Meeting Report

Understanding COVID-19 through Adverse Outcome Pathways – 2nd CIAO AOP Design Workshop

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Abstract
The CIAO project (Modelling the Pathogenesis of COVID-19 using the Adverse Outcome Pathway framework) aims at a holistic assembly of knowledge to deliver a truly transdisciplinary description of the entire COVID-19 physiopathology starting with the initial contact with the SARS-CoV-2 virus and ending with one or several adverse outcomes, e.g., respiratory failure. On 27-28 January 2021, a group of 50+ scientists from numerous organizations around the world met in the 2nd CIAO AOP Design Workshop to discuss the depiction of the COVID-19 disease process as a series of key events (KEs) in a network of AOPs. During the workshop, 74 such KEs forming 13 AOPs were identified, covering COVID-19 manifestations that affect the respiratory, neurological, liver, cardiovascular, kidney and gastrointestinal systems. Modulating factors influencing the course and severity of the disease were also addressed, as was a possible extension of the investigations beyond purely biological phenomena. The workshop ended with the creation of seven working groups, which will further elaborate on the AOPs to be presented and discussed in the 3rd CIAO workshop on 28-29 April 2021.

1 Overview

1.1 Adverse outcome pathways
“An adverse outcome pathway (AOP) is a conceptual construct that portrays existing knowledge concerning the linkage between a direct molecular initiating event and an adverse outcome at a biological level of organization relevant to risk assessment” is the well-known definition from the seminal paper (Ankley et al., 2010) that many consider the “birth certificate” of the AOP framework.

AOPs provide a mechanistic understanding of complex biological systems and pathways of adversity. The AOP regulatory framework (launched in 2012 by the Organisation for Economic Co-operation and Development (OECD)) captures in a structured way the causal relationships linking the initial perturbation of a biological system, i.e., the molecular initiating event (MIE), mostly resulting from chemical interaction with biomolecular targets, to an adverse outcome (AO) through a sequential series of key events (KE), which are cellular, anatomical, and/or functional changes in biological processes.

Not only can the development and application of AOPs pave the way for the development of improved evidence-based approaches for hazard and risk assessment, it also promises a significant impact on animal welfare with a reduced reliance on animal-based methods (Burden et al., 2015).

While AOPs are the central element of a toxicological knowledge framework originally built to support chemical risk assessment based on mechanistic reasoning, AOPs are in fact “stressor-agnostic”. The pathophysiological stories they tell start at the moment of first molecular interaction of a “stressor” with the organism, without specifying the nature of the stressor. Recent ly, nanoparticles and even radiation were introduced as possible stressors. The next logical step is now to expand the AOP scope to viral pathogens as potential stressors triggering an AOP.

1.2 The CIAO project
Coronavirus disease 2019 (COVID-19) is caused by a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and is an ongoing global health emergency. A plethora of data is being made available on a daily basis concerning the population impacts of the COVID-19 pandemic around the world. These have formed the basis for multiple approaches to modelling the trajectory of the disease, which in turn have been used to support
critical policy and healthcare decisions. What is missing, however, is the linking of this information on population effects with an understanding of the pathogenesis of the disease. In particular, there is an urgent need to develop insights into why some population groups are more vulnerable than others as a result of biological, epidemiological and socioeconomic factors. The nature of the vulnerability will be crucial in determining appropriate prevention and mitigation measures.

The CIAO project is based on the assumption that AOPs can play an important role in improving the interpretation and efficient application of the scientific understanding of COVID-19 (Nymark et al., 2021). Knowledge from different disciplines and specialists will be consolidated in a way that is understandable and actionable by relevant stakeholders (e.g., medical professionals, public health officials and policy makers, managers in the healthcare sector). Moreover, this mechanistic knowledge will be used to develop an understanding of the biological modulating factors that determine different clinical outcomes and will be exploited to develop mathematical models that simulate the pathogenesis and health impact of COVID-19.

Furthermore, AOPs allow us to look beyond the somewhat limited layer of mere apical endpoints (e.g., respiratory failure) and facilitate the use of non-animal assays that represent one or more KEs in a COVID-19 AOP when detecting, measuring or further examining the disease. Therefore, like in toxicology (Parish et al., 2020), this approach could enable more rapid, efficient and relevant assessment of the suitability of new approach methodologies (NAMs) tailored to relevant KEs.

CIAO is a crowdsourcing project steered by the European Commission, the Physicians Committee for Responsible Medicine (PCRM), and the Humane Society International (HSI). In CIAO, scientists from many different organizations (government, academic, research, industry, non-governmental organizations (NGOs)) work together to assemble AOPs that will help elucidate the biological mechanisms behind the COVID-19 pathophysiology.

On 1-2 October 2020, in its first online AOP Design Workshop, the CIAO crowd had formed five working groups (Fig. 1) that focused on KEs, sorted by the timeframe within which these events occur during a COVID-19 infection (early events, middle events (non-inflammation), middle events (inflammation), late events, modulating factors). In the three months following this first workshop, the working groups have elaborated on the KEs and modulating factors they identified (via literature research) and assembled first COVID-related putative AOPs.

On 27-28 January 2021, the CIAO project held its 2nd AOP Design Workshop online with 50+ parties (see Annex A). The workshop was facilitated by Laura Viviani and Clemens Wittwehr. After a presentation by Brigitte Landesmann on the goals of the workshop and a presentation on collaboration practicalities by Catharine Krebs (e.g., on how to fully exploit the functionalities of reference management and communication platforms like Slack and Zotero), the working group results were presented, discussed and further elaborated. This report summarizes the scientific highlights of the workshop as well as the AOPs CIAO will work on until the next workshop scheduled for 28-29 April 2021.

2 Highlights from the workshop

2.1 Scientific findings from the October 2020 - January 2021 phase

The early events group (presentation by Maria João Amorim) detailed the mechanisms leading to viral entry, viral replication and exit. The presentation included the identification of the host factors that the virus uses to establish an efficient infection inside the host cell that leads to successful production of viral particles. It also concentrated on how viral proteins modulate the cellular environment and escape immune detection. It became clear that

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1 https://ciao-covid.net
2 doi:10.14573/altex.2102221s
3 https://slack.com/
4 https://www.zotero.org/
three KEs could be prepared from the work developed and that additional curation of the literature would be necessary to address immune activation in the host cell – in particular inflammasome, interferon, reactive oxygen species, and cytokine production. In addition, viral replication in specific tissues is still incomplete.

The middle events (non-inflammatory) group (presentation by Natália Garcia-Reyero, Shihori Tanabe, Jorid Sørli, Catharine Krebs, and Julija Filipovska) detailed mechanisms leading to ACE2 dysregulation (overlapping with the middle (inflammatory) and early groups), increased coagulation (overlapping with the middle, (inflammatory) group), cell death, decreased lung surfactant function, decreased alveolar membrane integrity, and cytokine release leading to epilepsy and neurodegeneration. There was discussion about the alveolar membrane, and viral entry coming from both the air through the lungs and the blood through the capillaries. The lymph system via the gut could be another route of entry. The gut’s role is supported by high ACE2 expression in digestive tract organs, high SARS-CoV-2 concentrations in sewer systems, and gastrointestinal symptomology. This work laid the foundation for at least six KEs, with more likely to emerge from filling in putative pathways.

The middle events (inflammatory) group (presentation by Penny Nymark) detailed the mechanisms leading to perturbed immune responses and hyperinflammatory state. The work has entailed detailed unravelling of factors involved in the early immune response, such as ACE2 dysregulation, bradykinin activation, imbalances in the coagulation and fibrinolytic pathways, as well as induction of oxidative stress and NLRP3 inflammasome activation. Existing information contained within the AOP-Wiki\(^5\) was utilized to model the next steps of inflammation, including three previously developed KEs describing tissue resident cell activation, increased pro-inflammatory mediators, and leukocyte recruitment. In addition, neutrophil-platelet interactions and the circumstances leading to hyperinflammation were described. Finally, the group looked into the possibilities of modelling multi-scale factors using the AOP framework, including, e.g., involvement of multi-system, recurrent and advantageous factors affecting neuroinflammatory outcome. The work resulted in more targeted development of ten sets of KEs. Furthermore, in support of the KE developments, the group also established a set of harmonized approaches for a systematic/scoping literature review.

The late events group (presentation by Adrienne Layton, Vangelis Daskalopoulos, Sandra Coecke, Anna Price, Francesca Pistollato, Magda Sachana, Sabina Halappanavar, Mathieu Vinken, and Roos Masereeuw) detailed the COVID-19-related AOs and covered the following areas: 1) associated coagulopathy and thrombosis, 2) lung injury (including acute respiratory distress syndrome (ARDS)), 3) neurological effects, 4) liver injury, 5) cardiovascular effects, and 6) kidney injury. The presentation included emerging evidence that clearly shows a major risk of thrombosis linked to severity of disease caused by SARS-CoV-2, with strong association to disseminated intravascular coagulation (DIC) and risk of sepsis, leading to multi-organ failure and increased mortality. The cardiovascular AOs associated with COVID-19 covered myocardial infarction, hypertension, and associated coagulopathy (blood clotting abnormalities) that lead to heart failure. During the presentation, the role of the renin-angiotensin-aldosterone system (RAAS) was explained and its importance highlighted. The presentation also included mechanisms leading to the AO lung fibrosis. Evidence for these mechanisms linked to other groups investigating earlier KEs, for example hyperinflammation, a major “crossroads” for many events that will be established across the COVID-19 AOP network. With regard to the neurological effects, AOPs were proposed for short-term anosmia (loss of smell), and the role played by the regeneration of sustentacular cells was explained. The involvement of the neuro-olfactory bulb and the disruption of the blood-brain barrier, which potentially link to many other neurological AOs of COVID-19, was presented. These include encephalitis, stroke, multiple sclerosis, epilepsy and seizures associated with COVID-19. Established evidence for both pathology- and therapy-related AOs on liver (such as cholestasis and steatosis) in COVID-19 was presented, as well as the incorporation of KEs from existing AOPs. Critical mechanisms (both directly and indirectly) involved in liver injury included inflammation, thrombosis and sepsis, closely linking to other groups to establish key event relationships (KEs) by ongoing review of emerging literature. Renal injury associated with COVID-19 is a critical part of the CIAO project. As 90% of COVID-19 patients suffer acute kidney injury with dialysis being a common requirement, the KEs leading to these outcomes were elaborated, including the linkage to KEs across other therapeutic areas. The proposed AOP sketches will be taken forward and become the base for further advancing with KER development and network building by receiving input from the other groups.

The modulating factor (MF) group (presentations by Kristie Sullivan, Brigitte Landesmann, Nuria Amigó, Anna Berenius, Vangelis Daskalopoulos, Alberto Mantovani, and Sandra Coecke) reported that a great variety in the progression and outcome of COVID-19 has been observed. Besides co-exposure to non-viral stressors and existing comorbidities, a wide range of other factors can influence the severity of the disease, and the task of the MF group was to explore their role. Of the numerous factors, group members had chosen specific factors according to their expertise, namely chemicals like per- and polyfluoroalkyl substances (PFAS), vitamin D, diet, sex, lipid-related aspects, genetics, aging and immune suppression, and cardiovascular disease. After exploring the evidence for an actual modulating capacity, it was investigated in which way this modulation might occur and how and where the modulation might interact with the main AOP(s) developed by the other groups. KERs are the actual point of interaction where MFs shape the response-response relationship.

\(^5\) https://aopwiki.org
6. ACE2 receptor binding in liver cells leading to liver injury
7. ACE2 receptor binding in kidney cells leading to kidney injury
8. ACE2 receptor binding leading to gastrointestinal injury
9. ACE2 receptor binding in olfactory epithelium leading to anosmia
10. ACE2 receptor binding in olfactory epithelium/ACE2 receptor binding in CNS (endothelial, neuronal and glial cells)/hyperinflammation leading to seizures/epilepsy/encephalitis/multiple sclerosis
11. ACE2 receptor binding in pericytes leading to stroke/cerebrovascular disease/anosmia
12. Modulating factors
13. Multi-scale impact AOP

In addition, 74 KEs (see Annex B) were identified within these 13 AOPs. These KEs have been given project internal identifiers (CIAO IDs) and will be the basis for further development. Each KE is linked to one or several project members who are responsible for continued development (or alternatively merging or retiring the KE if deemed redundant with other KEs).

The 13 AOPs were further grouped into six related areas by organ function, which will be treated as working groups, as shown in Figure 3 and Table 1, and an additional working group was constructed to investigate the development of approaches for systematic literature review. After the workshop, members of the CIAO crowd were asked to self-assign to one or several of these seven groups.

The scientific focus of the first four “biological” AOP groups will be as follows:
SARS-CoV-2, including the kidneys, liver, cardiovascular and gastrointestinal system, connecting direct infection and viral replication in cells and tissues outside the lung with systemic inflammatory and other sequelae of COVID-19 disease.

- The “Neuro AOP” group will investigate the pathways associated with the most common neurological syndromes following SARS-CoV-2 infection such as anosmia, encephalitis, and stroke. The group will also explore the role of SARS-CoV-2 infection of non-neuronal cells in the olfactory epithelium and olfactory bulb and the involvement of astrocytes and microglia in COVID-19 infected brain.

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**Fig. 3: AOPs and future CIAO working groups**

**Tab. 1: Current CIAO working groups (WGs)**

| WG #     | Short name             | Purpose                                                                 |
|----------|------------------------|-------------------------------------------------------------------------|
| AOP WG 1 | Hub group              | This group will concentrate on systemic effects central to all other AOPs, i.e., mostly inflammatory processes, thrombosis and coagulation processes. |
| AOP WG 2 | Lung AOP group         | This group will deal with the respiratory AOs.                           |
| AOP WG 3 | Other organs AOP group | This group will cover liver, kidney, heart and gastrointestinal aspects. |
| AOP WG 4 | Neuro AOP group        | This group will also include anosmia, seizures, epilepsy, encephalitis, multiple sclerosis, etc. |
| AOP WG 5 | Modulating factors group | This group will deal with modulating factors and observe and support the AOP groups. |
| AOP WG 6 | Multi-scale impact group | This group will develop a multi-scale impact AOP, looking to expand the AOP concept beyond systems biology from “pre-MIE” influences, such as socioeconomic factors, other vulnerabilities and resilience, through to final prevention or cure of the disease. This could be a way to escalate the AOP framework concept to other areas. |
| AOP WG 7 | Literature review group | This group will cover the development of approaches for systematic literature review. |
AOP network developed from the CIAO project, similar to the COVID-19 disease map publication (Ostaszewski, et al., 2020) to describe all AOPs that are developed in the project. In the weeks following the workshop, a subgroup of the CIAO crowd will further elaborate this strategy.

### 3 Publication(s) and promotion

During the workshop, a basic strategy for publication and promotion of CIAO work results was agreed upon (Fig. 4). The first CIAO manuscript (Nymark, et al., 2021) gives an overview of how AOPs can be used to systematically organize COVID-19 data, which can improve interpretation and facilitate scientific understanding. Next, a meta-level paper describing the application of the AOP framework and the crowdsourcing effort is planned as well as papers on individual aspects of the CIAO project. The main publication will be an overview of the overarching AOP network developed from the CIAO project, similar to the COVID-19 disease map publication (Ostaszewski, et al., 2020).

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### 4 Conclusion and next steps

At the 2nd CIAO AOP Design Workshop several previously formed groups described the AOPs under development, and these were discussed and debated in breakout groups until 13 AOPs
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Clemens Wittwehr¹, Maria João Amorim², Laure-Alix Clerbaux¹, Catharine Krebs³, Brigitte Landesmann¹, Donna S. Macmillan⁴, Penny Nymark⁵, Rebecca Ram⁶, Natália Garcia-Reyero⁷, Magdalini Sachana⁸, Kristie Sullivan³, Jukka Sund¹ and Catherine Willett⁴

¹European Commission, Joint Research Centre, Ispra, Italy; ²Instituto Gulbenkian de Ciência – Fundação Calouste Gulbenkian, Lisbon, Portugal; ³Physicians Committee for Responsible Medicine, Washington, DC, USA; ⁴Humane Society International, Washington, DC, USA; ⁵Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden; ⁶Safer Medicines Trust, Kingsbridge, United Kingdom; ⁷U.S. Army Engineer Research and Development Center, Vicksburg, MS, USA; ⁸Organisation for Economic Co-operation and Development (OECD), Paris, France

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