

## Influenza-associated cardiac injury: A disease of the cardiac conduction system?

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Influenza has been recognised since the 16<sup>th</sup> century as an acute respiratory illness that spreads rapidly among communities in outbreaks<sup>1</sup>. It is typically caused viruses of the Orthomyxoviridae family that are divided in Influenza viruses A, B and C<sup>2</sup>. Influenza A viruses (IAV) have been responsible for seasonal epidemics but also sporadic pandemics resulting from distinct mechanisms of antigenic variation in the surface glycoproteins of the virus, known as antigenic drift and antigenic shift<sup>2</sup>. Virus replication typically takes place in the respiratory epithelium and causes lung inflammation<sup>3</sup>. Pneumonia may develop which might be followed by a secondary bacterial infection and in certain cases acute respiratory distress syndrome<sup>3</sup>.

IAV complications are not limited to the respiratory system but do occur in other systems and organs, such as acute myositis and central nervous system inflammatory syndromes such as encephalitis and meningitis<sup>3</sup>. Cardiac related manifestations of IAV infection have been previously characterised and may occur either due to direct effects of the virus on the myocardium or due to exacerbation of existing cardiac disease<sup>4</sup>. In the most significant recent Influenza pandemic, in 2009, myocardial injury and dysfunction were frequently observed and associated with high mortality<sup>5</sup>. The mechanisms of direct myocardial damage from IAV still remain to be elucidated.

The study by Filgueiras-Rama et al. aimed to address this knowledge gap by studying the infectivity of human IAV in the murine myocardium and to establish the relationship between infective capacity and molecular alterations<sup>6</sup>. Two recombinant viruses were used in these experiments; one with augmented (PAmut) and one with attenuated pathogenicity (PB2mut). Following intranasal IAV inoculation, viral titres were detected in both lungs and heart however the levels of the one did not correlate with the levels of the other suggesting that heart infection does not correlate with the extent of lung infection. The presence of viral mRNA (NEP) and proteins (NP) suggested myocardial replication of the virus. Infected mice demonstrated increased mortality prior to the established weight reduction of 75% of the original body weight which raised concerns about premature death of potentially arrhythmic nature.

Interestingly, myocardial histology did not reveal inflammatory infiltrates although an acute pro-inflammatory response was suspected by the presence of increased IL-1 $\beta$  and IL-6 mRNA levels in the infected murine hearts. There was also lack of increased fibrosis based on histological and magnetic resonance tissue characterisation. ECG acquisition revealed signs of significant cardiac conduction disease in the infected mice, including bradyarrhythmia, QRS prolongation that were present at the time of premature death. Viral replication was

confirmed in Purkinje Cells by the presence of the NP protein in the cytoplasm and nucleus. The observation of conduction disease was further supported by experiments of IAV transfection of human induced pluripotent stem cell derived cardiomyocytes which demonstrated decreased levels of Cx43 and NaV1.5<sup>6</sup>.

This study conveys novel insights into the pathophysiology of cardiac involvement in IAV infection and the researchers should be commended for their efforts. Interestingly, there was a lack of significant myocardial fibrosis and inflammatory infiltrates in the infected mice in contrast to reports from patients with Influenza<sup>4</sup>, but it should also be noted that histopathology analysis was performed only at 4 days post-infection. Accordingly, the authors report increased transcription of cytokines such as IL-1 $\beta$  and IL-6, suggesting activation of innate immunity, which can also indirectly affect cardiomyocyte function<sup>7</sup> and induce a status of metabolic stress, as reported in the study. The ability of these cytokines to alter the heart conduction system remains unexplored.

Another unanswered question concerns the mechanism of spreading of the infection from the respiratory system to the heart, given that the lung damage is limited and lung titres do not correlate with those measured in the heart. The authors suggest that endothelial cells, which become infected by the virus, might contribute to the spreading, presumably because of their anatomical location (Figure 1).

The demonstration of premature death and cardiac conduction disease in infected mice that were lacking significant cardiomegaly or ventricular dysfunction is a key finding but should be interpreted with caution. The occurrence of bradyarrhythmias and heart block is very uncommon in patients with influenza<sup>5</sup> but it has been reported in cases of patients immunocompromised or in cardiogenic shock<sup>8, 9</sup>. Further studies are needed to better characterize the cardiac phenotype of patients suffering Influenza infection and specifically to quantify the burden conduction disease.

Conflicts of Interest: None to declare

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**Figure 1. Key knowledge gaps in the pathophysiology of Influenza mediated cardiac disease addressed by Filgueiras-Rama et al. <sup>6</sup>**

A. The route of the viral quick spread from the respiratory system to the heart remains unclear. It is suggested by the authors that this might occur via the endothelium, which becomes infected by the virus.

B. The role of direct viral infection of Purkinje cells and cardiomyocytes relative to the indirect effect by the cytokines produced as a result of the infection to the development of cardiac metabolic stress and arrhythmias remains unresolved.

