The CIAO project "COVID-19 Pathogenesis via the Adverse Outcome Pathways". Report of the 5th online Workshop.

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1. Introduction

1.1 The CIAO project

The CIAO project aims to make sense of the overwhelming flow of data available on COVID-19 pathogenesis by exploiting the AOP framework (Nymark et al., 2021; Wittwehr et al., 2021). The AOPs depict the causal relationships that link the initial binding of the virus to ACE2 receptor over a series of biological key events (KE) toward an adverse outcome (AO), such as lung injury or anosmia. The modular aspect of AOPs allows the development of AOP networks where shared KEs become evident and knowledge gaps can be identified (Villeneuve et al., 2019, 2014). Such mechanistic organization of the COVID-19 knowledge also helps to capture the various factors influencing the clinical outcomes. Developing AOPs modeling COVID-19 pathogenesis relies on interdisciplinary collaborative effort, synergizing exchange between experts from different fields. In addition, the application of the "toxicological" AOP framework to map a viral disease of high societal relevance provides novel outputs which can inform on potential needs for changes and adaptations of the framework itself (Carusi et al., 2018; Nymark et al., 2021).

Around 70 scientists from across the world are currently participating in the project. The work within the project is organized among working groups (WG) focusing on the different outcomes of the disease and on different aspects of the project (Table 1).

Table 1. CIAO working groups (WG)

Working group name	Focus
Hub and Lung AOP group	KEs common to multiple COVID-19 AOs (e.g. coagulation, hyperinflammation) joint with pulmonary AOPs
Multi-organ integration AOP group	AOPs and KEs specific to liver, kidney, heart and gut
Neuro AOP group	AOPs and KEs linked to neuropathological conditions (anosmia, seizures, epilepsy, encephalitis, …)

Literature Review group	Applying systematic literature review to support AOP development (neuro pilot study)
Modulating Factors group	Integrate modulating factors into KEs/KERs/AOPs
Multiscale Impact group	Elucidate the multiscale factors of COVID-19 across levels and time and evolve the AOPs to address those multiscale aspects
AOP network team	Build up an AOP network based on the COVID-19 related KEs and AOPs already entered in the wiki
Meta-level paper group	Evaluate how the AOP framework and the crowdsourcing effort were applied to a viral disease
CIAO ontology team	Ontology and controlled vocabulary within AOPs

On 1-2 October 2020, 27-28 January 2021, 24-25 April 2021 and 15-16 September 2021, the first four online CIAO workshops were held (www.ciao-covid.net). On 9-10 March 2022, the 5th CIAO workshop gathered around 40 participants over 2 half-days (Annex A). The workshop was facilitated by Laure-Alix Clerbaux, Laura Viviani and all the coordination team.

1.2 Goals of the 5th CIAO AOP Workshop

After a warm up session and welcoming the newcomer Paula Burkhardt, the goals to be achieved during this workshop were presented. In continuity with the previous CIAO workshops, the first goal was to work together towards harmonization to increase interoperability, to build a comprehensive network and to exploit at best the re-use principle of the AOP framework. The first agenda item was therefore centered on increasing harmonization by application of ontology and by leveraging modularity of the AOP elements. A second goal of the workshop was to share the update and progress from the different working groups. Finally, the objective was to build the future of CIAO together and decide about the directions into which CIAO should develop this year and beyond.

2. Working together towards harmonization

2.1 Towards harmonization by application of ontology

The presentation from the newly formed Ontology group initially focused on the importance of using standardized terminologies to describe the main concepts in an AOP. Using terminologies avoids fragmentation in the way we describe terms and will enable CIAO AOPs to communicate in a common "language" with other domains outside CIAO, such as the regulatory world or PubMed. Most importantly, standardization and harmonization of terms will enable the communication of facts and knowledge in machine-readable format, important for downstream applications, such as reasoning and network visualization.

The presentation then went ahead with explaining basic facts about controlled vocabularies and ontologies with examples. Following this introductory section, the work performed by the Ontology group was presented with content from the surveys performed for KE, AOP and Stressor Pages at the level of data included in tabular format. Observations and suggestions were made regarding content and UI. A prototype visualization web app (built in-house based on Biovista Vizit) containing an AOP was then presented with the scope of demonstrating the need to have consistent terms for all Biomedical concepts in AOPs and the need to fill as much as possible the information required in all AOP tables (after all, without content the network cannot display nodes and relations). The

presentation of the current state of standardized data was then followed by the effort to standardize the titles of AOs using MedDRA or other terminologies.
Finally, the presentation touched upon some initial observations from the parsing of AOP-wiki xml files and potential challenges faced by Authors when trying to enter data into AOP forms.

2.2 Towards harmonization by leveraging modularity of AOP elements

One of the pillars of the AOP framework is the modularity of its elements (OECD 2017). Modularity and re-use of existing AOP elements within the AOP framework facilitate building new linear AOPs and complex AOP networks leveraging existing AOP knowledge.

2.2.1. Hub viral AOP: a new concept

The different AOP elements were discussed. Besides the well-established KE as building blocks, single KERs were proposed to be formally recognized as core building blocks of knowledge assembly within the AOP (Svingen et al., 2021). In addition, re-using two of the three existing Hub KEs associated with hallmarks of inflammation and established to harmonize the way the inflammation response was depicted in AOPs (Villeneuve et al., 2019), the AOP392 was developed. This AOP is proposed to be seen as a *Hub AOP*, a segment useful for integration and connection of a simple unit of KEs. Similarly, we proposed a hub AOP to depict viral replication.

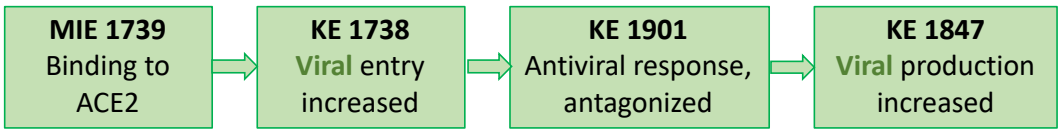


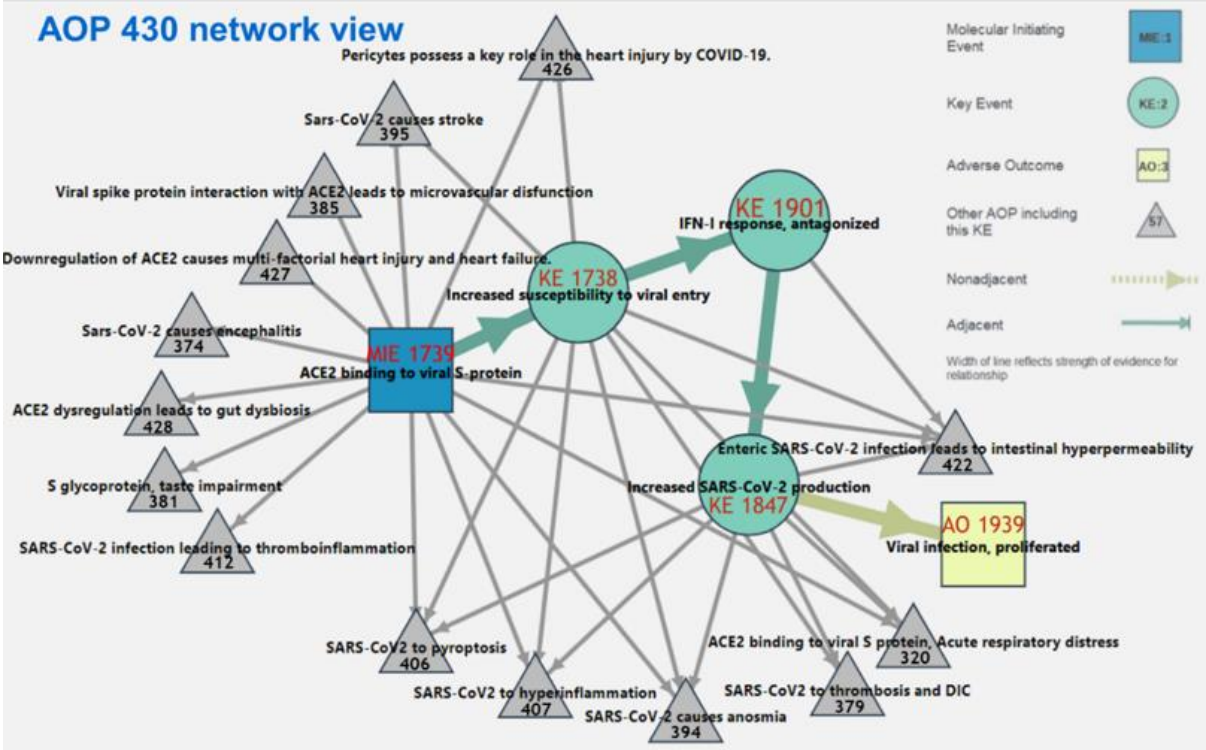
Figure 1. Proposed Hub viral AOP. CIAO COVID AOP Early KE harmonization: SARS-CoV-2 entry and antagonism of Interferon (IFN)-I antiviral response leading to replication (CIAO COVID-19 5th Workshop, March 9, 2022).

The level of details of an AOP was proposed to depend on the use of the AOP. In the context of AOPs depicting the pathogenesis a viral disease, here COVID-19, it was identified that developing such pathways allows to identify knowledge gaps and current inconsistencies in the literature guiding for further research as well as permits to propose biomarkers of the disease progression and severity. In that regard, a description of the viral biology is necessary and informative. Thus the proposed *viral Hub AOP* describes the biology of the virus within a simple unit, so that the principle of AOP being stressor aspecific is preserved.

2.2.2. Essential initial viral KEs shared by many CIAO AOPs

We proposed a potential hub module consisting of early key events documented in AOP 430: “SARS-CoV-2 Interferon-I antiviral response antagonism and increased viral production leading to viral infection proliferation”. The MIE is the same for many of the COVID AOPs, which is MIE 1739, the binding of the viral S, or spike, protein to the human angiotensin converting enzyme 2. The early key events include KE 1738 viral entry through TMPRSS2, cathepsins and in some rare cases assisted by Neuropilin-1, leading to KE 1901 antagonism of the interferon (IFN)-I antiviral response, allowing KE 1847 viral replication to generate the viral load. We propose that this hub AOP is the starting point for SARS-CoV-2 infection and biological alterations to the host response at this level are critical to establish the different outcomes of disease. However, as we will mention below, there are some AOPs that are caused by downstream events triggered by infection but independent of how well the virus manages to replicate/escape immune detection inside cells.

The network view for AOP430 shows all of the AOPs that are connected to MIE 1739. For eight AOPs (374, 381, 385, 395, 412, 426, 427, and 428), generally the MIE is leading to ACE2 dysregulation, then the adverse outcomes. Six other AOPs (320, 379, 394, 406, 407, and 422) require replication so are connected to the increased viral entry KE 1738 and increased SARS-CoV-2 production KE 1847. We propose the essentiality of KE 1901 for innate immune evasion and suggest insertion into these and any future AOPs requiring viral replication.



ACE2 and TMPRSS2 proteins allow viral entry but are not the only determinant of viral replication. The SARS-CoV-2 virus has evolved a repertoire of proteins that bind and block the proteins in the IFN cascade so the host antiviral proteins are not expressed, and the virus is free to replicate. The viral replication process may be going on at the same time, but in separate cellular compartments. Therefore KE 1847 is downstream based on essentiality: if IFN is suppressed, then viral load increases resulting in downstream inflammatory responses and disease and/or transmission. The IFN disruption cannot be merged into the viral replication KE 1847, because it is a specific antiviral process and more distinct to COVID-19 than to other viruses. Studies show that interferon expression is delayed by SARS-CoV-2 compared to other viruses like influenza, with an untuned or imbalanced response between interferons being initially low and inflammatory cytokines being elevated in moderate to severe cases (Blanco-Melo et al., 2020; Galani et al., 2021; Hatton et al., 2021; Rouchka et al., 2021). Several studies indicate that if IFN is administered just before or upon exposure, viral production is reduced or eliminated (Hatton et al., 2021; Hoagland et al., 2021). Also, some people have developed autoimmunity in which they produce autoantibodies that block IFN, resulting in more severe disease (Bastard et al., 2021; Lopez et al., 2021).

Further discussion questions include the issue of dosage: Is initial exposure load enough to trigger dysregulation so that replication is not essential? We propose the need to delineate between the AOPs where these early KEs are essential and where they are not. There is also the question of whether a later MIE than 1739 may make AOPs more stressor agnostic, but we need to consider how we can make good use of the AOP framework to characterize COVID-19 although the SARS-CoV-2 virus, and its immune evasion proteins, are a specific set of stressors.

2.2.3. Coaching strategy for compliance with the OECD AOP Framework

The CIAO project generated a significant number of putative AOPs and 13 of these were also included on the OECD AOP programme workplan (project 1.96). AOPs on the OECD wok plan, benefit from coaching support by members of the OECD Extended Advisory Group on Molecular Screening and Toxicogenomic (EAGMST) that help guide the authors in the development of AOPs consistent with the framework guidance (OECD, 2018) and compliant for future scientific review following the principles the Guidance Document for the scientific review of Adverse Outcome Pathways (OECD, 2021). The process ensures maintenance of the development of high quality AOPs fit for use in the regulatory and research science context.

An approach developed by a subgroup of CIAO members in consultation with the EAGMST coach was presented. It aims to leverage the modularity of the numerous CIAO AOPs and optimize the coaching support. Elements of the CIAO AOPs on the OECD workplan were analysed for their modularity based on (a) number of other CIAO AOPs (on the workplan or other AOPs developed/modified within CIAO) crossing paths; (b) number of shared elements (KEs) with the other CIAO AOPs.

Based on these criteria, four AOPs were identified as "core" AOPs to fast track in collaboration with the OECD AOP coach (bold in the table). It was agreed that the approach would enable consistent development of all AOPs within the CIAO and optimise the use of available resources.

CIAO AOP OECD project 1.96	**Crosses paths with AOP Wiki** (includes AOPs developed or considered within CIAO but not part of the project 1.96)																		***via* Shared KEs**
Wiki ID	320	374	377	379	381	382	385	392	394	395	406	407	412	422	426	427	428	430	173	Wiki ID
422	X			X					X	X	X	X			X			X	X	1739, **1738, 1847,1497**
374	X				X		X		X	X	X	X	X	X	X	X	X	X		1739
379	X		X						X	X	X	X		X				X		**1738, 1847, 1846**
382	X		X					X												**1496**
392	X		X			X								X					X	**1496, 1497**
320		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	**1738,** 1739, **1847, 1496**
394	X	X		X	X		X			X	X	X	X	X	X	X	X	X		1739, **1738, 1847**
395	X		X	X					X		X	X		X	X			X	X	1739, **1738, 1846**
412	X	X			X		X		X	X	X	X		X	X	X	X	X		1739, **1913**
302																			X	1498
428	X	X			X		X		X	X	X	X	X	X	X	X		X		1739
430	X	X			X		X		X	X	X	X	X	X	X	X	X			1739, **1738, 1847**

Figure 2. As of February 2020

Most shared KEs in these AOPs are KE1739 (ACE2 binding to viral S-protein), KE1738 (Increased susceptibility to viral entry), and KE1847 (Increased coronavirus production), outlining the first steps

of SARS-COV2 interaction with the host, hence their harmonised development under the guidance of the EAGMST coach is likely to add unique value to the AOP Framework approach and its application in biomedical sciences.

MIE: KE1739 - ACE2 binding to viral S-protein

AOP422: Binding of SARS-CoV-2 to ACE2 in enterocytes leads to increased intestinal permeability

AOP374: Binding of Sars-CoV-2 spike protein to ACE 2 receptors expressed on brain cells (neuronal and non-neuronal) leads to neuroinflammation resulting in encephalitis

AOP320: Binding of viral S-glycoprotein to ACE2 receptor leading to acute respiratory distress associated mortality

AOP395: Binding of Sars-CoV-2 spike protein to ACE 2 receptors expressed on pericytes leads to disseminated intravascular coagulation resulting in cerebrovascular disease (stroke)

AOP428: Binding of S-protein to ACE2 in enterocytes induces ACE2 dysregulation leading to gut dysbiosis

AOP394: SARS-CoV-2 infection of olfactory epithelium leading to impaired olfactory function (short-term anosmia)

AOP412: SARS-CoV-2 infection leading to thromboinflammation

AOP430: Sars-CoV-2 Interferon-I antiviral response antagonism and increased viral production leading to viral infection proliferation

MIE: KE1738 - Increased susceptibility to viral entry

AOP379: Increased susceptibility to viral entry and coronavirus production leading to thrombosis and disseminated intravascular coagulation

MIE: KE1851- Binding of agonist, Angiotensin II receptor type 1 receptor (AT1R)

AOP382: Angiotensin II type 1 receptor (AT1R) agonism leading to lung fibrosis

MIE: KE1866 - Fibrinolysis, decreased

AOP392: Decreased fibrinolysis and activated bradykinin system leading to hyperinflammation

MIE: KE1672 - Inhibition of lung surfactant function

AOP302: Lung surfactant function inhibition leading to decreased lung function (this AOP was on the OECD workplan previously but joined the CIAO crowd)

2.2.4 BO group discussions

Discussions continued in BO groups using four CIAO AOPs on the OECD Workplan (AOP320, 412, 379, 394) as examples to explore future progress with regard to coaching and development (i) leveraging the modularity of the AOPs, (ii) hub approach to the initial AOP elements describing viral replication (in essence AOP430, illustrated below), that can be latter embedded as complex modular element of other AOPs. Guiding questions for the BO discussion included (i) discuss the fast-track coaching strategy; (ii) does this proposed Hub viral AOP make sense; (iii) should MIE 1739 be the starting point; (iv) how could the proposed Hub viral AOP be implemented in your AOP.

BO discussions highlighted several important aspects for the future development of CIAO AOPs. It is important to fast-track the review and harmonize both the scientific content and compliance with AOP framework for the building blocks shared by many of the CIAO AOPs. Point was made that before developing any new standards or compliance rules, we need to carefully explore the existing ones (a work still in progress by all participants). Promote dialogue between the experts for different parts of the pathway *i.e.* explore the links between different organisational levels even if they are not necessarily adjacent. Ensure coherent AOPs, or development of hubs/parts that are truly modular and usable in other AOPs in a meaningful way. Keep in mind/follow the fellow developers at other organizational levels and users of your hubs down the road.

The hub of events covering the viral replication (essentially AOP430) was recognised as an important part of many of the putative AOPs described within the CIAO. Within the viral replication hub of KEs, viral replication and Interferon I antagonism (KE1901) was recognised as recurring KE which emerged as critical in many AOPs that did not even consider it initially, but the evidence keeps pointing out to its importance/essentiality. This aspect needs future careful examination. The upstream KE(s) to Interferon I antagonism (KE1901) need more work for better mechanistic anchoring. Noted that ACE2

binding (KE1739) is currently part of the viral replication hub (currently a MIE). Although evidence supports the essentiality of ACE2 binding for viral entry in many cell types, evidence for other non-adjacent links to KE downstream will need to be included. Aspect needing special attention is cell specificity of ACE2 binding and essentiality of this KE in different cell types/tissues, hence in different AOPs.
The time component of the progression of the hub elements (and more generally of AOP elements) needs to be considered. Toxicokinetic aspects have not been explored well so far in the AOP framework, so this can be a special contribution by the CIAO. Based on the analysis of the evidence, a putative AOPs may evolve from being initiated by ACE2 binding to being initiated by the viral replication hub (e.g. AOP412). Need to explore evidence for viral variants within the postulated AOPs. In the future, attention should be given and noted which variant the respective information comes from. These considerations may potentially provide greater valuable insight into the mechanisms of COVID19 pathophysiology. Evidence from other stressors (chemical/physiological/other viral, e.g hypoxia, influenza, etc) informing specific KER relationships and/or essentiality aspects is useful and continues to emerge during KER development and WoE analysis. The need for communication at controlled vocabulary level and potentially the Ontology group was highlighted.

2.3 Towards interoperability

2.3.1 The CIAO AOP network

The CIAO AOP network has developed through two steps involving i) a data-driven approach to include all AOP developments by all partners in the CIAO project, and ii) an expert-driven refinement of the AOP network.
The data-driven developments were initiated through a series of iterative processes aiming for compilation of a machine-readable file including all CIAO AOPs in development (see detailed description in the 4th CIAO Workshop Report (Clerbaux et al., 2022) Efforts were done to transparently harmonize terminology across MIEs and KEs with apparent sameness or similarity. Transparency refers to an approach where the IDs for the original MIEs/KEs were retained visibly in the visualization of the network. In addition, measures to FAIRify the data and information gathered were taken*, i.e.* to enable future researchers to Find, Access, Interoperate and Reuse the network and its components. the final computational generation of a directed network was done using the publicly available program Cytoscape v3.7.1.
The expert-driven developments of the AOP network entailed refinement of the computationally generated network, through inclusion of needed tissue-specific MIEs and KEs, grouping of MIEs/KEs in line with tissue-specificity and disease progression, and finally network-driven identification of gaps, i.e. missing KERs. The grouping of MIEs/KEs (nodes in the network) allowed for generation of a bird-view version of the network, which in turn supported a new level of insight in terms of identification of gaps and discrepancies.
The expert-driven gap-analysis led to identification of a row of 15 missing KERs in need of development (Table 2). The recently described pragmatic “unit approach” for development of single KERs and hub AOPs is considered for development of each of the 15 KERs and beyond (Svingen et al., 2021). The “unit approach” is coupled to diverse approaches for development and evaluation and can for instance be linked with activities in the CIAO Literature Group focused on systematic review.
Overall, the CIAO AOP network provides an infrastructure to contextualize the detailed complexities of COVID-19 and with its aim towards interoperability with other data sources has the potential to provide links to deepened detail, e.g. provided by the COVID-19 Disease Map project (https://covid.pages.uni.lu/; (Ostaszewski et al., 2021).

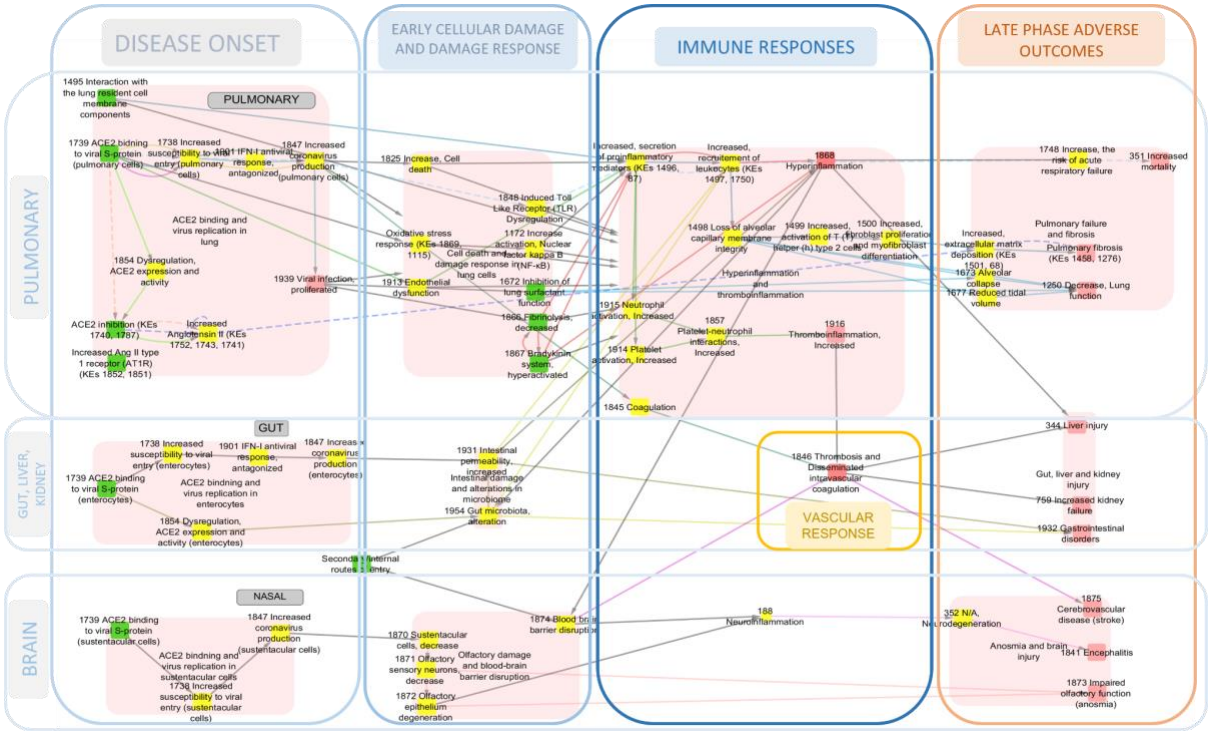


Figure 3. CIAO AOP network

Table 2. Fifteen newly identified KERs in need of further development

Upstream KE	**Downstream KE**
1847 Increased corona virus production	1825 Increase, Cell death
1825 Increase, Cell death	Increased, secretion of proinflammatory mediators (KEs 1496, 87)
1939 Viral infection, proliferated	1672 Inhibition of lung surfactant function
1939 Viral infection, proliferated	1866 Fibrinolysis, decreased
1868 Hyperinflammation	1748 Increase, the risk of acute respiratory failure
1868 Hyperinflammation	1954 Gut microbiota, alteration
1868 Hyperinflammation	1874 Blood brain barrier disruption
1868 Hyperinflammation	344 Liver injury
Oxidative stress response (KEs 1869, 1115)	Increase activation, Nuclear factor kappa B (NF-κB)
Alternative unidentified routes of entry	1954 Gut microbiota, alteration
Alternative unidentified routes of entry	1874 Blood brain barrier disruption
1872 Olfactory epithelium degeneration	188 Neuroinflammation

1916 Thromboinflammation, Increased	1846 Thrombosis and Disseminated intravascular coagulation
1846 Thrombosis and Disseminated intravascular coagulation	344 Liver injury
1846 Thrombosis and Disseminated intravascular coagulation	759 Increased kidney failure

2.3.2 COVID-19 WikiPathways-COVID-19 Disease Map

We had the pleasure then to welcome Dr. Martina Summer-Kutmon. She is an assistant professor at the Maastricht Centre for Systems Biology (MaCSBio) and the Department of Bioinformatics (BiGCaT) at Maastricht University. Since 2012, she is one of the two architects leading the WikiPathways project – an open, collaborative platform dedicated to the curation of biological pathways (Martens et al., 2021; Ostaszewski et al., 2021) (https://covid.pages.uni.lu/). Her research focuses on the development and application of network and pathway-based integrative systems biology approaches for the study of the molecular mechanisms involved in immunology and chronic diseases. As the project leader, she presented the "WikiPathways as a platform for COVID-19 pathway models" project and the international COVID-19 Disease Map project. A joint workshop between CIAO and WikiPathways will be organized on the 9th May toward increased interoperability between the two projects, to synergize expertise and bridge communities via case studies.

3. Sharing together

3.1 Update from Modulating Factors (MF) group

Based on clinical and epidemiological studies, we selected eleven factors modulating COVID-19 outcomes. Those MFs were representative of four different categories: (i) biological intrinsic factors like age, sex; (ii) co-morbidities such as pre-existing heart failure, dyslipidemia, obesity and gut dysbiosis; (iii) lifestyle associated factors such as diet or vitamin D deficiency and (iv) environmental factors like air pollution or exposure to chemicals. Finally therapeutic interventions against COVID-19 were also investigated. We then explored those eleven factors with evidence for modulating the outcome of COVID-19 mechanistically at the KERs level, based on the AOPs developed within CIAO.
By investigating the mechanisms of interference, we discovered that main points of intervention for our selected MFs were early KERs - related to viral infection and ACE2 dysregulation - and middle KERs related to the inflammatory process. Therefore, we focused our further research on these early and middle KERs. We did not develop an AOP, but added information to the AOPs that have been developed by other CIAO WGs. A better understanding on how the various MFs are interrelated will help to better understand the observed differences in the course of the disease. With this approach, we also identified current knowledge gaps and uncertainties orientating for further research. We also proposed early biomarkers for identification of high-risk patients. The manuscript is in its final stage and will soon be submitted for publication in a special issue related to risk factors in COVID-19.
In addition, we met with OECD EAGMST subgroups to discuss how MFs could be better integrated in the AOP framework and made more visible in the AOP Wiki. Our proposed solutions will be discussed in other committees and submitted for approval by EAGMST.

3.2 Update from Literature Review group

The Literature Review WG, coordinated by Donna Macmillan, provided an update to the pilot project which focuses on neurological outcomes related to COVID-19. The project began by downloading all of the COVID-19 literature available in PubMed (86,075 papers as of January 2021) and after screening

using Swift-Review and Swift-ActiveScreener (Sciome; Durham, NC, USA) at the title/abstract level, these articles were narrowed to ~2000 relevant articles. These articles have now been fully assessed at the full-text level and ~1000 relevant primary data-containing articles were found. The next steps of the WG are to complete the final "extraction" step on all screened articles. The group will then publish a systematic scoping review on the neurological effects of COVID-19, which will complete the initial task of the group, opening the possibility of applying this protocol to the topics of the other WGs. Also presented by the group (Joshua Breidenbach), was an approach of applying machine learning to automatically screen the same batch of articles, based on criteria learned from the initial manual screening, in order to expedite the systematic review process and simplify the incorporation of (the many) more recent publications. It is a support-vector machine-based method built on a bag-of-words model, and has so far achieved a balanced accuracy of 92%, a sensitivity of 96% and a specificity of 87% using a balanced subset of the PubMed articles. Validation of the model is underway.

	Manual assessment	
Model assessment	Included	Excluded
Included	772 (TP)	101 (FP)
Excluded	31 (FN)	702 (TN)

3.3 Update from Neuro group

The CIAO Neuro AOP group updated the workshop participants about the progress made since the last workshop in September 2021. At that workshop, although no direct update of the group activities was done, the draft neuro AOP network was presented as part of the whole CIAO AOP network and selected KERs developed by the Neuro AOP experts were presented and discussed during break-out sessions. Since then the group worked through expanding the network by integrating and linking to the putative AOPs on COVID-19 induced seizures and Alzheimer's disease. Furthermore, a KE that plays a central role in the network, namely hypoxia, was developed and now is included in the network.

These developments were the outcome of discussions at virtual meetings and contributions to the drafting of a paper on AOP framework application to neurological symptoms of COVID-19, which is close to completion. At the paper, the role of not only traditional modulating factors (i.e., gender, development stage, disease stage, environmental factors etc.) that currently considered by the AOP framework are explored as regards the presence and the severity of the symptoms, but also the role of more individual parameters such as psychological and social stressors is discussed. In addition, the paper introduces other initiation events that expand beyond the classical molecular initiation events of the AOP framework such as poverty, food insecurity etc. as an approach to provide more adaptive and predictive outcomes.

Parallel efforts from selected members of the group are directed to a more in depth analysis of available mechanistic knowledge to enhance the understanding of the olfactory neuroepithelium involvement leading to short-term anosmia in COVID–19 by applying the AOP framework.

Short term plans of the CIAO Neuro AOP group include further development of the KERs, using the output of the CIAO Literature group and population of the AOP-Wiki with the evidence collected. Within the long term plans of the group, efforts are envisioned to develop AOPs for Long COVID neurological symptoms, including exploration of vaccination status and recovery SARS-CoV2 groups, as recent study indicates that mental or cognitive symptoms are among the most frequently reported findings in patients who survived after Intensive Care Unit treatment for COVID-19 (Heesakkers et al., 2022).

3.4 Update from Multi-Organ Integration and Gut group

Kristie Sullivan briefly presented the Multi-Organ Integration group calling for scientists interested in COVID-19 related outcomes in kidney, liver, heart or microvascular system to join.

Laure-Alix Clerbaux, on behalf of the Gut group, presented the process and progress on the development of four AOPs depicting COVID-19 related perturbations of gut structure and function. The lessons learned from the approach were also discussed. COVID-19 patients experience gastrointestinal disorders, such as diarrhea, and show alteration of gut microbiota. Besides, the SARS-CoV-2 cellular receptor ACE2 is highly expressed in enterocytes. Thus, it has been proposed in the literature that SARS-CoV-2 enteric infection leads to intestinal barrier disruption, inflammation and dysbiosis. However, the underlying mechanisms are poorly understood. We applied the AOP approach to investigate the evidence behind the biological plausibility. The first pathway outlines SARS-CoV-2 enteric infection leading to intestinal barrier disruption via cytopathic effects. While SARS-CoV-2 infection of human enterocytes *in vitro* is supported by high evidence, it differs in animal studies and in a (healthy) human gut either due to timely interferon response limiting viral replication or due to the multiple layered barrier. Moreover, while the biological plausibility was high, currently, there is not enough evidence to support enterocyte massive cell death following SARS-CoV-2 infection. A second AOP considers ACE2 dysregulation. ACE2 plays a key role in intestinal homeostasis, notably in the uptake of dietary amino acids, such as tryptophan. Evidence supports high plausibility for intestinal ACE2 dysregulation due to S protein binding, however with inconsistencies regarding direction and magnitude. In addition, further research is needed to assess tryptophan alteration, which regulates secretion of antimicrobial peptides, impacting gut microbiota composition. Another putatively involved pathway is the one that proposes a new lens for understanding COVID-19 transmission with SARS-CoV-2 infecting gut bacteria. However, currently, inconsistencies in literature exist regarding detection of replicating SARS-CoV-2 in feces calling for additional research. Thus, proposing mechanisms leading to intestinal adverse outcomes in COVID-19 permits investigating the gut as another potential entry route for the virus. This approach also highlighted significant inconsistencies and knowledge gaps guiding for further research.

3.5 Update from Multiscale group

After a hiatus, the group is switching to a rotating coordinator system to reduce the workload for any single coordinator. The group is in the process of revisiting their goals and scope and potentially preparing a report on their meetings and a multiscale framework for COVID-19.

4. Building the future together

CIAO will soon go into its third year. The pandemic is finally showing first concrete signs of loosening its grip on society (at least in some parts of the world), but on the other hand new challenges are arising. A discussion about the direction into which CIAO should develop is therefore timely. Kristie Sullivan and Clemens Wittwehr led through an initial presentation laying out possible ways forward.

2022.

For this year, most of the work to be executed is already agreed and planned: Finalizing the planned peer-reviewed manuscripts and further developing the CIAO-related AOP-Wiki entries is well under way and on schedule. What the project could profit from, however, is increased visibility and acknowledgement in the non-scientific world, *i.e.* among policy makers and the wider public. Becoming a recognized brand even beyond the research community could certainly increase the chances for further financial support. Appearing in the “morning paper” gives street credibility. In a

Slido poll, ideas were collected on what messages could be sent out to the broader public, and they crystallized around these issues:

- Inform in an understandable way when we publish a scientific paper, show how this impacts people's lives;
- What can one do to reduce one's risk (= modulating factors in layman's terms)?
- How the power of the crowd helps to address a global problem;
- Interdisciplinarity, bridging silos, …
- CIAO as sense-maker, as filterer of the information tsunami

Participants were also asked (Slido word cloud) what would make them proud of being a CIAO crowd member on New Year's Eve 2022.

2023 and beyond.

From 2023, CIAO might have to reorient and refocus on upcoming issues. A Slido Poll collected ideas on what these 2023+ topics should be. Again, a word cloud was produced. The collected terms were then used for a ranking poll.

Finally, the question of funding was addressed. A submission to a 2023 Horizon Europe call is an option to receive funding, provided a suitable call is identified (in time) and a consortium is standing by to immediately react once the call is opened. A Slide Poll showed that all participants would be interested in being part of such a consortium. A Slido poll identified alternative funding schemes.

Breakout group sessions followed which examined the topics of the presentation in greater detail.

"Morning Paper" topics. In addition to the topics already collected in the main session, the BO groups created more ideas.

- How does vaccination work? What is the real contribution of vaccines?
- How do you know you are susceptible?
- What does the disease really look like? You're not getting Alzheimer!
- (Long-)COVID and the "real risks"
- Why are you asymptomatic?

More topics for CIAO 2.0. The BO groups came up with additional topics that could be treated in a CIAO follow up project.

<u>For 2022.</u>

- Virus variants and their impact on the pandemic
- Long COVID (which are the Modulating Factors influencing Long Covid onset and prognosis?)
- Adaptive immunity
- BOP: Beneficial Outcome Pathway

<u>For 2023 and later:</u>

- Preparedness for **future** health challenges: Using the AOP Framework to tackle the overload of information (seen now in CIAO)
- Use the lessons summarized in the meta-level paper and promote them to other disease areas
- How AOPs help in the identification of biomarkers
- Use the AOP network developed in CIAO, specially WoE for drug repurpose in future (non-COVID) health challenges
- Mental impact of COVID – as MF as well as consequence of Long-COVID

What would be seen as indicators for success of CIAO?

- Visualization of **all CIAO-**AOPs, ideally together with the non-CIAO ones
- AOP-Wiki: create **meta data and networks**
- Contribute to the **improvement of the AOP-Wik**i
- CIAO as an **acknowledged showcase** of AOP Framework and the AOP-Wiki
- **Standardization** of data (+AI/Machine learning)
- Observable **impact** on policy
- Set up a Model that could be **re-used for other pandemics**
- Use of AOPs in **other diseases**
- **Worldwide** collaboration
- CIAO project leads to **improvements** in AOP development/review process
- Showcasing CIAO's application of the AOP framework for medical research/questions can leads to a **further expansion of AOP** concept beyond toxicology
- **Become AOP evangelists** - how do we encourage our colleagues, people in medical sciences/research to contribute to AOP elements?

Funding. The following ideas were generated concerning the possible application for Horizon Europe funding: (i) Create a team of funds seekers that will proactively leading grant submissions; (ii) Prepare a template; (iii) Set up the consortium in advance. Such coordination will need a strong institution and established position, or a contractor with coordinating experience. The agreed way forward was to send a survey to give people the opportunity to provide their potential contributions (experiments/methods, interest, time, activities, expertise) to a call so that when a call comes along we have a sense of what we have and do not have.

Possible other funding sources identified were:

- Cost Action https://www.cost.eu/cost-actions/ Evidence-based medicine
- https://www.ihi.europa.eu/apply-funding/future-opportunities
- ONE Health, exposome, reinforcing modulating factors or investigating new ones
- Crowdfunding: may require large publicity effort and/or efforts WRT simplifying our messages/explaining our impact
- Personal grants? Marie Curie network? Trainee grants? PhD-post-doc exchange?
- Funding for FAIRification of CIAO knowledge, data interpretation, data reuse, translation of science to policy, open science. How do we make (re)interpretation of existing data attractive to funders?
- Looking for funding may require additional partners (e.g., pharma)

It was agreed that a **new working group** looking for funding will be established.

5. Wrap up and ways forward

At the 5th CIAO AOP Workshop, further work was done toward harmonization. Progress and current status of the work in the different groups were presented and the directions to take for this year and beyond were explored together.

Table 3. Next steps for CIAO

	Next steps	Timing
Tasks	Consolidation of AOPs/KEs/KERs within the group	
	Implement integration of the MFs in the AOPwiki	
Publications	**Neuro.** The Anosmia and the Neuro network manuscripts will be submitted in the Special Issue of the journal *Cells* "Neurological Symptoms and COVID-19 Pandemic"	Summer 2022
	MF. The MF manuscript will be submitted in the Special Issue of *the Journal of Clinical Medicine* "COVID-19: Special populations and risk factors".	20 April 2022
	Gut. Two manuscripts will be submitted in the Special issue of the *Journal of Clinical Medicine* "COVID-19 and Gastrointestinal Disease: Current Insights and Future Management".	20 June 2022
	Literature review – protocol publication	To be defined
	Multiscale approach.	To be defined
	Meta-level. "CIAO: a living experiment in interdisciplinary, crowdsourced collaboration"	To be defined
	AOP network. Building of the COVID-19 AOP network and drafting of the manuscript.	June 2022

Annex A. Participant list.

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