Host factor-based early stage diagnosis of high risk Covid-19 patients

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This research document is intended for scientists and healthcare professionals involved in the 2020 Covid-19 pandemic

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Current situation

In EU countries with significant Covid-19 outbreaks, the standard protocol is that people experiencing possible symptoms of Covid-19 infection are to remain at home and contact their family doctor. In Italy and the Netherlands patients with Covid-19 symptoms are specifically not expected to present themselves at hospitals or other care centers.

Sample collection for Covid-19 diagnosis is performed at home or at specific diagnostic locations.

As a standard practice, both Covid-19 patients and suspected cases are sent back home where they are supposed to remain in quarantine and be treated at home, unless or until their conditions would require hospital admission. This practice is born out of necessity because most Covid-19 patients will recover without any major difficulties and hospital admission would as such be a waste of capacity and resources.

At this moment it is only possible to identify potential high-risk patients based on age and underlying conditions. Such patients can subsequently be monitored more closely and receive extra care when needed. However disease outcome is not fully predictable based on simple criteria of age and underlying conditions. Even younger patients may in some cases develop serious complications and currently no diagnostic tools are available to assist in the identification of such higher risk patients.

Current situation

High risk patients could benefit considerably from early diagnosis and extra care during the early stages of infection, preventing an escalation of the disease that might necessitate the treatment in Intensive Care units.

Due to the increasing number of infected subjects requiring Intensive Care, some countries are setting up contingency plans that contain exclusion criteria for patients with unfavourable disease outcome.

This illustrates the necessity to develop improved diagnostic methods capable of identifying high risk patients at an early stage. This presentation will discuss the possibility of a diagnostic method based upon the expression of specific Covic-19 viral host factors.

(table below: recommended exclusion criteria for IC admission in case of Covid-19 pandemic in the Netherlands)

Patient Condition	Age Group
Metastatic malignancies	Adult and pediatric
Hematologic malignancies with poor prognosis	Adult and pediatric
End-stage organ failure with expected survival < 1 y, such as end-stage cardiac failure (NYHA class IV), severe chronic lung disease, advanced hepatic failure (MELD score > 20)	Adult and pediatric
Very advanced age	Adult
Advanced and irreversibly immunocompromised, such as drug-resistant AIDS	Adult and pediatric
Congenital anomalies with expected survival < 1 y	Pediatric

MELD = Model for End-Stage Liver Disease; NYHA = New York Heart Association.

Scientific Hypothesis

Some cellular host factors have so far been identified for CoVid19 and have been shown to either support or contrast viral replication at different stages, whereas other host factor targets can be hypothesized based on similarity with other Coronaviruses.

Host factors like Dead box helicases DDX5 and ACE2 have been shown to support viral replication, and modulation in vitro of their levels impacts viral replication. As such these host factors can be both drug targets and disease biomarkers.

It can in fact be hypothesized that high or low levels of host factors can influence the prognosis of the disease and can help in identifying patients at high risk, obviously in conjunction with other parameters and markers.

DEAD Box helicase expression in healthy tissues can vary substantially. In this hypothesis, if a Patient A would have high levels of Covid-19 host factors in his/her lung tissue, considering the fact that coronaviruses are host factor dependent, he/she would be a high risk patient.
Patient B on the other hand has exceptionally low levels of Covid-19 host factors, resulting in a significantly reduced replication of the virus in the lungs.

Scientific approach

First Health Pharmaceuticals is not interested in a commercial development of diagnostic methods for Covid-19, but is only hoping to contribute to the fight against the coronavirus epidemic by starting up this discussion about the possibilities of improved diagnostic methods capable of identifying high risk patients in an early stage.

The clinical studies that are cited and discussed in this document were not carried out by First Health Pharmaceuticals and are presented for illustrative and discussion purposes only. Some studies have been performed from January 2020 till now, focusing on inflammatory markers, eg. IL6, or on comorbidities markers, e.g. Cardiovascular diseases. *(see slide 6 to 8)*

In order to test the hypothesis on host factors levels as prognostic stratification markers, it might be sufficient to start with evaluation in blood samples via ELISA or RT-PCR tests, perhaps on already existing patient blood samples from the first visit (admission).

Peripheral blood biomarkers are preferred for practical reasons rather than lung tissue samples.

Projects may be submitted to the Ethic Committees of the Medical centers in question; In several territories, comunication to regulatory authorities is requested but not mandatory in case of exceptional urgency.

Examples of performed investigation (1).
IL6, lymphocytes, inflammation: a study at Wuhan (Lancet 2020)

191 patients:
137 were discharged and 54 died in hospital.
From end of December 2019 to February 2020

Conclusion
Potential risk factors:
Age, high Sequential Organ Failure Assessment-sepsis and blood levels of d-dimer protein (marker for coagulation) **on admission to hospital .**

Lower lymphocyte count, elevated levels of Interleukin 6 (biomarker for inflammation and chronic disease), and increased high-sensitivity troponin I concentrations (a marker of heart attack), were more common in **severe COVID-19 illness**

Author declaration
The study was approved by the Research Ethics Commission of Jinyintan Hospital (KY-2020-01.01) and the requirement for informed consent was waived by the Ethics Commission as described previously

Examples of performed investigation (2). The potential role of IL-6 in monitoring severe case of coronavirus disease 2019 (Liu 2020)

69 patients : 30 out of 69 had sampling before and after treatment

Conclusions

On admission, the baseline level of IL-6, CRP, LDH and ferritin was closely related to the severity of COVID-19, and the elevated IL-6 was significantly related to the clinical manifestation of severe type patients. The decrease of IL-6 was closely related to treatment effectiveness, while the increase of IL-6 indicated disease exacerbation.

Author Declarations

This is a retrospective observational study for 2019 novel coronavirus disease. **The study has not been registered in light of the urgent need to collect clinical data.** All relevant ethical guidelines have been followed; any necessary IRB and/or ethics committee approvals have been obtained and details of the IRB/oversight body are included in the manuscript.
I understand that all clinical trials and any other prospective interventional studies must be registered with an ICMJE-approved registry, such as ClinicalTrials.gov. I confirm that any such study reported in the manuscript **has been registered and the trial registration** ID is provided (note: if posting a prospective study registered retrospectively, please provide a statement in the trial ID field explaining why the study was not registered in advance).

Examples of performed investigation (3). Hypokalemia and Clinical Implications in Patients with Coronavirus Disease 2019 (COVID-19)

175 patients:
Recruitment by 15th February.

Conclusions
93% of severe and critically ill patients had hypokalemia
The end of urine K+ loss indicates a good prognosis and may be a reliable, in-time, and sensitive biomarker directly reflecting the end of adverse effect on RAS system.

Author Declarations
All relevant ethical guidelines have been followed; any necessary IRB and/or ethics committee approvals have been obtained and details of the IRB/oversight body are included in the manuscript.
I understand that all clinical trials and any other prospective interventional studies must be registered with an ICMJE-approved registry, such as ClinicalTrials.gov. I confirm that any such study reported in the manuscript **has been registered and the trial registration** ID is provided (note: if posting a prospective study registered retrospectively, please provide a statement in the trial ID field explaining why the study was not registered in advance).

Known or potential host factors to be investigated as potential markers

Viral stage	Name	Virus	Role
Replication	DDX5	SARS-CoV	Support replication; binding SARS helicase; Potential role in transcription and translation
Innate response	DDX3	hCoV-OC43	Antiviral effect via IFN pathway; Potential role in transcription and translation; inflammatory response
Attachment and entry	ACE2	SARS-CoV, CoVID19, h CoV-NL63	Cellular receptor
Replication	DDX1	MHV-JHM	Facilitate synthesis of genome RNAs
Infection/ Innate response	RIG-1 and TRIM25	SarS-CoV	intracellular, should contrast viral replication, but TRIM25 is inhibited by viral protein N
Transcription, replication	APOBCEs	HCoV-22E; (HIV)	Can be packed in virion-like particles

Next steps

1. **Identify Clinical Investigators and groups that might be interested and capable to conduct this investigation**
    1. China? USA? Europe?
    2. Groups that already published similar studies?
    3. Are there associations?

2. Establish the assays

1. Compare reproducibility and sensibility of RT-PCR or ELISA on blood cells and/or plasma samples
2. compare costs, time and required operators of the two techniques
3. set up the assay

3. Protocol design

1. group and number of patients
2. sampling
3. informed consent, dossier for the Ethic Committee

First Health Pharmaceuticals does not intend to take a lead in this discussion but would be glad to facilitate contact with clinicians and scientists of its network and offer support in any scientific aspect related to Dead Box RNA Helicase viral hostfactors.

Appendix

table of additional potential Coronavirus host factor targets

Host factors of human CoV mediating viral entry and virions release

Viral stage	Name	Human CoV	Role
Entry and Infection	ACE2	CoVid19, SARS-CoV, HCoV-NL63	Receptor
	APN	HCoV-229E	Receptor
	Cathepsin L	SARS-CoV	Protease
	DPP4	MERS-CoV	Receptor
	Furin	MERS-CoV	Protease
	IFITMs	SARS-CoV, MERS-CoV, HCoV-229, HCoV-NL63	Restriction factor
	VCP	HCoV-229E	Endosomal protein
	DDX3	hCoV-OC43	Promote innate response (& translation)
Virion assembly	Tubulin	HCoV-229E, HCoV-NL63	Facilitates assembly and release

Host factors of human CoV mediating transcription and translation

Viral stage	Name	Human CoV	Role
Transcription and Translation	DDX5	SARS-CoV	Binds viral helicases
	GSK3	SARS-CoV	Kinase
	hnRNPA1	SARS-CoV	Viral synthesis
	N-linked glycosylation enzymes	SARS-CoV	Modify structural proteins
	ER chaperones	SARS-CoV	Folding and maturation

Host factors of non-human CoV

Viral stage	Name	Non- uman CoV	Role
RTC assembly	Annexin 2	IBV	Ribosomal regulation
	GBF1 and ARF1	MHV	Vesicles formation
Virion assembly	B-Actin	IBV	Facilitates
	Vimentin	TGEV	Facilitates
	Filamin A	TGEV	Facilitates
Genome replication and transcription	DDX1	MHV-JHV	Facilitates synthesis
	ZCRB1	IBV	Binds to genome
	Mitochondrial aconitase	MHV	Binds to genome
	PABP	Bovine CoV	Binds to genome

Data shown in the three tables are from Chen et al., 2018, Shen et al., 2016, Fung et al., 2019)