbacks of targeted diagnostic methods. Current diagnostic procedures are complicated by the extensive and growing list of viral pathogens that cause encephalitis, and in up to 50% of cases the etiology remains unknown. The need to specify targets in advance is eliminated by sequence-agnostic metagenomic next-generation sequencing (mNGS), which is thus highly promising for the diagnosis of encephalitis and other forms of nervous diseases caused by infectious agents. However, the low relative abundance of virus-derived material in clinical specimens compared to host-derived material makes viral mNGS a difficult approach. To ensure reliable detection and accurate identification of viruses in a sample, it is essential to improve this ratio and achieve a sufficient amount of viral reads [15-19]. We set a procedure to enhance the sensitivity of the assay, trying to detect viral agents that were not revealed by classical molecular tests. In this case, the patient was first hospitalized, due to pneumonia, and RSV was detected as the etiologic agent. After a few days, the disease worsened and the man began to show neurological problems. Analyses were then focused on pathogens infecting the central nervous system, without success. Therefore, NGS was performed on the patient's CSF in order to assess the presence of a virus. The blood-brain barrier (BBB), the essential homeostatic bridge to neural function, plays a vital role in neural protection and molecular passage of microorganisms. Hematogenous spread of viral pathogens is the common mechanism allowing cellular migration of the virus, therefore neurotropism is necessary for viral pathogens to invade the CNS. Before the onset of GB syndrome, most patients showed an antecedent illness, most commonly an upper respiratory tract infection [12]. Extrapulmonary manifestations of RSV involving neurologic, cardiovascular, and hepatic organ systems are well reported [20]. However, neurologic abnormalities, such as convulsions, following cardiopulmonary arrest, apnea, and nuchal rigidity have been mainly reported in children infected with RSV [8]. Saravanos et al. [21] undertook a systematic review that included articles concerning patients <15 years old with RSV-associated severe acute neurologic complications. However, due to the high incidence of RSV viral infection and low prevalence of complications, host factors must also play a role [21]. By contrast, scant evidence exists in the literature implicating RSV as a neuropathogen in the adult population. RSV was detected in the CSF of some patients, as shown in this report, making direct viral invasion of the CNS plausible. A case of Respiratory Syncytial Virus-associated encephalopathy, in which the virus was detected in cerebrospinal fluid and intratracheal aspiration despite negative rapid test results, has been reported [22]. The exact mechanism of CNS penetration and pathologic response of RSV are unknown; however, it is fundamental for diagnosis to verify whether the subject presenting neurologic complications has or had previous respiratory infection, and to enlarge the panel of viruses to look for, including respiratory viruses. While in children the virus antigens seem to trigger an inflammatory response producing a 'cytokine storm', which mediates immune cell over activation and cellular dysfunction within the CNS, in adults the virus seems a direct cause of the disease, as shown in this case. The detection of RSV in CSF suggests that it could have potential neurotropism and play a role in the pathogenesis of the nervous system. Therefore, the pathogenesis of respiratory virus-associated encephalopathy is likely diverse and may include both direct and indirect pathways. To prevent and treat infections that affect the nervous system, the responsible pathogens must be identified, and NGS could be a valid tool to aid the diagnosis of these infectious diseases. This case underscores the importance of physicians being mindful of the clinical presentation of GBS, and the importance to also investigate on the presence of respiratory viruses, such as RSV, as etiologic agents.

Author	Age	Clinical manifestation	Diagnosis	Therapy	Clinical course
Gupta M et al. (2023) doi: 10.1016/j. pediatrneurol.2023.06.013	2y	Croup and Guillain Barrè syndrome	Positive PCRfor RSV in the Respiratory viral panel	IVG treatment racemic epinephrine + dexamethasone	Benign
Helgeson S.A. et al. (2018) [12] doi: 10.4103/ijccm.IJCCM_171_17	81y	Cough, runny nose, and sore throat followed by Guillain Barrè syndrome	Positive PCR for RSV in the nasopharyngeal swab	Ribavirin plasmapheresis	Complicated
Erler T. et al. (2008) [21] doi: 10.1055/s-2007-971050	10m	Respiratory infection by RSV followed by Guillain Barrè syndrome	Positive PCR for RSV in the nasopharyngeal swab	Corticosteroid + IVG treatment	Benign
Push E. et al. (2018) [22] Review doi: 10.1007/s15007-018-1580-4	Children<5y and adults>65y	Respiratory virus-induced heterologous immunity (HI)	Humoral and cellular Cross-reactivity	Structured literature search	Structured literature search

Table 1: Literature cases of Guillan-Barrè syndrome associated with RSV infection.

A summary of the literature cases of Guillan-Barrè syndrome associated with RSV infection. For each article we have registered the following data: first author, clinical manifestation, molecular diagnosis, therapy and clinical course.

Acknowledgements: This study was supported by the Italian Ministry of Health- CUP B93C2200121000, CCM 2022 'SURVEID'.

Ethical approval and consent to participate: The study has been performed in accordance with local ethics committee requirements and has therefore been performed in accordance with the ethical standards laid down in the Declaration of Helsinki in 1964 and in its later amendments. Written informed consent was obtained from the patient.

Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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