# The case for Chronotherapy in COVID-19 induced Acute Respiratory Distress Syndrome (ARDS).

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**Abstract**

COVID-19, the disease resulting from infection by a novel coronavirus: SARS-Cov2 that has rapidly spread since November 2019 leading to a global pandemic. SARS-Cov2 has infected over 2.8 million people and caused over 180,000 deaths worldwide. Although most cases are mild, a subset of patients develop a severe and atypical presentation of Acute Respiratory Distress Syndrome (ARDS) that is characterised by a cytokine release storm (CRS). Paradoxically, treatment with anti-inflammatory agents and immune regulators has been associated with worsening of ARDS. We hypothesize that the intrinsic circadian clock of the lung and the immune system may regulate individual components of CRS and thus chronotherapy may be used to effectively manage ARDS in COVID-19 patients.

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COVID-19, the disease resulting from infection by a novel coronavirus: SARS-Cov2 that has rapidly spread since November 2019 leading to a global pandemic. SARS-Cov2 has infected over 2.8 million people and caused over 180,000 deaths worldwide. Although most cases are mild, a subset of patients develop a severe and atypical presentation of Acute Respiratory Distress Syndrome (ARDS) that is characterised by a cytokine release storm (CRS). Paradoxically, treatment with anti-inflammatory agents and immune regulators has been associated with worsening of ARDS. We hypothesize that the intrinsic circadian clock of the lung and the immune system may regulate individual components of CRS and thus chronotherapy may be used to effectively manage ARDS in COVID-19 patients.

Since December 2019, SARS-Cov2 has spread rapidly leading to a global pandemic of COVID-19 (Guo et al., 2020). Although COVID-19 is mild in the majority of cases, a subset of patients quickly develop acute respiratory distress syndrome (ARDS), a clinical presentation of acute lung injury (ALI), that leads to respiratory failure requiring mechanical ventilation (Fung, Yuen, Ye, Chan & Jin, 2020). This unprecedented crisis is equal only in magnitude to the 1918 influenza pandemic (Taubenberger & Morens, 2006). Regrettably, despite important medical and technological advancements since then, our approach to treating patients with acute lung injury following influenza or SARS-Cov2 infection remain palliative at best, with no proven pharmacological therapies (Mehta, McAuley, Brown, Sanchez, Tattersall & Manson, 2020).

A central challenge for the development of therapies that target ARDS is the myriad of pro-inflammatory mediators that are released during ARDS (Conti et al., 2020). This response in COVID-19-induced ARDS has been termed as a cytokine release storm (CRS) (Mehta, McAuley, Brown, Sanchez, Tattersall & Manson, 2020; Ruan, Yang, Wang, Jiang & Song, 2020). The CRS seen in severe cases of COVID-19 include high numbers neutrophils and low levels of lymphocytes, as well as elevated serum levels of IL-1β, IL-2, IL-6,

IL-8, IL-9, IL-10, IL-17, G-CSF, GM-CSF, IFNγ, TNFα, IP10, and MCP1 (Huang et al., 2020; Ruan, Yang, Wang, Jiang & Song, 2020). Thus, understanding the mechanisms that modulate the release of these pro-inflammatory mediators in ALI is paramount to developing effective strategies to treat ARDS.

There is an outstanding paradox that COVID-19 causes a CRS that is associated with increased lethality. Yet, reports suggest that anti-inflammatory drugs such as ibuprofen could aggravate the progression of disease. Further, recent studies in respiratory infections have shown that while anti-inflammatory agents could alleviate symptoms, they can also promote increased viral shedding (Walsh et al., 2016). Even though this possibility has not been confirmed with COVID-19, it is very plausible that this is the reason why NSAIDS could be detrimental. In this sense, ideal management of COVID-19 would entail a reduction of harmful inflammatory mediators that damage the host but maintain expression of key mediators that target the virus.

An emerging venue of therapeutic development impinges on the circadian clock, the biological timer that has been shown to control the rhythmic expression and release of many cytokines in inflammatory settings (Labrecque & Cermakian, 2015; Thompson, Walmsley & Whyte, 2014). However, despite the known effects of the circadian clock in lung diseases such as asthma (Clark, 1987), how the circadian clock influences the progression of ALI remains largely unknown.

Studying the circadian rhythm of lung injury secondary to ventilation therapy (Ventilator induced lung injury, VILI) is a current concern for COVID-19. Circadian rhythm disruption was seen in a rat model of VILI with high and low tidal volumes by studying the expression of Bmal1, clock, Per2 and REV-ERBα mRNA expression. REV-ERBα was found to have an important role in VILI and inflammation. That is, circadian rhythm disorder in inflammation response may be a novel pathogenesis of VILI.(Li, Wang, Hu & Tan, 2013) Club cells have been also found to have a role in lungs circadian rhythm. Selective ablation of these cells resulted in the loss of circadian rhythm in lung slices, further demonstrating the importance of this cell type in maintaining pulmonary circadian rhythm in one murine and human lung tissue study (Gibbs et al., 2009) .

The immune system displays circadian rhythms, for instance at the beginning of daily activity there is increased expression of pro-inflammatory mediators such as interleukin(IL)-1β, IL-6, IL-12,TLR9 and TLR4, CCL2, CXCL1, CCL5, as well as macrophage and leukocyte activity, which leads to potential damage in injured tissues. By contrast, anti-inflammatory mediators and other growth or angiogenesis factors, such as the vascular endothelial growth factor (VEGF), peak during the resting phase (Curtis, Bellet, Sassone-Corsi & O'Neill, 2014; Koyanagi et al., 2003; Liu et al., 2006; Nakamura et al., 2016; Scheiermann, Gibbs, Ince & Loudon, 2018; Vgontzas, Bixler, Lin, Prolo, Trakada & Chrousos, 2005). Moreover, both CD8 and CD4 T cells cytotoxic activity against viral antigens reach the highest levels during the resting phase (Bollinger et al., 2011; Nobis, Laramée, Kervezee, De Sousa, Labrecque & Cermakian, 2019) while the cytotoxic activity of natural killer (NK) cells was most severe at the beginning of the active part of the day (Logan et al., 2012).

The lungs also have an intrinsic circadian clock which plays a key role in inflammation and leukocyte migration in the lungs, as well as in many lung diseases including viral pneumonia (Nosal, Ehlers & Haspel, 2019; Sundar, Yao, Sellix & Rahman, 2015).

Circadian rhythms in viral respiratory illness have been so far examined in mice for parainfluenza and influenza A viruses (IAV), which cause bronchiolitis, and pneumonia in humans respectively. With either virus, acute inflammatory responses, but not the peak viral load, vary with the time of inoculation in wild-type mice (Ehlers et al., 2018; Sengupta et al., 2019). Similarly, deletion of the clock gene *Bmal1* worsens acute lung injury and lung inflammation in response to parainfluenza or IAV, suggesting that circadian clocks may play a role (immunologic or otherwise) in the resolution of viral pneumonia. Additionally, there is strong evidence from animal models that the circadian regulation within the lung is important in the likelihood of developing chronic lung disease such as pulmonary fibrosis in the aftermath of the infections.

The time of the day in which a viral infection occurs affects the survival. For instance infections at the

beginning of the activity phase are more fatal than infections that occur at the beginning of the resting phase (Sengupta et al., 2019). Evidence indicates an important role of the circadian rhythm of NK cells underlying this temporal pattern.

Due to circadian variations of the immune system and the lungs, the effect of immune modulators and anti-inflammatory agents on the activity of cells and cytokines in injured tissues also depend on the time of the day in which these medications are taken (Al-Waeli et al., 2020). It is plausible that a proper circadian timing of anti-inflammatory drugs (chronotherapy) can target the detrimental inflammatory cascade in COVID-19 patients without interfering with the fight of the immune system against the virus. For instance, the circadian time of drug administration will differentially affect various cytokines involved in viral immunity and COVID-19, including among others IP10, IL-1b, IL-4 , IL-8, IL-10, TCR, INF-α, CIITA, TNF-α, and TLR as well as the activity of T cells (CD8), NK cells, and B cells (Al-Waeli et al., 2020; Canan et al., 2014).

Based on the known circadian *peak* (point of culmination of an oscillatory function) and circadian *through* (lowest value of an oscillatory function) for known detrimental (**Table 1** ) or beneficial (**Table 2** ) inflammatory mediators identified in COVID-19 patients, treatment could be optimized for chronotherapy. Hereby, adjusting the timing of the day in which the medications are given to result in highest drug levels at the time point when detrimental inflammatory mediators reach their Peak (**Figure 1** ). This would mean that afternoon is the preferred time window for drug administration whereas nighttime intake should be avoided.

Table 1. Detrimental inflammatory mediators in the cytokine release storm (CRS) in COVID19 (RED).

| | PEAK | Trough | Reference |
|---|---|---|---|
| IL-1β | Bedtime | Early morning | (Einhorn, Majeska, Rush, Levine & Horowitz, 1995) |
| IL-2 | 1200 h | | (Young, Matthews, Kanabrocki, Sothern, Roitman-Johnson & Scheving, 1995) |
| IL-6 | First Peak 1900 h Second Peak 0500h | | (Vgontzas, Bixler, Lin, Prolo, Trakada & Chrousos, 2005) |
| IL-8 | First peak 1000 h Second peak 2100h | | (Rahman et al., 2015) |
| IL-10* | First peak 0730 h Second peak 1930 h | | (Young, Matthews, Kanabrocki, Sothern, Roitman-Johnson & Scheving, 1995) |
| G-CSF | 2200 h | | (Jilma, Hergovich, Stohlawetz, Eichler, Bauer & Wagner, 1999) |
| GM-CSF | First peak 1330 h Second peak 1930 h – 2330 h | | (Young, Matthews, Kanabrocki, Sothern, Roitman-Johnson & Scheving, 1995) (Rahman et al., 2015; Young, Matthews, Kanabrocki, Sothern, Roitman-Johnson & Scheving, 1995) |

| | PEAK | Trough | Reference |
|---|---|---|---|
| TNFα | First Peak 0730 h Second Peak 1200 h –1330h | | (Young, Matthews, Kanabrocki, Sothern, Roitman-Johnson & Scheving, 1995) (Young, Matthews, Kanabrocki, Sothern, Roitman-Johnson & Scheving, 1995; Zabel, Linnemann & Schlaak, 1993) |
| MCP1 | 0200 h | | (Rahman et al., 2015) |

*involved both in the "cytokine storm" and in the anti-viral response

Table 2. Beneficial inflammatory mediators in the cytokine release storm (CRS) in COVID19 (Green).

| | Peak | Trough | Reference |
|---|---|---|---|
| B cells | 0200 h – 0300h | 1100 h | (Born, Lange, Hansen, Molle & Fehm, 1997) |
| T cells: Naive, central memory, effector memory CD4+ and CD8+ T cells | 0200 h | 1400 h | (Dimitrov, Benedict, Heutling, Westermann, Born & Lange, 2009) |
| Effector CD8+ T cells | 1400 h | 0200 h | (Dimitrov, Benedict, Heutling, Westermann, Born & Lange, 2009) |
| NK cells | 1100 h – 1400 h | 0200 h | (Born, Lange, Hansen, Molle & Fehm, 1997) |
| IL-10* | First peak 0730 h Second peak 1930 h | | (Young, Matthews, Kanabrocki, Sothern, Roitman-Johnson & Scheving, 1995) |
| IFNγ | 0000 h to 0300 h | 0800h to 1100 h | (Petrovsky & Harrison, 1998; Petrovsky, McNair & Harrison, 1998) |

*involved both in the cytokine storm and in the anti-viral response

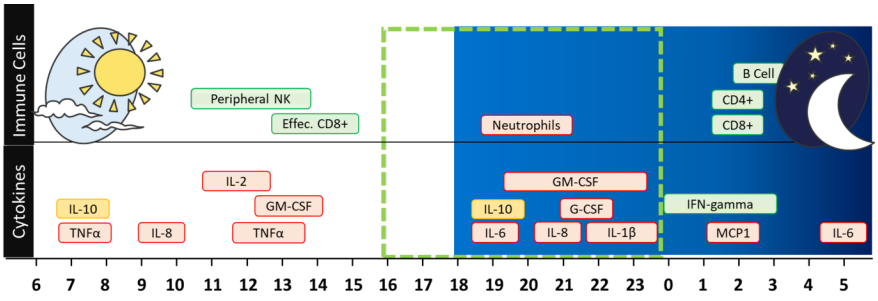

***Figure 1****. Diagrammatic representation highlighting the peak of cytokines and immune responses involved in the cytokine release storm (CRS) of COVID-19 . Peak* detrimental inflammatory mediators (red) and beneficial adaptive immune response against viral infections (green) are shown during a 24 h time period. The dotted line and the red clock indicate the period between 4:00 pm and midnight in which the detrimental effects of CRS outweighs the beneficial effect of the adaptive immune response. This period of time could be the ideal target for anti-inflammatory treatments. On the other hand, the green clocks indicate the periods of anti-viral activity of the immune system, these are the periods in which anti-inflammatory therapy should be avoided.

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