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Webinar

Stand on the Same Side Against Covid-19 – Clinical Management of Covid-19

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"STAND ON THE SAME SIDE" Videoconferences

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"Implementing a science-based lockdown exit strategy is essential to sustain containment of COVID-19. China's experience will be watched closely, as other countries start considering—and, in some cases, implementing—their own exit strategies"

The Lancet, Volume 395, Issue 10232, 18–24
April 2020, Pages 1305-1314

This phrase expresses the purpose of this program called "Stand on the Same Side against Covid-19" that takes advantage of the new and rapid digital technologies to put together several experts worldwide. It's a global space were many countries hit by SARS-COV-2 can share only scientific information in order to face the pandemic.

May, 29th 2020,
CHINA-EUROPE VIDEOCONFERENCE

"STAND ON THE SAME SIDE AGAINST COVID-19
– CLINICAL MANAGEMENT OF COVID-19"

Prof. Shiyue Li: Ladies and gentlemen, welcome to this third international video conference entitled Stand on the Same Side Against COVID-19, Clinical Management of COVID-19. Good morning, good afternoon and good evening, depending on the part of the world you come from.

I would like to express my gratitude to Professor Corbetta for organizing this very interesting series of conferences. Very informative. Today we have the privilege to have top class speakers and experts, and to make the introduction as short as possible, I'd like to pass the chair, the lead, to Professor Ling. I have to propose to you the apologies of Professor Sergio Romagnani, who was unable for health reasons to attend this conference, but sends his regards and wishes. Professor Li Jing**.**

Professor Li Jing: Okay, I'm so glad that we have this again. We have several outstanding speakers with a very interesting topic today, so I would like to introduce the first one, Professor Bin Cao, and he's the Vice President of China-Japan Friendship Hospital, and also the Vice President of Chinese Academy of Medical Science. I want to put it out that he went to Wuhan early in December last year, and worked in the front-line to treat patients with critical illness in the intensive care units until the last minute of this year. I am glad he's very well today. He has published several outstanding papers on prevalence about the disease and the treatment including lopinavir and ritonavir, a clinical comparative study in the Journal of Medicine of very outstanding doctors. He's going to give us a talk on one of these studies with highlights on treatment, the LOTUS China trial and more, and I want to clarify that the Chinese interpretation has some misunderstanding of the trial, that's not the Chinese traditional medicine, but it's the anti-virus medicine. Okay, let's welcome Professor Bin Cao.

Professor Bin Cao: Okay, thank you for the kind introduction. Yes, so I have been working in Wuhan and other cities in China for the last nearly five months, and today is my second afternoon in Beijing during the last five months. Yes, this afternoon I enjoyed a tea, you can see, this is my teacup. There's a dragon picture on the teacup, and I read a book, the book is by a philosopher from Germany, and maybe you can figure out who he is. Yes, so it's the first easy afternoon for me in the last five months, so I wish you all stay safe and to take care of your patients in your countries. I will share my slide. In the next fifteen minutes I will share a recent public paper of the LOTUS China trial.

I will introduce the LOTUS China trial. This is a lopinavir–ritonavir trial for severe COVID-19 in China. (https://www.nejm.org/doi/full/10.1056/NEJMoa2001282) In these, we have used primary outcomes very often used for severe viral pneumonia, so we also used the ICU stay, 28-day mortality, and the reach of clinical improvement at day fourteen and day 28 as our secondary outcome. Here is the flow, and you can find that among 357 patients assessed for eligibility at last 199 went into the randomisation, 99 in the lopinavir trial and 100 in the control group, but unfortunately three patients died very early without use of lopinavir. So, if we exclude these three patients out of the lopinavir group, we can define this group as modified ITT patients.

We also carefully studied 60 of the patients who received lopinavir-ritonavir. So, we can find that the lopinavir group can have a much faster recovery compared to the control group. **It seems that the patients who received lopinavir within twelve days have a trend of less mortality compared with the control group.**

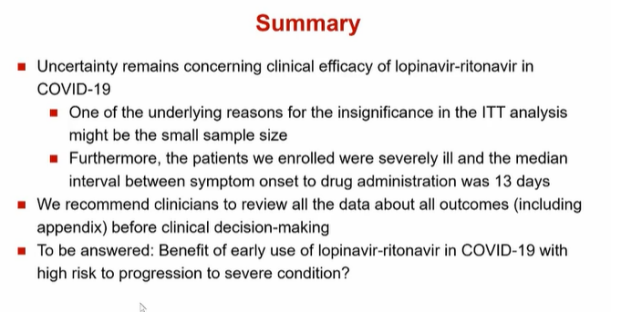
When we look at the secondary end points of the ITT population, for those with the lopinavir group the mortality is nineteen, and the standard care group, the control group, is 27, so there is a trend of less mortality of the lopinavir group. When we look at the clinical improvements of the secondary outcome on day fourteen, the difference was significant. That means that the patients in the lopinavir group reached much higher clinical improvement on day fourteen, and for the ICU length of stay it seemed that the lopinavir group is the median of six days and the standard care group of eleven days, so there's still a difference between the two groups. When we look at the quantitative RNA detection of these patients, it seems that the slope of the RNA decrease is similar, but we have to keep in mind that for all these patients, from the symptom onset to the treatment is about thirteen days. We also looked at the adverse events of the two groups.

It seems that the lopinavir group has much, much more gastrointestinal adverse outcomes such as vomiting and nausea, but when we look at the severe side effects of the two groups it seems that the control group had much more serious adverse outcomes.

So, after this study was published, I am happy to learn that this month another group in Hong Kong published a paper in The Lancet. In this paper, this is a phase two study, they used the triple combination therapy for treatment of mild to moderate COVID-19, and they used the interferon beta 1b combined with lopinavir and ritonavir and the lopinavir group as the control. The data said that the triple group can have a much faster viral clearance compared with monotherapy. So, when we look at all the data it seems that certain things still remain concerning the clinical accuracy for lopinavir-ritonavir in COVID-19 especially for severe or critical cases.

The ITT analysis might be a small sample size, and another limitation that the symptom onset to drug was thirteen days, so we recommend clinicians to review all the data in this study and other data such as I mentioned, the data from Hong Kong to help us understand the benefit of lopinavir is there, especially for those with early treatment of lopinavir-ritonavir, because in the Hong Kong study, the symptom onset to the treatment is five days, and in our study the symptom onset to treatment is thirteen days. **So, it seems that the earlier use of antivirus should have more benefits compared with later use of antivirus**. At last I will appreciate all my colleagues in this clinical trial and all my cooperators in Wuhan City and also I thank the contribution of all healthcare workers including doctors, nurses, in the treatment of patients in Wuhan. Thank you very much for your attention.

Professor Li Jing: Very nice, thank you for Professor Bin Cao, very interesting study. We still need to study more in terms of increasing the sample size or maybe the window to start the treatment. It needs to be discussed later. Okay, thank you very much. Then we move to the second speaker, Professor Giancarlo Agnelli, and he's Professor of Internal Medicine at University of Perugia and Director of the Division of Internal and Cardiovascular Medicine and Stroke Unit at University Hospital of Perugia, President of the Ethical Committee of the Umbria Region and Director of the Research Department of Italian Federation of Hospital lists, and member of the research committee of the European Federation of Internal Medicine, and he's going to give us the topics of information and coagulation of COVID-19.



The lecture will be given by Dr Michela Giustozzi and Professor Agnelli will say a couple of words.

Professor Giancarlo Agnelli: I simply would like to thank you for this outstanding opportunity to be with you today. It's a nice experience for us to share our experience on this disease, and concerning Michela, Michela is a young co-worker of mine working in the thrombosis service and the emergency medicine service of our university, so it's indeed a pleasure to introduce her and to give her the opportunity to present the data and her vision about this pathophysiological condition. Michela?

Dr. Michela Giustozzi: Okay, thank you very much, Professor Agnelli and Professor Li Jing for the introduction. I'll share my slides. Okay, I've no disclosure to declare for this presentation. The title of my presentation is Coagulation and Inflammation in Patients with COVID-19 Disease. The COVID-19 disease is causing significant morbidity and mortality worldwide. The majority of patients usually have a respiratory tract infection, however, a proportion of patients may develop more severe, aggressive COVID-19 disease. That is usually characterised by the presence of fever, resistance to treatment, acute respiratory distress syndrome, a state of shock, and often a multi-organ failure. So, the first question that you should know is what is the meaning of severe COVID-19 disease and what happens in these patients? The definition of severe COVID-19 disease is very heterogeneous amongst studies. Usually it's defined as the composite of the combination of the need of mechanical ventilation, the admission to the intensive care unit and patients who died. In these patients, in patients with severe COVID-19 disease, there is a severe uncontrolled generalized immuno response. The presence of the SARS-CoV-2 in the lungs induces an activation of immune cells such as macrophages, lymphocytes and dendritic cells that actively produce cytokines and chemokines, and the severe overproduction of cytokines is usually called a cytokine storm. (https://link.springer.com/article/10.1007/s00134-020-05991-x)

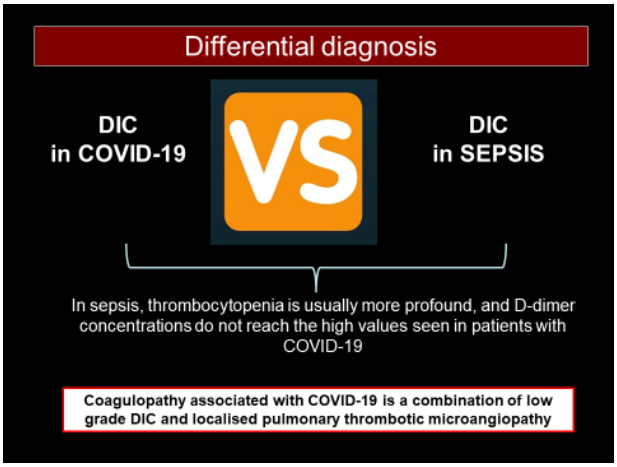
The cytokine storm is a hyper-immune phenomenon, leading to uncontrolled release of pro-inflammatory cytokines that usually lead to a systemic inflammatory state. We can observe in this important state of inflammation the increase of the level of interleukin 6 and the C-reactive protein. This is a study of 150 patients with COVID disease admitted to the Wuhan Hospital in China. The aim is to evaluate the predictors of mortality, and as you can see, the high levels of interleukin 6 and the C-reactive protein were significantly associated with the increased risk of mortality. This means probably that mortality might be due to virally-driven hyper-inflammation. Similarly, these are the new results of meta-analysis including eight studies. The increased levels of interleukin 6 are associated with complicated COVID-19 disease and need of intensive care unit admission, and also with severe and critical COVID-19 disease. The concept of

immunothrombosis is already known: for the concept of immunothrombosis we mean that there is a strong association between coagulation and inflammatory response.

This is exactly what happened in cases of infection by SARS-CoV-2. In presence of the virus there is an overproduction of cytokines, in particular interleukin 1, interleukin 6 and tumor necrosis factor. The interleukin 6 usually leads to expression of tissue factor on mononuclear cells that activate the coagulation cascade and thrombin generation. Similarly, tumor necrosis factor and interleukin 1 usually are the main mediators for depression of inhibitory systems, and often in severe COVID-19 patients we observe an inhibition of fibrinolysis. During an inflammatory state the mechanism can be impaired, so we can have an imbalance from anticoagulant and coagulant products. That determines an increased risk of coagulopathy. In clinical terms, the coagulopathy observed in patients with COVID-19 is usually characterized by high levels of D-dimers and high levels of fibrinogen. There is a minimal prolongation of prothrombin time and activated thromboplastin time, and a mild thrombocytopenia. For mild thrombocytopenia we're finding that platelet counts were lower than 150,000. Only a minority of patients, and these are more severe and critical COVID patients, may develop a disseminated intravascular coagulopathy. For disseminated intravascular coagulopathy even for COVID-19 patients, we can use the DIC score, according to the ISTH definition. So, if a patient has a DIC score of 5 or more, the patient has a diagnostic of disseminated intravascular coagulopathy.

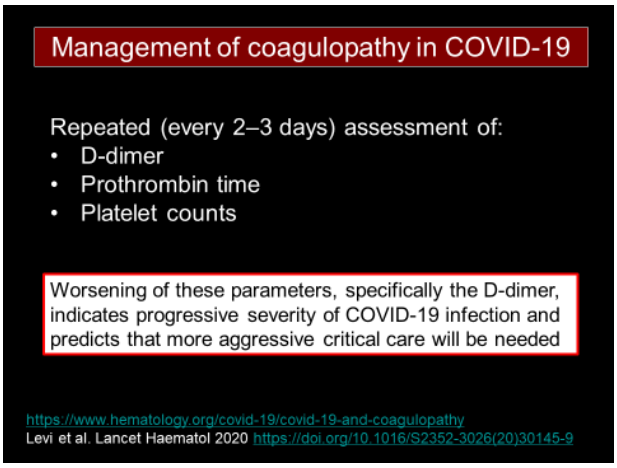
How is the prevalence of coagulopathy in patients with severe COVID-19 disease? This is probably one of the largest studies published in China. It's 1,099 patients included in the study, and as you can see, high levels of D-dimer and a low number of platelet counts are associated with more severe COVID-19 disease. What is the meaning in terms of prognosis? In this retrospective study of 183 consecutive patients admitted for pneumonia by COVID-19, patients who died had increased levels of D-dimer and fibrinogen degradation products, as well as there is a prolongation of prothrombin time at admission, and moreover, in patients who died, 71.4% of these patients had the criteria for disseminated intravascular coagulopathy according to the ISTH definition, compared with 0.5% of patients who survived. The median time from admission to developing disseminated intravascular coagulopathy in these patients was four days. That means that increased levels of D-dimers and prolongation of prothrombin time at admission were a predictor of poor prognosis in these patients. What about thrombocytopenia? In severe thrombocytopenia, that is defined by platelet count of 100,000 platelets, usually it's observed only in a minority of patients with severe COVID-19 disease, about 5%, whilst the milder thrombocytopenia is often present in severe COVID-19 patients.

Here in this table I report the mean result of the meta-analysis including nine studies. These studies was very heterogeneous and different to each other, for example, the sample size is varied from twelve patients included in one study to 1,099 patients included in another study, but also the definition of severe COVID-19 disease is very heterogeneous, the most commonly used is that already said, so the composite of the need of mechanical ventilation, the intensive care admission and the



patients who died. If you turn to the pooled results of this meta-analysis, the lower number of platelet count was significantly associated with severe COVID-19 disease. However, I would like to highlight the high heterogeneity (92%). That means there are several differences amongst the studies included. In severe COVID-19 patients we'd also observe other laboratory abnormalities, for example, in this study, in patients who died there were increased levels of lactate dehydrogenase as well as serum ferritin compared to patients who survived. So, even the lactate dehydrogenase and serum ferritin may be parameters for full prognosis in these patients, and we have usually this in a thrombotic microangiopathy. In fact, several expert opinions define coagulopathy in severe COVID-19 patients as a combination of low grade of disseminated intravascular coagulopathy, and the localised pulmonary thrombotic microangiopathy. What are the main differences between disseminated intravascular coagulopathy caused by COVID-19 and that caused by sepsis?

Usually in the disseminated intravascular coagulopathy observed in sepsis there is more severe thrombocytopenia, but we do not reach the high levels of D-dimer observed in patients with severe COVID-19 disease. According to that I have just said, there is a hypercoagulable state in these patients, which is an important risk factor for thrombosis. In cases of endothelial dysfunction by cause of viral infection, or blood stasis, for example, in patients hospitalized the risk of thrombosis rapidly increases, and in several core studies we observed a high instance of thrombotic complication in these patients. Lastly, what is the management for coagulopathy in

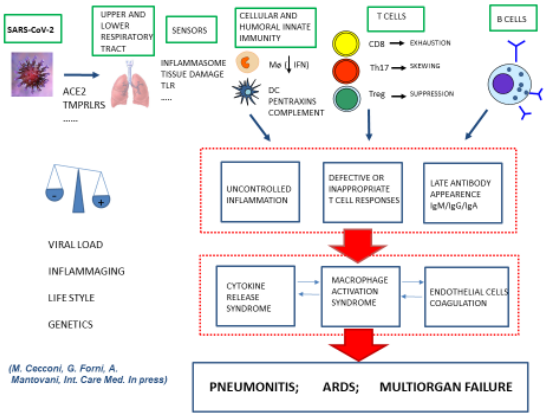


patients with severe COVID-19 disease? The experts suggest monitoring every two or three days laboratorial parameters such as D-dimers, prothrombin time and platelet counts. In case of worsening of these parameters, specifically with D-dimers, probably aggressive critical care will be needed.

These are my conclusions. Inflammation and coagulation in severe COVID-19 patients are linked by a two-way association. The most common coagulopathy is characterized by elevated D-dimer and fibrinogen levels. COVID-19 associated coagulopathy or disseminated intravascular coagulopathy leads to a severe prognosis. In severe COVID-19 patients D-dimers, prothrombin time and platelet counts should be repeated every two to three days. Thank you for your attention.

Professor Li Jing: Very interesting talk on the information and the coagulopathy in the COVID-19 patients, and you introduced a lot of studies and insights of this issue. Thank you very much, and then our next speaker, Professor Alberto Mantovani and he's the scientific director of Istituto Clinico Humanitas and emeritus Professor of Pathology at the Humanitas University in Milan. He is considered one of the most influential Italian scientists in his field, and he's going to give us the a talk with the topic of immunologic response to the SARS-CoV-2 infection and treatment implications. Now, please, Professor Mantovani.

Professor Alberto Mantovani: Good morning all, good evening all, goodnight, depending on where you are, or good afternoon. It's a pleasure and I thank the organizers for having made me part of this, and I will start with a Greek philosopher, Socrates, and he stated, 'I know that I don't know.' I must say, from the point of view of immunology of COVID-19 I feel like Socrates, since there are many things that we don't know. And what I will try and do will provide you with an overview of progress that has been made in the field and of the many question marks. And if you are interested in a short, very concise review, we have one coming up, it's not actually submitted, it's in press in intensive care medicine. So this is just histology and we are all familiar with the radiological appearance and the corresponding histology, the evolution of the lesion, the thrombosis that were discussed, the microvascular pathology and thrombosis, and the inflammation. Plenty of leukocytes, plenty of macrophages there. And this is actually the paper in press in intensive care medicine, and this is a summary slide and I will go through the various steps.

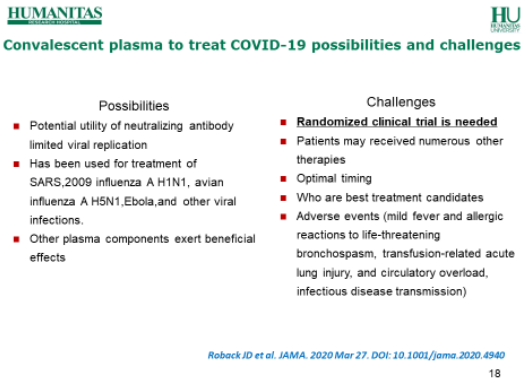


Starting from left, the virus entering the upper respiratory airways, innate and inactive immunity and then the inflammation and then the cytokines, so the macrophage activation syndrome. Viral load, ageing, inflammaging, lifestyle and genetics are key determinants. So we learnt a lot from SARS and much of what we know is based on SARS, and here you have the classic evolution with innate immunity, the acute infection, the adaptive immune system coming up, and pneumonitis and cytokines and the cytokine store. And we know much less because of lack, for instance, lack of a suitable pre-clinical model, but still we are making progress. And this, again, is SARS. And they used to say this and it's reported in the papers, that maybe skewing at the very beginning you have interaction with the innate immune system, and there is inhibition of effective antiviral immunity based on blocking of interferon production. There is a molecular basis for this. And there is very recent work published, for instance, in Cell, and with evidence indicating that, indeed, suppression of interferon mediated innate resistance plays a role and is a key determinant in the pathogenesis of COVID-19.

And this is, again, a schematic representation of the various steps: innate immunity, sensing. I will get back to sensing, and then activation of adaptive immunity with T cells and B cells. So sensors here, of course there are sensors of tissue damage and there is some evidence for SARS and there is now some evidence for COVID-19, that the key driver of innate immunity and inflammation is sensing by inflammasomes. Here is a schematic representation of the NALP3 inflammasome, and the molecular matrix here is unclear. It remains unclear whether it's tissue damage or, actually, because I should have said that there is evidence for virus sequences in innate immunity cells in particular, in macrophages where it's not clear whether disease reflects replication, and the same is true for endothelial cells. And downstream of inflammasome and caspase activation is IL-1. My lab has long been involved in the dissection of the molecular components of the IL-1 system. Let me just remind you that at a local level, there is a driver of amplification of innate and adaptive immunity, higher concentration, systemic and here, of course, we've heard about fibrinogen, acute-phase protein, C-reactive protein and fluid phase pattern recognition molecule. And I will get back to complement IL-1 that is also driver of adaptive immunoresponses, and this is from a review of ours on IL-1, and it is of TH-17. And I should remind you that TH-17 is an appropriate response for extracellular material, but an inappropriate response for viruses. And there is some evidence for skewing of the adaptive immune system to a TH-17, not terribly solid, but some evidence. In addition, and this is a slide taken from my cancer immunity lecture, there is evidence for exhaustion of CD8 T cells and there is evidence for regulatory cells for professional inhibitors of inactive immune responses, and then we get back to that. And, of course, we all know that during COVID-19 there is lymphopenia.

(https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30183-5/fulltext)

The reason we have a story now available on loss of eosinophil counts, inflammatory cytokines including IL-1, IL-6. And the drivers of the uncontrolled inflammation, as I said, include sensing of tissue damage, pyroptosis, inflammasome and IL-1 activation and it may include complement activation, and there is evidence for that. And this is Cecilia Garlanda, my colleague, this paper in Nature Reviews Immunology. And there is evidence for uncontrolled complement activation, virus endothelial cells and direct activation of the lectin pathway. And, of course, there are immune complexes and that may also be a driver. And there are implications of that and there are, of course, ongoing trials, for instance, John Lambris, and here in Italy there are at least three sets of trials. John is an old friend of mine, targeting C3. Of course, the obvious thing is targeting C5a based on a syndrome activity of anti-C5a blocking the C5a pathway, and then there is a study by targeting the lectin pathway. So don't forget, humoral innate immunity and complement. And we are working on that because we discovered some of the molecules involved in humoral innate immunity. Of course, you have antibodies, we are all familiar with that, antibody production, and this is one of the early studies by Lou et al but, in essence, we have similar data in China, Europe, USA. The antibody response is late, as late as after twenty days after exposure, fifteen days after symptoms it came to exist with a virus. And, unfortunately, this virus has not studied immunology in the textbooks, and so you don't have the usual clear definition, distinction between IgM and IgG.
(https://www.medrxiv.org/content/10.1101/2020.03.23.20041707v1)
And I should say that we have just made the available in the literature, I didn't have time to put the reference, a large serological survey in a hospital population that is located in different areas of the Lombardy region, Bergamo, one epicentre of the epidemics in Northern Italy and Milan is now available in the literature. And this is, again, the same story from the Nature paper, again, the immunasome coming up late, and the same, of course, paper taught us that the upper respiratory tract is a major source of virus.
(https://www.nature.com/articles/s41586-020-2196-x)
Of course, antibodies have obvious implications, including plasma therapy, and there are hints that it may work, more than 2,000 subjects have been treated in USA, there is a study ongoing in our country in Italy.



But I think that the heart of the matter is, as we heard from Doctor Bin Cao in the first presentation, is that we need to have results from randomised clinical trials. And this is the take-home message of my talk, let me move this so that I can say it. This is an attempt to summarize the old story, everything starts with interaction of the virus with the upper and lower respiratory tract. Replication in other organs, we now know, sensors, and there is evidence that sensors may include inflammasomes, tissue damage, sensors of tissue damage, toll-like receptors, activation of cellular and humoral innate immunity, including pentraxins, we have cloned members of the pentraxin family, complement, I discussed complement, with inhibition of effective antiviral innate immunity, interferon. Then, further downstream, T cell responses, the directors of the immunological orchestra. Of course, tremendous progress has been made in the last few weeks in terms of identifying recognition, what is recognised by T cells, and there is evidence for exhaustion, inappropriate skewing and suppression. Then the B cells, of course, would assume the antibodies. And downstream is uncontrolled inflammation, associated or because of defective inappropriate T cell responses and late appearance of antibodies. We are also familiar with the cytokine disease syndrome, macrophage activation syndrome and we just talked about endothelial cells and coagulation and the clinic, and Doctor Cao has beautifully commented on a very important clinical trial.

And here you have regulators, viral load, springtime, summertime, all upper respiratory tract infections tend to go better in spring and we are seeing that. Ageing, lifestyle, smoking and more, and then genetics. And we published, I think possible the first report of genetics of the human population and the presence of peculiar variants of TMPR, LR-5 in the human in the Italian population. We are now linked with a large European study on genetic polymorphism and we are linking to USA. And, again, here is the review, concise, very concise review if you are interested. And the final take-home message is, again, I know that I don't know, there is plenty of things we don't know about the virus which have implications for treatment. And I want to end with a picture of mine at work on COVID-19, and this is me, trying to decipher the immunology of COVID-19. Thank you for the pleasure to be here with you.

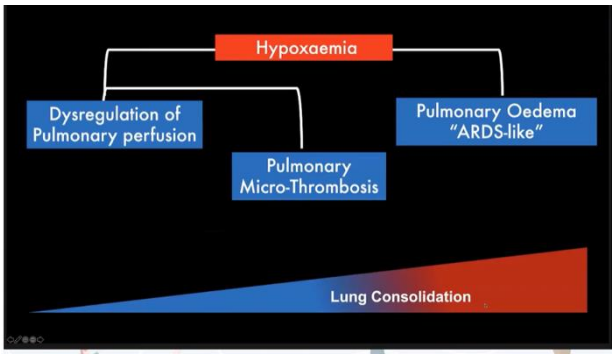
Professor Li Jing: Thank you very much. It's a very incisive talk on the immune response to the SARS, COVID to infection, including varied kinds of immunology components, including cytokines, complements, antibodies and the immunology cells. Yes, very nice talk, thank you very much. And then, the next talk is given by the professor from UK, Professor Luigi Camporota, he's the consultant in critical care, Guy's and St Thomas' NHS Foundation Trust, London, UK, and an honorary senior lecturer in Kings College London. And also he's the associate editor of Journal Intensive Care Society and the visiting research fellow, Department of Critical Care, University of Göttingen and visiting research fellow in the Institute de Chirurgie Guidée par L'Image. And also he is the deputy chair of the Section Acute Respiratory Failure of European Society of Intensive Care Medicine. And he is going

to give us the talk with the topic of intensive care management. Let's welcome Professor Camporota.

Professor Luigi Camporota: Good afternoon, many thanks, and I'm sorry if my names and various titles have made life complicated for you and for everyone listening. Well, I just say, it's a great pleasure and honour, and of course I'm very grateful to you and Professor Corbetta for the kind invitation. And I'm just going to talk a little bit about the intensive care management of these patients and, particularly, mechanical ventilation, and touch on briefly at the end on ECMO, so the Extracorporeal membrane oxygenation. So we know from various literature about 20% of hospitalised patients and maybe 70% of critically ill patients develop what we call ARDS or ARDS-like syndrome. And a proportion, which varies quite a lot between one third and almost 90% of these patients require invasive mechanical ventilation, with a mortality that ranges quite widely as you see in the literature, between 16% and 62%. And, obviously, this contributes to what Professor Mantovani was saying about we don't know what we don't know, or sometimes we know what don't know, about the characteristics of this disease. But what we know for sure, in terms of the syndrome that we see in the intensive care, that COVID-19 has atypical characteristics.

This is because there is an onset of respiratory failure normally later than, for example, flu, about eight to fifteen days from the insult. And we found a dissociation between radiology and symptoms. We'll see later on some of the examples, a dissociation between the degree of hypoxaemia and dyspnoea, degree of hypoxaemia loss of lung volume and, therefore, compliance and, therefore, the response to PEEP, which makes it slightly different from the 'typical', I use here with inverted comma, typical, ARDS. And so, first of all, radiology, you can see this is a study, it came out in the Lancet Infectious Disease, 81 patients from China, and you can see that when the symptoms go from subclinical to two, three weeks, the clinical picture and the radiological picture changes from a unilateral to a bilateral, from a multi-focal to a diffuse.

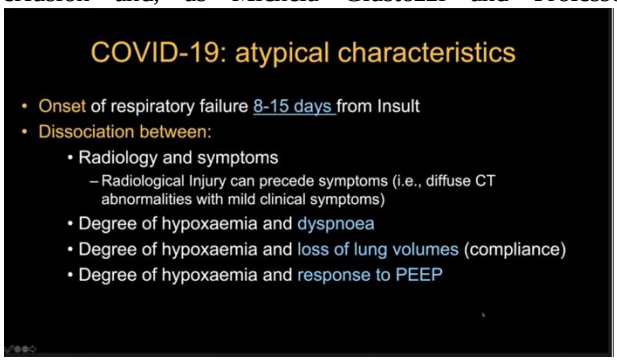
The ground glass goes down over time but the amount of consolidation increases. And this is because it is important, in my view, because it can tell us a little bit about the cause of the hypoxaemia and, whereas, in ARDS, there is a great component which is due to consolidation and oedema. In this group there is more of a dysregulation of pulmonary perfusion and, as Michela Giustozzi and Professor

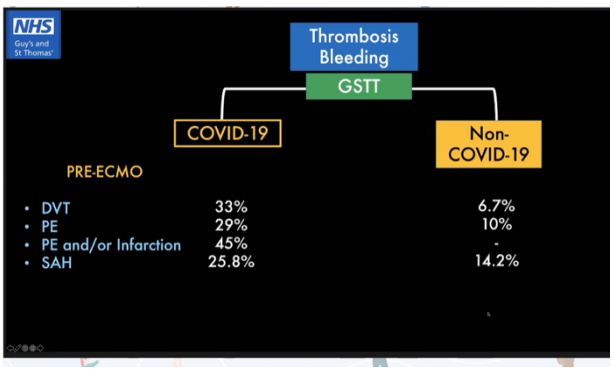


Mantovani have said, there is a great degree of immunothrombosis and microthrombosis.

And so you can see here, this is a picture where you can see this dual energy CT scan and you can see at the bottom, the one in yellow, sort of, a bright yellow, are dilated vessels. So this is dilated vessels in poorly ventilated area, which, obviously, they give an increase shunt fraction. And the one on the bottom left, you can see that, sort of, red area, which is low perfusion. So you can see that within the same lung field, there are areas of increased perfusion and there are areas of reduced perfusion due to vasoconstriction, or sometimes microthrombosis. And this is quite clearly you can see that cross-sectional histological image of small intraceptal and medium-size arteries which are completely occluded by thrombi. And on the right-hand side you can see how patients who have a worse outcome have also higher D-dimers and ferritin and IL-6, indicating a role of inflammation in the generation of this thrombi. And this is one of our own images, you can see some presentations have minimal parenchymal changes but large pulmonary emboli, and you can see that there in one of the pulmonary entries. And this is our own data, just our fresh data of all the patients who came to us pre-ECMO. You can see we made a comparison between COVID-19 and non-COVID-19, and you can see that there are one in three patients who come to us prior to receiving ECMO that got evidence of DVT, compared to 6.7% in a non-COVID cohort. We're talking about the non-COVID cohort is about 500 patients and we've got about 60 patients pre-ECMO in the COVID-19 population.

Almost one in three have a pulmonary emboli, you can see it's 10% in the non-COVID and about almost half how they combined either pulmonary emboli or an infarct that we diagnose on a dual energy CT scan. And also the rate of minor bleeding has also increased in the COVID-19, so that goes to the idea that, perhaps, the gas exchange difficulties that we



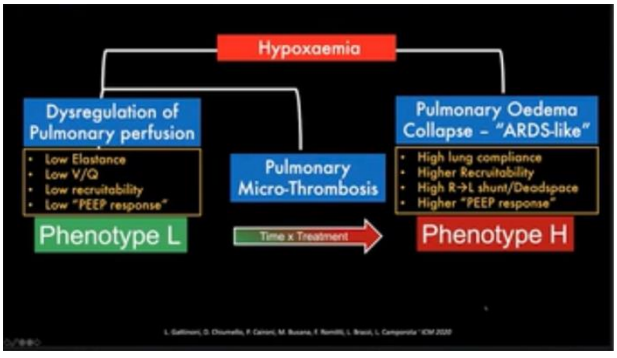


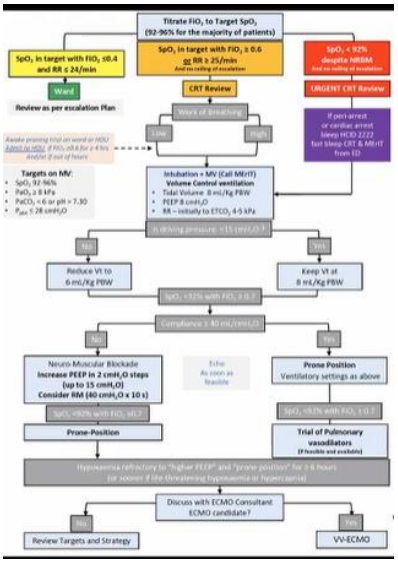
see in intensive care patients is not exclusively due to consolidation. And you can see these are two images, you can see on the top there, the rotating 3D image of a lung of a patient, and the one at the bottom, you can see that they are hugely different. One looks like normal lung volume, the other one is reduced lung volume, the rest that you cannot see at the back is all consolidation. And on a, sort of, cross-section imaging, you can see the two at the bottom and the top and the bottom, what you can clearly see is that the amount of volume is reduced at the one at the bottom, and also there is a large component of oedema and consolidation. (https://link.springer.com/article/10.1007/s00134-020-06033-2)

But phase two patients have the same gas exchange, the same degree of hypoxaemia. So that led us to investigate a little bit and try to understand why this hypoxaemia is the same, with a different degree of mechanical impairment. And so, on one hand, we've got the reduced pulmonary perfusion with the microthrombosis, in the other hand, we've got a different phenotype which is more typical of a collapsed ARDS. And we, sort of, code that as a way of distinguishing these two as a phenotype L, because L stand for 'low', they've got low elastane, so i.e. high compliance, low V/Q, low recruitability, so in other words, the response to PEEP is minimal. Whereas, on the other hand, there is the high lung compliance, there is higher recruitability, so the response to PEEP, and this is important because they're obviously two extreme of a large spectrum. But time and treatment can interact in the determination of the phenotype H or L. And you can see, this is a study where, essentially, they find that 45% of the intensive care patients might have a phenotype H when there is about 75% of consolidated tissue, and 55% of the patients have very small amount of consolidated tissue.

And, obviously, there is a large group in between these two phenotypes. So, it's not meant to be mutually exclusive but it's a representation of the two possible causes of hypoxaemia in these patients and that's why we came to ventilate our patients in a very protocolised way that tends to identify these two groups very early. So, we want to do, first of all, an assessment of hypoxaemia and shunt fraction prior to presenting to intensive care, usually the emergency department.

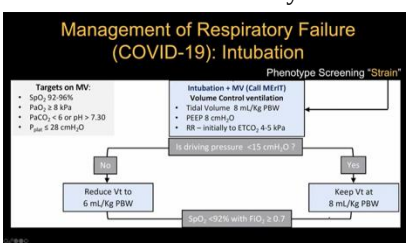
We want to see whether these patients can do well with non-invasive support and the risk of patient self-inflicted lung injury and I'll talk very briefly about this in a second. We want to see whether the tidal volume is appropriate for the lung size, just all non-invasively. And we want to do a

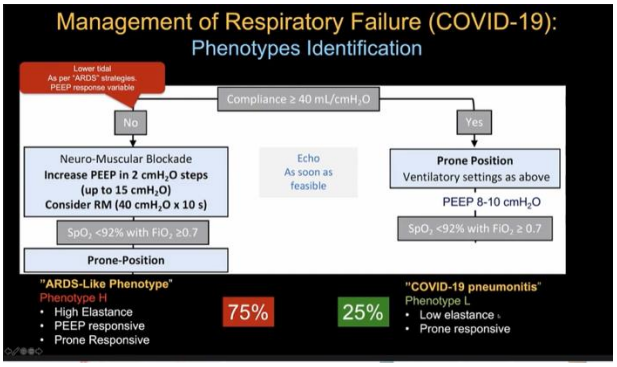




treatment that is based on phenotype so trying to individualize the mechanical ventilation of these patients and then finally, early recognition of failure and escalation. So, the first thing we want to do is making sure that the patient will not cause self-inflicted lung injury so don't stay too long on CPAP or non-invasive ventilation and we normally do a very brief trial, one or two hours and I think the Chinese protocol that I've seen published online they do exactly the same as we do. And then, if there is an intubation that is required then we go for volume control, low PEEP, because most of the patients might not need high-level of PEEP or indeed might not respond to an increased level of PEEP and then the second thing we look at the driving pressure.

If the driving pressure is low then tend to maintain 8ml/kg predicted body weight and if the driving pressure is below fifteen that means the lung volume is reduced and therefore they have maybe a more typically ARDS and therefore we need to reduce the tidal volume to make sure that the strain of that lung is not excessive. The second thing we look at, we look at compliance just to identify the two phenotypes that I just alluded to a few moments ago and if the compliance is less than 40 then we often do neuromuscular blockade. We do a PEEP trial, increasing the PEEP up to fifteen or we consider short, moderate recruitment manoeuvres and certainly we employ prone positioning very early and that is 75% of our population. The moment they come to the intensive care we'll satisfy that criteria and whereas we need to do-, well we tend to use the ARDS strategies so this is more like a typical ARDS. On the contrary these patients who represent a quarter of the ones that are admitted to intensive care and we need to think that by the time they get to us they're already two, three weeks in their illness. So, about five days of illness then presenting somewhere else and usually a smaller hospital and



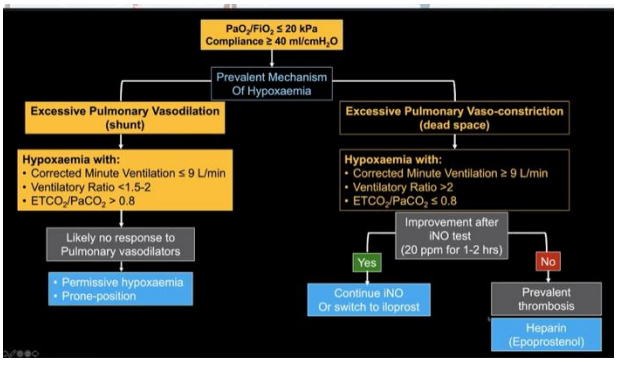


stay there for three, four days and then they come to us. They're already about day ten which might modify something to do with this ratio of 75:25. So, in this group of patients we tend to use a more liberal tidal volume. So, lower tidal volume that often are necessary might lead to hyperventilation but also at the same time higher PEEP is in effect it doesn't recruit, there is very little consolidation as we've seen.

It might affect blood flow, so more of a redistribution of blood flow rather than alveoli recruitment. So, this is one of the studies that basically I would like all you to concentrate on the recruitment inflation ratio which is a ratio to say how much can we gain in terms of lung volume by just increasing PEEP. And we can see that it's hugely varied, you can see the confidence sinks over there and anything above 0.5 means the PEEP is effective. And you can see that in some patients it's completely ineffective in others, more effective but essentially what we see is a huge amount of dead space. This is very important.

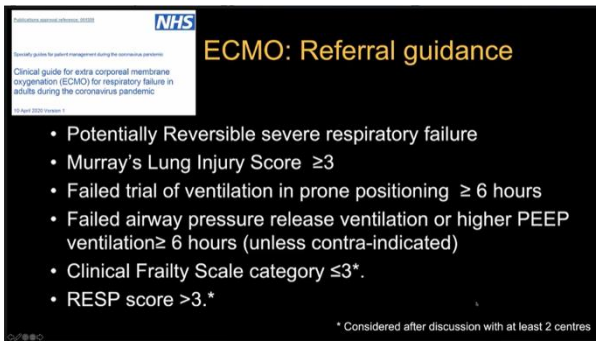
(https://journals.lww.com/ccmjournal/Abstract/9000/Potential_for_Lung_Recruitment_and.95675.aspx)

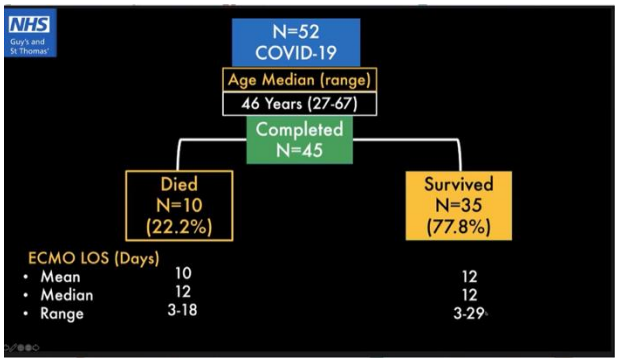
Now, what I want to show you very quickly is just this data from our patients. You can see driving pressure on the Y-axis and tidal volume on the X-axis and you can see that a large proportion of patients have actually very high compliance compared to the normal ARDS with the same PF ratio. So, what we do for these patients with low compliance-, sort of, high compliance but low PF we look at a nitric oxide test, you can see in the bottom. If the nitric oxide, which is a pulmonary vasodilator as you know, is able to increase the gas exchange then what we'll find that normally the major mechanism is excessive pulmonary vasoconstriction and dead space and is dynamic. Whereas if they don't improve then often the dead space is due to prevalent thrombosis which is not obviously reversible with a pulmonary vasodilator. In that case we go for full heparinisation or sometimes prostaglandin infusion which have anti-platelet and also increase the vasodilation so it might have some additional effect to heparin.



But obviously, not everything goes according to plan and you'll see shortly what has been our experience. We are a big ECMO centre in the UK and probably a big one in Europe and you can see that some of the patients were not improved by the maximal treatment that we could provide in intensive care. And you can see from the chest x-ray that it's quite obvious that the patient on the right has got very little chance of improving with just conventional ventilation. There is no aeration in that lung at all and so this is our number of ECMO referrals. Now, what you can see these are the four units in the UK and we had between the month of April and the first two weeks of May we had 1,186 referrals for ECMO. Now, you can see on the right-hand side that has been a number of referrals per day and the line is where we are normally. This is our normal, usual number of referrals so you can see the activity of an ECMO service has been incredibly high and so the NHS or the National Health System in the UK has come up with a guidance and I think this is quite interesting. It may be some point for discussion at some point during this meeting. That we would consider ECMO if the lung injury score is greater than three, if the patient had failed, trialled and failed ventilation in prone position or higher PEEP level for at least six hours, they were not clinically frail, and I'll show you the clinical frailty scale. If the RESP score, which I'll show you in a second, was greater than three. Now, if any of these considerations were not met then we were asked to consult another centre and to get an agreement whether or not this patient was a candidate for ECMO.

This is the RESP score, it's a well-validated score although in a non-Covid population. You can find it online and calculate the RESP score for any of your patients and essentially if a typical patient is young, has been ventilated for up to seven days and I'll say here they've got viral pneumonia. Even if Covid-19, as you all well know, might not fit that definition because of the calibration of the RESP score is mainly with influenza virus. This is the clinical frailty score, so a three and below. Patients who are essentially autonomous and they have well-controlled medical problems and this is our final outcome in terms of the ECMO patients that we've seen in the last six weeks essentially. We had 52 patients on ECMO, median age about 46. Of these patients we had a good survival of 77.8% and on average they stayed on ECMO about twelve days between three and 29. We had quite long, long stayers.





So, this is my summary which is also last slide, I would suggest that perhaps we need to consider the different phases of the illness where the initial part is the low oedema and therefore there is need for low level of PEEP, the lung volumes are normal. Then through a period where the oedema increases and the lung is responsive to PEEP and then the lung volume reduces further with an organising pneumonia or sometimes fibrosis where the lung no longer is recruitable. So, at the top there, the use of prone position is very important, maybe liberal use of tidal volumes and low PEEP at the beginning.

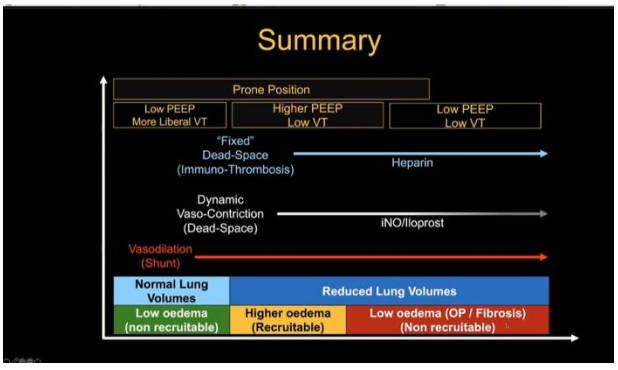
Higher PEEP and low tidal volumes in the middle when there is the recruitable lung and the use of high PEEP in the later stages is clearly not effective when organizing pneumonia or fibrosis are the predominant histology. And then something in the medical management in the middle and with that I'd like to thank you for your attention

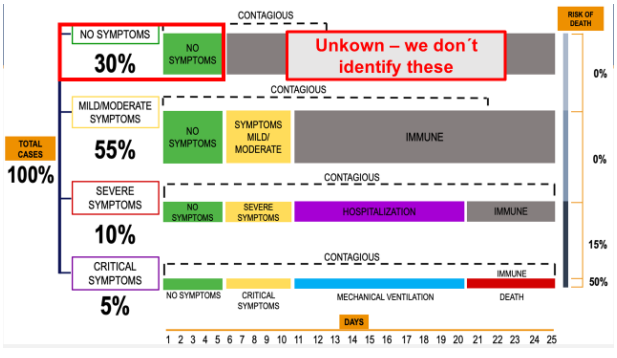
Professor Li Jing: Thank you very much for the very nice talk. You introduced your experience in the treatment of the very critically ill patients with ARDS and other critical situation in Intensive Care Units, and use of the ventilation and ECMO, and other supportive treatments. A very incisive talk. And we'll move on to the next one, Professor Luis Adrian Rendón, Specialist in Internal Medicine and sub-specialist in Pneumology and Intensive Care, Professor – University Hospital of Universidad Autónoma de Nuevo León, Head of CIPTIR, Center for Research, Prevention and Treatment of Respiratory Infections, President of the Mexican Pulmonology Society.

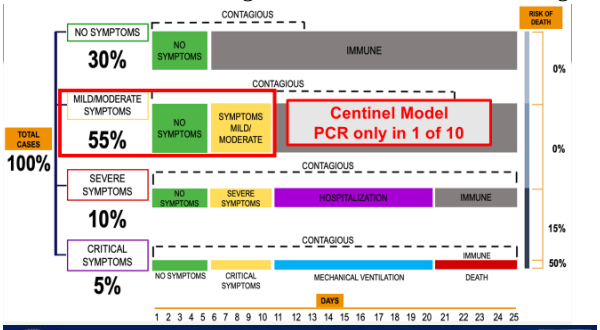
Professor Adrian Rendón: Hi, hello. First of all I would like to thank you for the invitation to be part of this, it is an honour for me. When I was invited to talk about the management of COVID in Mexico, I said yes, but I've got a problem to decide what to present, because there is a huge amount of information around. And for me, the problem was to decide what to present in the lack of published evidence from Mexico. So what I'm going to do in the next few minutes is to give you the big issue of the pandemic, the COVID pandemic in my country. We are a large country, with a medium population, and we have a lot of situations that could make worse the COVID epidemic. Poverty, Diabetes, Smoking, and Overweight. So we start in a fine position to fight against COVID. The first case we had was in February 28th, before the pandemic was declared. And in Mexico it was a sanitary emergency since March 30, with lockdown.

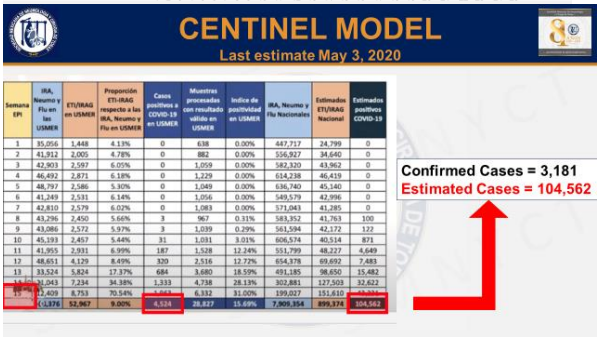
The problem we had was how to define the non-essential activities. So everybody interpreted it in different ways, and we are supposed to restart activities in two more days. In Mexico, the diagnosis of COVID was mainly based on PCR. But the problem it was a centralized PCR. From all over the country, we had to send the samples to Mexico City, to the official lab in Mexico. That was at the beginning. Later, we had labs in the states, at least one per state, but not in all the cities. Serology was was bad. This statement should be very familiar. It's a deductive table that was built from the original data from China, in the middle of February. Here we can see that there are four groups of patients. One asymptomatic, and three symptomatic. What I want to point out here is that we didn't look in Mexico for this group, the asymptomatic. For this, with my thesis, we followed the Centinel Model, so we went from PCR only on one of these suspicious cases. We were not counting all the cases.

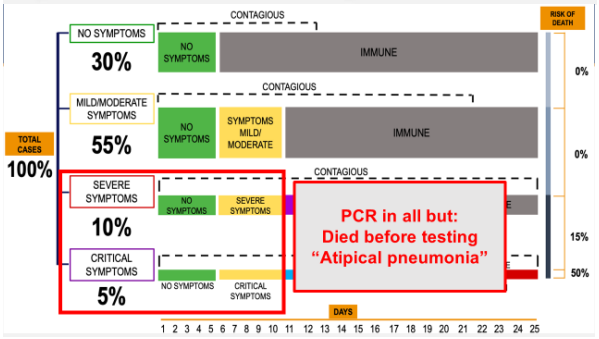
And for this group, the severe patients, we were supposed to perform PCR as well, but the problem that we had was that some of them died before we got the testing, and weren't classified as atypical any more. For this model, the Centinel model, this is the last official data we have in Mexico. It was











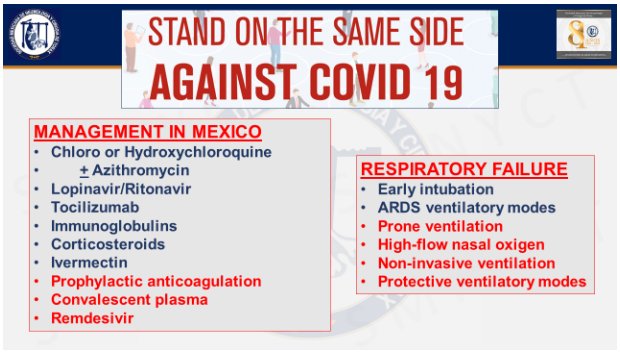
presented on May 3rd. You can see the number of estimated cases, I mean the number of counted cases, confirmed cases, about 3000 but the estimated number was much higher than this. So, we are not counting all the patients we have. This is the curve we have in Mexico, it's supposed to be flat and the last information we have is from yesterday. 81,000 confirmed cases by today, only by this year and we are getting close to the 10,000 deaths. For prevention we were focusing on those active patients to avoid transmissions. And we used the regular, the standard recommendations and we were not using anything for this, the incubation period or the patient who had an asymptomatic disease because the masks were not recommended in my country. With these recommendations how are we doing in the country? Well, it's a shame for me to say that Mexico's place in the bottom of this graphic according to the number of tests performed. So, the conclusion of that for sure, we have more cases than we are counting.

This is the Mexican curve, this is the global curve. I would like this to be as flat as this. If we compare the global numbers confirmed with cases, Mexico is in 17th place, you can see here. For deaths, Mexico is in 8th place and we compare it with Latin America we are the number four place for cases and we are the 1st place for deaths. We are paying the toll of this huge amount of morbidities. The bottom of this, this is high in Mexico and it for sure is related with the high number of deaths that we are having in my country. The other problem we had in Mexico is as in other countries, the healthcare workers. The healthcare workers represent almost a quarter of all the COVID confirmed cases in Mexico. I was invited to talk about the management of COVID in Mexico. In Mexico we usually follow what the WHO says. In these guidelines from the WHO you can see at the bottom of the recommendations is the specific anti COVID treatment. So, if we open that the specific recommendation is that there is no specific anti COVID treatment. So, in Mexico we have to follow that. No specific treatment. They recommend thrombo prophylaxis and they also recommend, at the beginning they didn't, high flow nasal oxygen and non invasive ventilation. What about PAHO, the branch of WHO in Latin America? In these squares, the summary here. PAHO does not recommend any antiviral agents or any anti immunomodulator or convalescent plasma, chloroquine or steroids. So, there is no recommendation for treatment.

What about the Mexican guidelines? In Mexico, guidelines, the Mexican Secretary of Health does not recommend any antiviral therapy but we are specialists. We belong to some of these large societies. IDSA, ATS, well, on their guidelines they don't have any specific recommendation. Lastly the NIH from the USA published these guidelines where they recommend the use of remdesivir and they have a position against the use of chloroquine combined with azithromycin and they don't recommend the other HIV protease inhibitors.

So, facing the lack of recommendation, specific recommendation for Mexico we have done whatever we read or whatever we have, what is available. Every institution has its own guidelines.

Sometimes they are very different from each other and what we're representing here is not published information. It's personal communication, observational data. At the beginning of the pandemic here in Mexico everybody was using chloroquine along or combined with azithromycin. In some centres they have available lopinavir or ritonavir. Some of them have some of the other mentioned, not recommended, drugs or medicines but most of the people right now are moving to the three last ones. Using prophylactic anticoagulations. Low molecular weight, using convalescent plasma and remdesivir. These last two are being used in clinical trials. Multi centre clinical trials mainly with NIH. What about the management of respiratory failure? At the beginning most of the patients they had ARD secondary to influenza. Now we are moving to a more personalized approach using prone ventilation, high flow nasal oxygen, non invasive ventilation and protective ventilatory modes. We still need more information to decide what is the best to use in my country. My country is a mass of states, of cities. The north region is very different from the south region. Most of the medical facilities are in the capitals of the states, not in the small cities. So, we need to decide what is the best in each region and each location and who is going to handle the patients. Right now we are putting the patients in the hands of internal medicine doctors, a few pulmonologists, a few intensive care doctors.



So, we need to improve that lack of specialist in my country. Thanks for your attention and the greetings from the Mexican Society of Pulmonary Medicine and Critical Care

Professor Lorenzo Corbetta: I am here with my colleague Semra Bilaceroglu: we are in the same board in the European Association Bronchology and Interventional Pulmonology (EABIP) and I asked her to introduce and to stimulate the discussion, please.

Professor Semra Bilaceroglu: Hello friends, colleagues. It's a pleasure to be on this panel as a moderator. I will ask the first question to the first speaker, Professor Bin Cao, is he here?

Professor Bin Cao: Yes, I'm here.

Professor Semra Bilaceroglu: Okay. As you said we should give this lopinavir with another treatment earlier to these patients. Can you describe the phenotype or the future of the patient to give this drug combination at an early phase? What type of patient? Is it a pre ICU patient or is it moderate case? As Prof. Mantovani said, we don't know many things in treatment, in diagnosis, in immunology but you have a lot of experience and you have done the study, can you give the profile of the patient to give this combination drug early enough?

Professor Bin Cao: Okay, I think it's a great question about the antiviral because everyone knows that the COVID 19 is caused by a new virus, the SARS corona two. We have learned a lot about the pathogenesis of the COVID 19 caused by SARS corona two.

Yes, I do believe for the fight against the COVID 19, antiviral is one of the main choice of the treatment of such disease. The problem as you mentioned is that the phenotypes and the timing of the antivirals. So, in my opinion the antiviral should be given as early as possible but I don't think it's a question of phenotype but is a question of the high risk of patient who we are aware have a severe condition. So, I think that the nations, not only China but in UK and in Italy, in Turkey, in Mexico, that we have to work together to pick out the high risk patients who can develop to the severe disease and for some populations already known such as the elderly patients. The patients with the underlying disease. The patients with immune suppression and also partly the obesity. So, for such patients the earlier use of antivirals would be

better. As I mentioned from the proof of researcher from Hong Kong, the professor Ivan, the professor Ming, the start of the first dose of antivirus is five days. In Luigi Camporota lecture he mentioned that from the onset to the progression of the ICU patients is averaged between eight and fifteen days. So, I think the beginning of antivirus for high risk patients should be less than one week. So, this is what we have learned and I think another question that maybe some patients who are unlucky who go to the hospital very early such as within one week and what is more problem with that is the patient may have already developed significant issues such as ARDS. So, how can we do if the patient comes to the hospital very late? I think the modern therapy of antivirus such as lopinavir or even remdesivir is not enough and you have already read in the paper in UN journal of medicine by NIH. The first author of the NIH remdesivir trial, doctor John Bygoe sent me an email after he published the paper, the preliminary remdesivir trial and he sent an email to me and told me, he said about this, I think it is not enough. He has to give remdesivir to rescue the patient who has already developed ARDS. So, in this severe condition patients I do believe that the combination therapy while the antiviral, the other is the whole target therapy such as the R 1 antibody or R 6 or some kind of other choices. So, the combination maybe two drugs or maybe three drugs or even four drugs.

For the severe condition patients, the combination therapy, antivirals and the new immuno modulators should work together to help the patients together with the standard care such as the mechanical ventilation or even ECMO.

So, for high risk patients early use of antivirus, for severe cases combination therapy including antivirus. That's all, thank you.

Professor Semra Bilaceroglu: Thank you Doctor Bin Cao. Another question to Doctor Michela Giustozzi. What is the rate of large vessel thrombosis in your hospital in COVID patients? Or you may give, if you have, the rate for whole of Italy. Why I ask this question, in many countries, some countries say there is pulmonary thromboembolism, large vessel thrombosis in these COVID patients but then I look at my country, yes we see some patients but the rate is not so high. In my hospital, even in ICU patients, we have seen not so many. My hospital by the way is a COVID hospital. I am working in a COVID department. So, can you give me the approximate rate of large vessel thrombosis in COVID 19 patients in your hospital or in Italy?

Dr. Michela Giustozzi: Okay, thank you for the question. I also work in a COVID-19 centre. So, I can report our experience. Fortunately the Umbria is a region with a low incidence of COVID 19 patients compared to other regions in Italy. We have collected data from about 120 patients overall and we observe that the incidence of thromboembolic events was about 15%. This is probably lower compared to the evidence, to the literature, but I think that one reason was the difficulty that we have to perform for example CT scan in our hospital for logistic question. However on the other hand we are emergency specialists but we are skilled to perform ultrasound lower limb. In the majority of patients we perform the ultrasound of the lower limb and we observe that the majority of these patients, the majority of thromboembolic event that we have observed were deep vein thrombosis and in particular distal deep vein thrombosis and often this distal vein thrombosis were asymptomatic. So, the real meaning of distal asymptomatic vein thrombosis in this patient remain to be declared and in our patients, according to the guidelines we started at the initial low dose of prophylactic with low molecular-, we started thromboprophylactics with low molecular weight and in case, when we found for example a distal vein thrombosis, even if it was asymptomatic, we adjusted and we increased the dose according to the therapeutic dose. And this is probably our experience. I don't know if Professor Agnelli wants to add something more.

Professor Giancarlo Agnelli: You described exactly the situation. Many of these events are asymptomatic because they were screened lower limb ultrasonography. The rate is high but it's not so higher when compared with what you see in patients with sepsis. So, I have some doubts that it's a specific reason to use a particular technique for screening in this patient that in ICU. With some more increase but not that much. That's how it's been.

Professor Lorenzo Corbetta: There are some questions from the faculty? Professor Fabbri has a question. Thank you.

Professor Leonardo Fabbri: Thank you Lorenzo, thank you Semra. My question is actually directed to the panel and particularly starting from Prof. Camporota and to the other speakers, Doctor Giustozzi and Professor Mantovani particularly. One of the intriguing clinical feature of this disease is the inability to predict patients that are admitted with respiratory symptoms, mild and fever, maybe cough and some within three, four hours, maybe twelve hours, go into respiratory failure. So, the question to you from your perspective and also from your expertise, I know it's a difficult question but we need to address difficult questions. I mean, are any biomarkers, you mentioned some but any biomarkers that might be, or physiological marker, Doctor Camporota that might be meticulously monitored to predict this evolution? And if that is the case from your experience do you think that if you get them early you are able to improve the prognosis of these patients? Thank you.

Prof. Luigi Camporota: Thank you very much. I think you've put your fingers on the really difficult question because some of these patients they come to the emergency department and it's very difficult, the triage system but I think what we've seen, I think the combination of radiology, PCT and CRP, I think they're really good markers because if they're at the hyper inflammatory end of the spectrum I think it's very likely they'll get pulmonary infiltrates quite quickly and we tend to use them and not send them to the ward but a little bit more well controlled. I think that once they've got low inflammatory markers and low infiltrates they tend to improve but there is no absolute in that discrimination but I'm very concerned about the patient with high COP, maybe low PCT, low basophils and lymphocytes.

Those are the ones that really concern me.

Professor Leonardo Fabbri: Thank you. Doctor Giustozzi please?

Dr. Michela Giustozzi: I agree with Professor Camporota. In my experience often in COVID 19 patient the procalcitonin is usually low or negative but in case the procalcitonin is high, for me that means that patient has a severe prognosis, probably because other than COVID 19 disease there is something else or sepsis or some other infection that may determine a poor prognosis of this patient.

Professor Leonardo Fabbri: Professor Mantovani?

Professor Alberto Mantovani: Yes. Well, of course it's a key question. We just said everything I think and I have little to add except that we are working on it, looking for early biomarkers as associated with good outcome. But I think it's the key question and I think it's key also for clinical trials because of course, we would like to be able to select the patient. For instance we are part of an anti IR map trial and of course you would like to select those patients that are more likely to benefit from that.

Professor Leonardo Fabbri: Yes, if I may ask you specifically Alberto, if I may ask you specifically. One of your slides, you showed that one of the earlier events is lung injury and maybe that a triggering factor of the following cascade. Is there any evidence that people at risk are developing a more severe, quick parenchymal injury or vascular and in other words that the early stage of the attack, viral attack is actually determining the following inflammatory cascade?

Professor Alberto Mantovani: I'm not aware of data. My prejudice is that we understand very little of what is going on in the battlefield. So we had a sense of what is going on around, but I mean there is now some single cell, it is very hard to do. At least, we find it very hard. There is some single cell analysis, I think it's one of the open questions. What's going on in the beta field, and how that dictates the following.

And I have a general question for all of you. I mean, glucocorticoids. I mean, is there a window for gluco, I know the negative stories. Do you have a feeling that there is a window for glucocorticoids?

Professor Luigi Camporota: Well, we have to say, we started very cautiously because obviously we saw the experience from China and other reported risks of delaying the viral shedding and prolonging it. But I have to say we use quite a lot of steroids at the moment. We have sign that the lung inflammation at least gets better very quickly. And now, in retrospect, we wished we had used that a little bit sooner in the disease. Just because, as I said, by the time they come to us, they're already two or three weeks in their illness. And I think sometimes they're go into early fibrosis. But we tend to use them now earlier than we were. And anecdotal response is quite interesting radiologically. We've got some data there, hopefully will come out soon.

Professor Semra Bilaceroglu: I'll comment. In my department, at the beginning maybe we didn't know Covid and how it caused so severe a disease, we tried to give many patients to the ICU department and they were very reluctant to have those patients. And we tried to keep them in our department and maybe for ten or fifteen of the patients, we gave loads of steroids on a short course and they benefited. Again, anecdotal, there is no evidence-based data, but some patients appeared to be benefiting from steroid treatment. And I would also ask the whole panel, can you compare tocilizumab and steroids? Because nowadays tocilizumab is not so recognised but used. But this drug also has a lot of side effects and there are some case reports mentioning terrible side effects that takes the patient to mortality. What does the panel think about steroids versus tocilizumab in severe patients that are going to ICU or ICU patients?

Professor Giancarlo Agnelli: We need to try it. We need a trial, a good trial, properly done. If I can make a short comment, and this is the last time I'm going to be speaking, I am merely involved in clinical trials. I am a so-called clinical trialist, I must say that after the presentation of Professor Mantovani and the presentation of Professor Camporota, I really feel ridiculous. Because we are-, well, actually I do, for several reasons, not just for the sample size of our studies, but I think we-, I feel ridiculous because we are targeting one small piece of the story. Both concerning the pathogenesis, and this was shown by Professor Mantovani, and those of the stage, and this was actually shown by Professor Camporota. So, we are using the drug A, whatever it is, in all the faces of this patient, and honestly if I have understood globally what Professor Mantovani said, what Professor Camporota said, this is ridiculous. That's why I feel ridiculous right now. Because we need to actually be a little bit more relaxed and taking care of the basic science and making the proper target in the proper patient in terms of targeting the proper part of physiology and targeting the proper moment, time of the treatment of the disease. So this is, I believe, why we are in trouble, and we are having positive and negative results simply because we are confused. We, the clinicians, the clinical trials, are confused. We want to do something, but we don't know what to do. That's my main consideration, honestly.

Professor Bin Cao: Thank you. Yes. I want to give some comments on steroids, yes. We have a submitted paper to the authorities and a review about our experience of corticosteroids in the early stages. Yes, although they didn't show that there is no benefits of the mortality but the risk of the secondary bacteria, fungal infection is not worse in our trial. But it is because that we use low dose corticosteroids and for a short duration.

And yes, I agree with the panelists that, I think, yes, we are talking about the clinical trial, but we have to keep in mind that one clinical trial cannot answer all the questions of corticosteroids, yes. I think the key point of a clinical trial is

that what population and what timing and what dosing of corticosteroids in one clinical trial, yes, in our experience, I believe there are two time windows for the use of corticosteroids. The first time window is when there is a rapid deterioration of a disease.

So it is a question from Professor Fabbri, that what is the marker, the bio-marker? I believe the idea when we find the right biomarker and there's an increase of the bio-marker within 24 or 48 hours, it is a time point, yes, to initiate corticosteroids. I think it is the timing that the patients should be admitted to ICU. Just one or two days before ICU or the first day of being in ICU, I think it is the time point, it is the first time window. And I believe that the second time window is at the later stage. The later stage, during the repair of the lung injury sometimes they have the pathological changes of-, during this stage there is the pneumonia due to the infection. It is because of the repair of the damage of the lung. And at this time the steroids are helpful for the faster repair of the lung injury. So, I do believe that there are two time windows for the use of corticosteroids. Over. Thank you.

Professor Semra Bilaceroglu: Thank you. Okay, Professor Adrian Rendon?

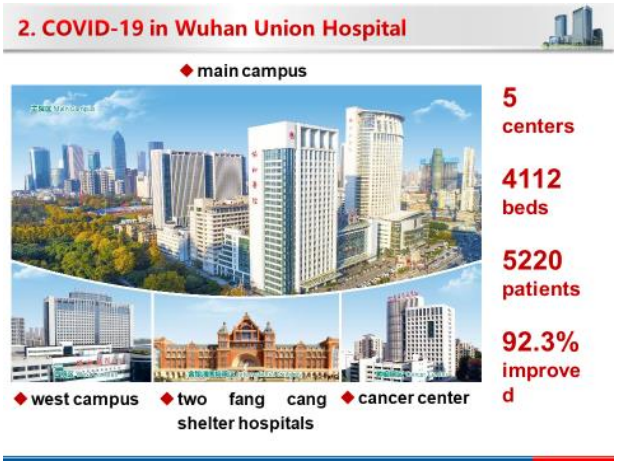
Professor Adrian Rendon: Yes, I would like to comment about the bio-markers. I think that instead of looking for one or two bio-markers, what we need from the clinical point of view, for a personalized approach to the patient is a list score that combines clinical data, vital signs, bio-markers and co-morbidities. And we need a score that can be flexible that we can measure at the beginning and that we can follow, so I think that's the best thing we have to look for in order to improve the treatment of the patients, because right now everybody's doing whatever they think is right.

Professor Semra Bilaceroglu: Thank you, Doctor Rendon.

Professor Lorenzo Corbetta: There is Professor Jin Yang who is ready for his presentation. Maybe now it will work better.

Professor Jin Yang: Thank you. I'm Jin Yang from the Department of Respiratory and Critical Care Medicine, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology. Just finishing with the job against the Covid-19 in Wuhan with my colleagues. And today I'm very glad to share some experience about how we established a mantle of early warning models for Covid-19 in Wuhan. The trend figures shows that a number of confirmed Covid-19 cases in Wuhan increased sharply before March, but now under control.

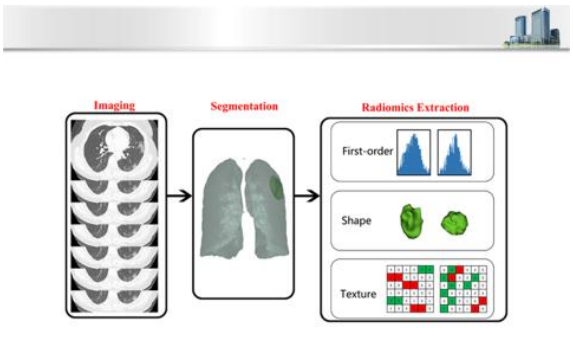
Introducing some conditions of Wuhan Union Hospital. You have five centres involved in Covid-19 fighting in Wuhan Union Hospital including the main campus, the west campus, two shelter hospitals and a cancer centre. And we provide more than 4,000 beds for patients with Covid-19. And now of more than 5,000 patients who were treated about 92.3% of them have improved.



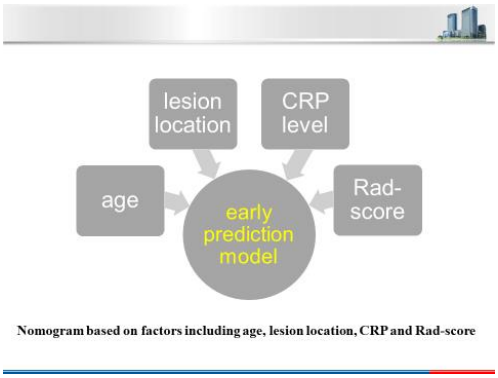
In order to fight Covid-19, our group tried to build early risk models, tried to predict the fourteen-day outcomes for improving efficiency. We also tried to predict 28-day outcomes for reducing mortality and we also want to evaluate the outcomes of intubation.

First, development and validation of a predictive model based on radiomics, to predict fourteen-day outcomes for patients with Covid-19 in Wuhan. This is the processing flow of the first model. Patients with NCP (Novel Corona Virus) were evaluated on the fourteenth day after admission to hospital. Divided into two groups, one is good, the other not, one with patients with a good prognosis, and another, with patients with a poor prognosis. Good prognosis means symptoms improved and no respiratory support needs. And two test, over 24 hours apart, with matching results. Patients with poor prognosis means condition deteriorated, or in respiratory support. According to selected factors associated with patients' outcomes by univariate and multivariate analysis, we have started a nomogram based on the multivariate analysis, and using it in the training set the ROC is 0.88. And then we use multicentre validation, the ROC means 0.88.

This is the flow chart of the extraction of radiomic features to calculate the Rad-score. We're supposed to collect certain images. Then four radiomic features with the highest correlation with the prognosis mostly of patients are extracted. Using the nomogram system, we carried out an early prediction model based on factors including age, lesion location, CRP levels and the Rad-score.



Flowchart of the extraction of radiomic features to calculate the Rad-score



Nomogram based on factors including age, lesion location, CRP and Rad-score

Here is the calibration curve of the nomogram scoring system in training and a validation set. The ROC shows that the model gets better with (mw 02.01.13) outcome prediction.

This model indicates to us that, according to the radomics, future extraction mastered a Rad-score system and nomogram scoring system based of Covid-19 patients which could factually predict a short-term outcome.

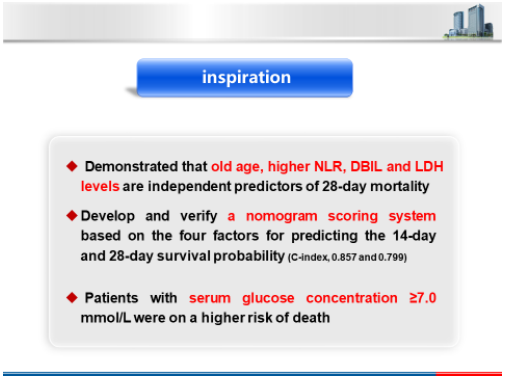
Second, we also tried to develop the risk of factors based on a system to predict 28 days' mortality in patients. So, for patients with Covid-19 in Wuhan Union Hospital with a sub-training cohort, independent predictors of mortality, including age, LDH, NLR and the DBIL we constructed a nomogram scoring system to predict the fourteen-day and 28-day survival probability. Then we would use the validation cohort including the internal analysts on a core validation cohort.

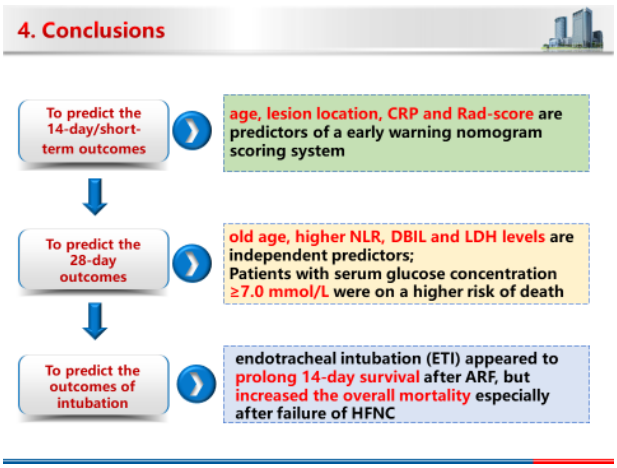
There is the mortality prediction model, including age, NLR, LDH level and the DBIL level.

Here is the training cohort calibration plot of survival probabilities at fourteen days and 28 days. The curve of predicted fourteen-day and 28-day survival probability, the observed probability, which means the nomogram system shows good calibration.

The plot also demonstrated good calibration of observing the predicted fourteen-day and the 28-day survival probability in the validation cohort, as shown in (d) and (e). So, the external cohort true validation results from the final 28-day model, as shown, in (f) and (g).

The model was clear that old age, higher NLR, DBIL and LDH levels are independent predictors of 28-day mortality. This model was developed and verified a nomogram scoring system for between the fourteen-day and 28-day survival probability.





Still, we also want to know the outcome of Covid-19 patients on intubation. Consecutive critical Covid-19 patients in two designed hospitals in Rome, according to the oxygen index level, below 150. Some patients used ETI, some patients intubated, some patients not intubated, use NIV, NIV and HFNC, or HFNC. And for those patients who were intubated, some patients had early ETI or late ETI.

Here is the comparison of Covid-19 patients, with different oxygen definition, we saw that the overall mortality and the 28-day mortality was higher in the intubated patients and was in the fourteen-day mortality lower in the intubated patients compared to NIV or HFNC alone, but no benefit was found in 28-day mortality and final mortality.

In conclusion, we tried to predict fourteen-day short-term outcomes, so we used age and lesion location, CRP and Rad-score as predictors for an early-warning nomogram scoring system. And second, to predict 28-day outcomes, we also used age, higher NLR, DBIL and LDH levels. These independent predictors constructed a nomogram system. And finally, to evaluate the outcome of intubation, ETI appeared to allow the prolonged fourteen-day survival but increased the overall mortality, especially after failure of HFNC.

After the use of the models in Wuhan Union Hospital, the mortality rates reduced apparently. The reduced rate is merely 60%.

Thank you very much. And, I'm sorry again.

Professor Semra Bilaceroglu: Thank you Doctor Jin Yang for this talk on prognosis and early-warning models. I think it also relates to the previous questions, predicting the severity of the disease, earlier in the course of the disease is important in the management of the patient. Are there any comments from the panelists, Prof. Fabbri and others, any questions?

If not, I will ask a question to Professor Mantovani, is he here? Or maybe, the panelists can also answer. As we are entering the reopening phase in many countries, how can we use serology, antibody tests, to manage Covid-19 in the reopening phase? When we are loosening the containment measures. What is the rational way of using these antibodies? And also, not having it very clear in my mind, maybe he mentioned about immunology, it can give us some comment on it.

Professor Leonardo Fabbri: Now, there is a programme in

several countries called the Phase 2 reentry that regards particularly the healthcare workers but also the other industrial workers and the programme is based on regular screening of antibodies. The bottom line is that there doesn't seem to be any different time course or function of the antibodies. But the positivity to the antibody may identify people who are potentially carriers of the virus, particularly asymptomatic. So, as a filter from the population, usually the top percent positive to IGG, unfortunately Professor Mantovani had to leave, but he had made the presentation a few days ago, it's a maximum 5%, it depends on the area the population's coming from. And within this 5%, 20% of them are positive to the PCR. So, as a screening, I mean, the antibodies are not diagnostic, are just for screening, but they allow you a cascade to identify the 1% of PCR positive subjects in the general population who are asymptomatic.

Professor Semra Bilaceroglu: Thank you Prof. Fabbri.
I understand that it is not diagnostic, the antibody tests, but we are opening a new phase and in this phase I think it was also show whether there is-, what is the percentage of immunity in the population. If we are sure of immunity. I am not very sure of immunity because it's also evidence-based, but maybe Chinese colleagues can comment on it? The antibody tests in the opening phase, how can we use it logically? In Turkey we were not doing any PCR tests for the asymptomatics, so we were doing the tests for only symptomatic cases and antibody tests only to the doctors, healthcare workers where needed. I think they will increase the antibody tests now in Turkey to see what is the rate of immunity we have, because most of us are infected. We don't know the real infection rate in Turkey. We only know the rate from the population who are tested. Does anyone want to talk on it? Okay, Doctor Bin Cao.

Professor Bin Cao: Yes, I want to make comments on the antibody testing, yes. For the other, the acute units, I don't think the antibody testing is helpful for making the diagnosis. We trust it for the other acute detection of the disease. But I believe that there is some advantage of the antibody testing. I think the advantage is that it is retrospective, as a way, especially for the special population, as you mentioned, the healthcare workers, and in time, yes, we have done the analysis of the association of the antibody with the clinical outcome, but we do not find any positive or negative association between the antibody and the clinical outcome. But from the animal model of the monkey infect with the SARS-coronavirus-2 infection, it said that the monkey is not likely to be reinfected. So, I think it's a good clue, we have the antibody response of a person such as care workers, so it will be unlikely to get reinfection of the areas. I think it is this advantage of the antibody testing.

Professor Semra Bilaceroglu: Okay, thank you. Any comments, any questions, or do the panelists have any questions? Yes, Prof. Rendon, please.

Prof. Adrian Rendon: My concern about serology is which brand are we going to use? There are too many outside. There are one from blood, and there are one from the finger. We don't know the sensitivity or specificity of each one, so we have to learn how to use those tests. Which is the best for our country, our region, our population, and the other thing, having a positive test, it doesn't mean that we have immunity. We have to determine antibodies, and not one of these tests is doing that.

Professor Semra Bilaceroglu: Oh okay. Thank you. Doctor Rendon, I read the history of Covid-19 in Mexico, from Wikipedia. I think it's a true story. I think it started in February, the first three cases, but the real precautions, measurements, were taken maybe one month later. You said the mortality rate is very high, so, do you think-, of course there are many factors affecting the mortality rate in your country, but one of the important factors in increasing the mortality is if the governmental policy's coming late, because the first three cases were defined in your country in February, I don't know, sometime in February, but in March, fourteen or fifteen, the containment and many measures for preventing the spread of the disease are taken. Can you comment on that?

Professor Adrian Rendon: Yes, sure. The first case was on February 28th. Then the next wasn't until March, and the third was in the middle of March and the government recommendations for lockdown were on the end of March, one month later of the first case. But I think that for sure could play some role in having high incidence of deaths. But the only very important thing is that we have a high bulletin of co-morbidities as I showed, and the other thing, we are counting only the civil cases. So if were counting the mild and the other rates, the rate of deaths would be diluted. So, most of the cases we have counting are civil cases. That's one of the reasons, the high rate of mortality.

Professor Semra Bilaceroglu: Okay. I just have to comment on one thing. In Turkey the first case was defined as Covid-19, PCR positive, coming from outside of Turkey, on March 11, after that precautions were taken in short time, but when I flash back to January, I now look at the CT of the patients, there are at least five patients in my department hospitalised, but we did not recognize they were Covid-19 patients. I gave the names to our infectionist and she went and saw them at their home, and they did tests. Of course, the patients did not have positive PCR but their relatives were having positive PCRs. So, we did not recognise that it started much earlier in my country because there was no case definition before March 10, but now I flash back and see. I just wanted to say this.

Professor Lorenzo Corbetta: Okay. I thank all the speakers, thank you very much for your precious time.

I thank Semra, I thank Luigi, it was a pleasure to meet you Michela, thank also Professor Agnelli, Prof. Mantovani, Professor Bin Cao, thank you very much, very interesting. Li Jing, Prof. Fabbri, Prof. Rendon, it was a pleasure to meet you, thank you Professor Jin Yang and my friend Prof. Shiyue Li.