

First utilization of BioFire FilmArray[®] Respiratory Panel 2 Plus in post-mortem diagnosis of Influenza A H1N1

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Summary

Post-mortem microbiology is an important tool in legal medicine and forensic pathology; this case report shows the importance of microbiological testing to establish the cause of death in infection suspected death. We refer a case of fatal Influenza A (H1N1) diagnosed only after death with *post-mortem* microbiology tool.

Introduction

Post-mortem microbiology is an important tool in legal medicine and forensic pathology and might help determine the death cause and mode. Sudden unexpected death is one of the most important matters in forensic medicine; making a differential diagnosis between internal infection and other causes is crucial (1-4). In our case, the *post-mortem* microbiological diagnosis has

been an essential tool for understanding the nature of the unexpected death and determine the disease aetiology by detecting H1N1 Influenza A virus. The main indications for its use are like a part of a standard protocol as sudden unexpected death or a clinically suspected but not confirmed ante-mortem infection (5,6). In Italy, forensic microbiology techniques are not included in the standard autopsy protocols. We report a case that shows the essential role of microbiological testing to establish the cause of death from suspected infection. We refer a case of fatal influenza A H1N1 virus diagnosed only after death with *post-mortem* microbiology tools.

Case Report

A 30-year-old man was admitted to the emergency ward due to respiratory syndrome. Chest x-ray highlighted bilateral basal opacity and a reticular interstitial thickening. An empirical antibiotic therapy was started with Levofloxacin 2×500 mg/dL. He was then transferred to the medical ward where was administered a continuous treatment; on the second day his clinical condition worsened, with an increase in temperature (41.3°C) and acute respiratory distress syndrome. Laboratory tests showed neutrophilic leucocytosis (WBC 10,400/μL, Neutrophils 79,8%), an increased C Reactive Protein (CRP = 161,00 mg/dL, normal values <0.5 mg/dL), normal level of Procalcitonin, negative Legionella and Mycoplasma antibodies. Unexpectedly, the same day the patient died.

An autopsy was requested and performed within 24 hours of death. The gross macroscopic investigation revealed that both lungs were mildly oedematous, the pleural and cardiac cavities were carrying a 250 and 50 mL of fluid respectively, abdominal organs and adrenal glands were normal. No signs of haemorrhage were found. The coroner, together with the forensic microbiologist, performed sampling of cardiac and peripheral blood, lungs, fluid of pleural cavity, heart and fluid of cardiac cavity, kidney, liver, intestinal matter and brain for microbiological and histologic examinations. All samples were analysed for bacterial detection, and intestinal matter, blood and cerebrospinal fluid were also collected to detect viral agents. Nasal, pharyngeal and lungs swabs were also taken with the aim to perform molecular research. All specimens were handled following Riedel's recommendations, using dedicated instruments and iodine scrubs to sterilize the whole-body surfaces. Tissue samples, homogenized in sterile PBS, and swabs underwent culture analysis using both non-selective and selective media (Columbia blood agar, Chocolate agar, MacConkey agar, Sabouraud agar, Mannitol agar): they were then

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incubated under aerobic and anaerobic atmosphere at 37° C up to seven days. The blood and cerebrospinal fluid were inoculated into a BACTEC blood culture system (Beckton Dickinson, Italia) by using paediatric bottles and incubated for seven days. Nasal, pharyngeal and lung swabs were analysed by FilmArray Respiratory Panel 2 Plus (RP2_{plus}) (BioMerieux, France) according to the manufacturer's instructions. The viral assay detected H1N1 Influenza A in nasal, pharyngeal and lungs swabs, while all samples were negative for bacterial organisms.

Discussion and Conclusions

We report here a *post-mortem* diagnosis of pulmonary and systemic post-mortem pathology of a patient fatally infected with H1N1 influenza A virus performed by Biofire Filmarray® Respiratory Panel 2 Plus (RP2_{plus}). Influenza A viruses cause respiratory infections that range from an asymptomatic picture to a fatal course picture. Influenza A viruses undergo continuous genetic variation as they replicate, enabling viruses with advantageous antigenic mutations to spread and cause disease in naïve or previously immune or vaccinated individuals. Human influenza A can date back to the “*Spanish flu*” in 1918, which infected almost 500 million people around the world and killed almost 60 million of them. In 2009, a triple reassorting strain of human influenza A H1N1 subtype caused severe outbreaks around the world and gradually replaced the old lineages (7-10). Respiratory Panel 2 Plus (RP2_{plus}) is a multiplexed nucleic acid test that can detect a broad spectrum of respiratory microorganisms like respiratory virus, such as Influenza A and B, Coronavirus or bacterial organisms like *Bordetella pertussis*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*. This tool have already shown benefits in terms of sensitivity gained and a greater range of detected pathogens in comparison with the conventional techniques (11-14). Particularly, the whole process takes only about an hour; compared with multiple PCR, the FilmArray RP has provided faster results. For some viruses, like Influenza A, a rapid diagnosis may help prevent secondary spread. In children and adults, neuraminidase inhibitors reduce time of symptoms resolution by 0.5-2.5 days when administered within 48 h of symptoms onset while a timely treatment with neuraminidase inhibitors reduces the development of complications, such as pneumonia (15,16).

No study in literature so far has highlighted the use of this technique in post-mortem diagnosis. To the best of our knowledge, our case is first report for scientific community.

Unfortunately, in Italy diagnostic microbiology is seldom applied to *post-mortem* samples, and there are no validated and standardized sampling protocols. In our case, we applied the ESCMID technical protocol for forensic and *post-mortem* microbiology. In April 2019, AMCLI has established a new study group (GLAMIFO - <http://www.amcli.it/associazione/gruppi-di-lavoro/>) that represents a first step towards a consensus in *post-mortem* microbiology procedures in Italy.

We hope that our report will stimulate a closer relationship between legal-medicine specialists of and microbiologists, with the aim of making *post-mortem* microbiology a notable part of the medical-legal investigations.

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