Institutional Ethics Committee

College of Medicine and Sagore Dutta Hospital
578, B T Road, Kolkata – 700058
Registration No. ECR/1210/Inst/WB/2019

Memo no. CMSDH/IEC/187/01-2021 Date:09.01.2021

To
DR. SUSMITA BHATTACHARYA,
Professor & Head of The Department,
Department of Microbiology,
College of Medicine and Sagore Dutta Hospital.

A meeting of the Institutional Ethics Committee (IEC), College of Medicine and Sagore Dutta Hospital (CMSDH) was held on 09.01.2021 at 11 am in the Examination Hall, 4th Floor, Academic Building, College of Medicine and Sagore Dutta Hospital. In this meeting, the research proposal titled **"IDENTIFICATION OF SIGNATURES FOR PROGNOSTIC AND THERAPEUTIC STRATIFICATION OF HOSPITALIZED COVID-19 PATIENTS"** of which you are the Principal Investigator, was reviewed.

After deliberations and review the committee thinks that methodology of the project conforms to the ethical principles, institutional rules and regulations. So the project is **APPROVED** by the committee.

The list of IEC members present in the meeting on 09.01.2021 are appended herewith:-

1. Prof. Mrityunjay Mukherjee, Professor, General Surgery, KPC Medical College & Hospital, Kolkata. – Chairman.
2. Prof. Parag Baran Pal, Professor & HOD, FMT, CMSDH, Kolkata. – Member-Secretary.
3. Swami Kamalasthananda, Principal (Actg), Ramkrishna Mission Vivekananda Centenary College, Rahara, Kolkata-700118. –Philosopher/ Ethicist/ Theologian.
4. Mr. Bikram Singh, PP-In-Charge, Barrackpur Sessions Court. – Legal person.
5. Mr. Swapan Biswas, Clinical Researcher, Jadavpur University. – Scientific Member.
6. Mrs. Soumya Bhattacharya, Social Worker and Singer – Social worker.
7. Mr. Subhash Guptabhaya, Retd Govt. Officer, Audit & Accounts, West Bengal. – Lay person.
8. Prof. Goutam Kumar Joardar, Prof & HOD, Community Medicine, KPC Medical College & Hospital, Kolkata. – Clinician.
9. Prof. Ranendra Kumar Roy, Prof & HOD, Pharmacology, SRK Institute of Medical Sciences & Sanaka Hospital, Durgapur. – Basic Medical Scientist.
10. Prof. Sujay Mistri, MSVP, CMSDH, Anatomist –Basic Medical scientist
11. Prof Arun Kr. Bandyopadhyay, Principal, CMSDH, Ophthalmologist – Clinician.

It is placed on record that the opinion of the committee was unanimous in this regard and did not require any voting procedure.

The committee expects that any amendments of the Study protocol or any other relevant documents would be brought to its notice.

Date: 09.01.2021
Place: Kolkata

Paragbaran Pal
09/01/2021

Prof. (Dr.) Parag Baran Pal
Convenor and Member-Secretary,
Institutional Ethics Committee
College of Medicine and Sagore Dutta Hospital
578, B T Road, Kolkata – 700058.
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Title: Identification of signatures for prognostic and therapeutic stratification of hospitalized COVID-19 patients.

Principal Investigator: Dr. Susmita Bhattacharya, Professor& Head, Department of Microbiology, College of Medicine and Sagore Dutta Hospital

Co-investigators:

Prof.Dr. Partha Chattopadhyay, HOD, Department of General Medicine, College of Medicine and Sagore Dutta Hospital

Prof. Dr. IndranilChakraborty, HOD, Department of Biochemistry, College of Medicine and Sagore Dutta Hospital

Dr. Runa Das, Associate Professor& HOD, Department of Radiology, College of Medicine and Sagore Dutta Hospital

Dr. Ranu Roy Biswas, Associate Professor, Department of Pathology, College of Medicine and Sagore Dutta Hospital

Dr. Soumen Kanti Manna, Associate Professor, Biophysics and Structural Genomics Division, Saha Institute of Nuclear Physics, Kolkata

Dr. Raghunath Chatterjee, Associate Professor, Human Genetics Unit, Indian Statistical Institute, Kolkata

Dr. Sanghamitra Bandyopadhyay, Professor, Machine Intelligence Unit, Indian Statistical Institute, Kolkata

Introduction:

SARS-COV-2 has infected more than 91 lac people in India causing around 1.35 lac deaths so far.[1]Worldwide, it has been shown to cause mortality and morbidity in mostly elderly people (>60 years) and those suffering from co-morbidities such as hypertension, diabetes, lung and cardiovascular diseases. Patients are assessed upon admission and thereafter based on well-established clinical criteria for risk assessment. Widely used tools for such stratifications such as NEWS/NEWS-2 score[2] comprises of pulse rate, respiratory rate, oxygen saturation, requirement of oxygen supplementation, blood pressure, temperature, level of consciousness. NEWS2 score was found to predict mortality with 76.9% (95% CI 46.2–94.7) sensitivity and 80.1% (95% CI 68.0–90.6) specificity in a cohort of mostly elderly patients (median age = 71.5 years, n =66). [3]In view of highly variable clinical manifestation and outcome of COVID-19, development of new scoring algorithm such as 'ANDC' (takes age and biochemical parameters such as D-dimer, C-reactive protein into account)[4] or 'ANEWS' (which takes age and co-morbidities into account)[5] are being developed. Most of these algorithms tend to stratify younger patients without significant co-morbidity as low risk. However, in India, a significantly large number of people in the age range 26-60 years seem to be adversely affected. [6] The fact that adverse disease presentation, sudden deterioration and death have been observed in many young patients [7] is alarming.

This warrants a better method for stratification of hospitalized patients with respect to intensity of monitoring and urgency of critical care interventions.

Development of such a method which would be robust enough to be applicable across the board should, in principle, take into account every possible signature that is either known to be or may potentially be influenced by host-pathogen interaction. The human physiology is governed by genetic architecture as well as environmental exposures that both acutely and chronically affect gene expression and function. The response to invading pathogen is governed by the host genome.[8] For example, since SARS-COV-2 attaches through the ACE2 receptor; it is obvious that inter-individual differences in genetic architecture affecting ACE2 expression would affect disease susceptibility.[9]Similarly, genes regulating immune function would affect the response.[10] Interestingly, metabolism is increasingly realized to play an important role in function of immune cells.[11,12]Metabolic reprogramming of host cells is also known to be essential for viruses including zika and influenza to replicate and spread.[13,14]A large number of expressed genes codes for gene products that end up directly (as enzymes and transporters) or indirectly (non-protein or protein regulators of biochemical pathways) influencing metabolic processes. Metabolome, also gives a biochemical read-out of commensal microbiome that is emerging to be crucial in shaping host immunity and inflammation.[15,16]Circulating metabolites including those regulated by commensal microbiome have been implicated in pathogenesis of wide spectrum of diseases and disorders including cardiovascular and neurological problems, which are being widely reported in COVID-19.[17-19] On the other hand, lipids are important regulators of energy metabolism and signaling. They are also essential for viral envelope formation. Lipidome and metabolome also capture the nutritional status of the host that is known to affect immune system. Thus, metabolome is sensitive to both baseline physiologies as well as to the fall out of host-pathogen interaction upon infection. A tandem analysis of host genome, proteome, metabolome and lipidome can yield crucial information about the outcome of the host-pathogen interaction and may help to predict course of the disease.

It's interesting to note that co-morbidities in COVID-19 are associated with widespread derangement of metabolic pathways. In fact, few recent studies reported significant change in plasma metabolome, lipidome and proteome in COVID-19 patients.[20-23]Integrated analysis of plasma metabolites, lipids and proteins and leukocyte transcripts showed severity associated patterns. [24] On the other hand, integrated analysis of changes in plasma proteome, metabolome, PBMC transcriptome along with other demographic and clinical data revealed significant changes between mild and moderate patients related immune system.[25] However, no study has looked into systems-level changes beyond blood. The fact that COVID-19 that starts with respiratory infection goes on to affect to affect multiple organs is expected to be associated with release of signalling and effector molecules including proteins, lipids and metabolites in systemic circulation. Understanding the disruption of systemic metabolic homeostasis warrants knowledge of both metabolites in blood as well as other body fluids, particularly, urine, in tandem. Urine is an abundantly available sample that can be collected non-invasively and harvested to get a better picture of metabolic derangements. Till date, no study has investigated changes in urinary metabolome in COVID patients or its correlation with disease manifestation and outcome.

Thus, this study proposes simultaneous analysis of exome, plasma and urinary metabolites, plasma lipids, proteins and combine them with demographic, clinical, haematological, radiological and biochemical data to identify signatures that can predict disease course and outcome.

Objective:

Development of method for stratification for critical care of young COVID-19 patients combining demographic, clinical, biochemical, radiological, haematological features with genomic, metabolomic, lipidomic and proteomic signatures.

Materials and Methods:

Study type: Observational cross-sectional

Study population: Patients (18-65 years) being admitted at Sagore Dutta Hospital with RT-PCR confirmed COVID-19 diagnosis will be included in the observational cohort. The inclusion and exclusion criteria are mentioned in table 1. For consistency, patients will be recruited from Covid ward, HDU and CCU for mild, moderate and severe cases respectively from the dedicated COVID-care facility.

Table 1: Exclusion and inclusion criteria for recruitment of hospitalized COVID-19 patients

Inclusion criteria	Exclusion criteria
COVID +ve (RT-PCR)	Age > 65 yr, <18 yr Pregnant Malignancy or other terminal illness Admitted for or undergone surgery within 2 months

In order to assess the COVID-specificity of observed differential signatures, RT-PCR confirmed non-COVID in-patients will be recruited.

Sample size: The objective of the study is to characterize and estimate number of young patients requiring intensive and critical care as well as to identify signatures to stratify them accordingly as soon as possible following admission. A preliminary analysis based on past experience of 4 months on all the COVID-19 patients admitted in this institute, suggests that around 60% of admitted patients show severe disease manifestation and adverse outcome. Thus, the sample size for this observational cross-sectional study may be calculated assuming a 60% of patients shall require critical care, who need to be identified using the proposed predictive algorithm, using the following formulae.

$$n = \frac{z^2 \hat{p}(1-\hat{p})}{\varepsilon^2}$$

With a margin of error (ε) = 0.05 at 95% confidence interval (z = 1.96) and estimated 60% cases requiring intensive and critical care, the number of COVID-19 patients enrolled for the study ought to be minimum 369 qualified enrolled samples.

However, given the huge costs associated with untargeted multi-omic characterization analysis of each sample, it would be prudent to adopt a resource-limited sample size initially. So, initially, we plan to perform untargeted multi-omic analysis on samples from minimum 25 severe (severe/critical) and 25 non-severe (mild/moderate) patients based on availability of funds. This will be followed by targeted analysis of multi-omic signatures of interest identified through the preliminary analysis in all samples.

In addition, a set of age-, sex- and, preferably, BMI-matched non-COVID in-patients will be recruited to assess the non-COVID distribution of identified signatures. The sample size of this set shall be 100 so that they may, in principle, populate every percentile of the distribution for a continuous variable assuming random sampling.

Sample collection: A part of the fasting blood samples collected (in EDTA-containing tubes) for routine haematological and biochemical assessments in the morning of the second day, fifth day and eighth day of hospitalization will be used for the study. Fasting urine samples will also be collected from patients in 50 ml sterile polypropylene tubes on aforementioned days. All sample collection tubes will be pre-labeled with patient ID and sampling day (D2/D5/D8) before they are handed over to para-medical personnel for sample collection and the labels will be re-checked after sample collection during processing. From non-COVID patients, samples will be collected only once, preferably, on the second day of hospitalization.

Sample processing and storage: Total DNA will be isolated from whole blood. Plasma will be prepared as per standard protocol. These samples as well as urine will be stored at -20^{o}C for a maximum of one week within which they will either be processed for metabolite and lipid extraction or stored at -80^{o}C.

Clinical assessment:Body temperature, oxygen saturation, pulse rate, oxygen requirement as well as other clinical parameters and presentations will be routinely monitored and catalogued during hospital stay. EWS scoring will be performed as per existing protocol upon admission. Haematological and radiological assessment (X-ray or CT scan) will be performed as recommended by the clinician. Details of therapeutic interventions as per recommendation of the clinician will be recorded. Patients will be categorized as responder or non-responder to a therapeutic protocol as per existing guidelines. All major events including ICU admission, ventilation, cardiovascular event and death will be catalogued. COVID-19 cases shall be retrospectively categorized as mild or moderate or severe or critical as per existing guidelines.

Biochemical assays: Routine biochemical assays including serum urea, creatinine, liver function panel including LDH as well as, C-reactive protein, D-dimer, ferritin, cytokine and chemokine analysis will be performed using plasma/serum samples following established methods and using commercially available kits.

Metabolite and lipid extraction:Plasma/serum samples will be extracted by biphasic solvent extraction method involving methanol/water/chloroform to harvest metabolites from the upper aqueous layer and lipidsfrom the lower organic layer. Metabolites from urine will be extracted by monophasic solvent mixture comprising isopropanol, water and acetonitrile. Both methods would also inactivate the virus and denature all proteins.

Protein extraction:Abundant plasma proteins will be depleted using immune-depletion spin columns and precipitated using organic solvent.

Transport of samples:Extracted DNA samples will be transported to the laboratory of Dr. Raghunath Chatterjee at Indian Statistical Institute, Kolkata. Extracted lipid, proteins and metabolites will be transported to the Laboratory of Dr. Soumen Kanti Manna at Saha Institute of Nuclear Physics, Kolkata. All samples will be transported at sub-zero temperature, preferably, in dry ice. Containers will be thoroughly decontaminated upon arrival. All sample processing will be done with appropriate protective gears and inside fume hood.

Exome Sequencing*:*The isolated DNA will be eluted in 50µl of TE buffer. Whole-Exome sequencing libraries will be prepared using Illumina TruSeq Exome Library Prep Kit following manufacturer's protocol. The resulting enriched DNA libraries will be multiplexed by adding index tags by amplification, followed by purification. Indexed captured library DNA will be assessed to check the quality and quantity of the captured libraries. Finally, indexed captured library DNA will be sequenced on Illumina HiSeq2500 or 4000 to generate paired end 100 bp sequence reads with at least 100X sequencing depth. Sequenced data will be processed to generate FASTQ files. After quality control filtering, the high quality sequence will be used for further analysis.

Metabolomic analysis: Metabolites will be analysed as described in detail earlier. Briefly, extracted metabolites will be derivatized with MSTFA or ethylchloroformate to make them amenable to GC-MS analysis or be directly used for LC-ESI-MS analysis.Chemoinformatic pattern recognition analysis will be used to screen features of interest that can help to predict disease severity, requirement and length of ICU or ventilation or overall length of hospitalization or therapeutic response or death.Consistency and correlation of these features with patient status will be examined using samples collected at day 5 and 8. Analysis of fragmentation pattern and comparison of retention time with authentic standard (wherever available) will be used to ascertain chemical identity of features of interest.

Data Sharing and privacy: All patient samples, medical records, clinical, biochemical, haematological and radiological data will be coded and made anonymous. Only treating clinician and counsellors shall have access to personally identifiable information of patients which will be used strictly for treatment and follow-up. Anonymous patient data and processed samples will be shared with collaborators for research purposes only. No patient sample will be shared with any foreign individual or institute.

Statistics and Integrated Machine Learning:False discovery rate correction will be used to screen features for subsequent analysis. **Intra-feature correlation will be examined to identify features with correlation coefficient >0.9**. Among features showing such high correlation, the most robust, easily

implantable and affordable feature will be kept and others will be removed from subsequent analysis. Patients will be retrospectively categorized with respect to disease severity, requirement and length of ICU or ventilation, response to therapy and final outcome (discharge or death) during hospitalization. The demographic, anthropometric, clinical, haematological, radiological, biochemical, genomic, metabolomic and lipidomic signatures will be combined to identify signatures that could help in prediction of prognosis and therapeutic response on the day after hospitalization in all qualified enrolled samples, 75% for model training and rest 25% to see the prediction accuracy through machine learning. These signatures will be screened for consistency and correlation with disease course on Day 5 and Day 8 samples. Further screening of these signatures will be based on their distribution among non-COVID samples to select those showing significant specificity to COVID-19 phenotype. They will be validated in rest of the samples. A minimum improvement of sensitivity or specificity by 15 - 20% (without compromising the other) over existing EWS scoring will be used for selection of any signature(s). Signatures will also be screened based on ease of implementation and affordability to eventually develop an improved algorithm for prospective stratification of young COVID-19 patients at hospitalization.

Pathway Analysis:Integrated pathway analysis will be performed combining genomic, metabolomic and lipidomic signatures to identify molecular events and signalling pathways that may contribute to the disease progression, therapeutic response and long-term consequences. The contribution of these pathways will be examined by comparative analysis expression of related genes or proteins among patient categories that differ with respect to phenotype of interest.

Ethical Considerations: Study will commence after receiving approval from Institutional Ethics Committee (IEC). The study will be conducted in conformity with all ethical guidelines. Only those persons willing to participate in the study and sign an informed consent form will be included. Identity of all the study subjects will be kept confidential.

Impact: This study will help to develop an algorithm for identifying patients at risk of severe disease, poor therapeutic response and adverse outcome early during hospitalization. This should help clinicians to stratify patients for intense monitoring as well as requisite clinical and therapeutic intervention. Thus, it will immensely help to optimize utilization of resources while reducing improving clinical outcome.

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Questionnaire/Schedule/Case Record Form: Informed consent forms andcase record form for hospitalized patients are appended.

Funding: Routine sample collection and processing clinical, hematological, biochemical and radiological assessments are already part of the existing patient management protocol in the hospital. Exome sequencing analysis and machine learning will be partially supported by intramural funding of ISI, Kolkata through collaborations with Prof. Raghunath Chatterjee and Prof. Sanghamitra Bandyopadhyay, respectively. Metabolomic and lipidomic analysis will be partially supported by intramural funding of SINP, Kolkata through collaboration with Dr. Soumen Kanti Manna. Additional funding will be sought through DBT-DFG (Indo-German) call for proposal that Dr. Manna is preparing for. This shall cover expenses to be incurred for sample processing, storage and transport, employment of personnel for sample processing at the hospital, analysis of cytokines, chemokines as well as additional resources and personnel for genomic, metabolomic, lipidomic, gene and protein expression analysis.

Subject information sheet

Informed consent form for the study titled "Identification of signatures for prognostic and therapeutic stratification of hospitalized COVID-19 patients."

Principal Investigator: Dr. Susmita Bhattacharya

Organization: Department of Microbiology, College of Medicine and Sagore Dutta Hospital

Background:

The present study will be performed to identify signatures that can predict clinical course and outcome of COVID-19. A part of your blood and urine samples collected for routine investigations required for your treatment will be used for this research, if you kindly give consent. Subject to your consent, your case record comprising demographic and clinical info only will be used for this research.

Compensation: No compensation can be awarded in the present study.

Confidentiality: The sample collected will be kept anonymous throughout the study and will strictly be used for research purposes only. The data collected will be remain strictly confidential and only used for publication in a scientific journal with due consideration to maintain anonymity.

Participation: Your participation is completely voluntary. You are free to withdraw from the study anytime you wish.

Please enter your name and sign below if you give consent to participate in this study.

Consent form in English

I have read the foregoing information or it has been read to me. I have had the opportunity to ask questions about it and questions asked by me were answered to my satisfaction. I consent voluntarily to participate in this study and understand that I have the right to withdraw myself from the study anytime without giving any reason.

Name: _____

Signature: _____

If Illiterate;

I have witnessed the accurate reading of the consent form to the potential participant and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name of the witness: _____

Thumb impression of the participant:

Signature of the witness: _____

Thumb impression of the witness:

Date: _____

Place: _____

Consent form in Bengali

[illegible],
[illegible]

[illegible]: ____

[illegible]: ____

[illegible]:

[illegible];

[illegible]

[illegible]: __________________________

[illegible] :

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Consent form in Hindi

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COVID-19 Case Record Form

Patient ID:

Gender: M ◯ **F** ◯ **Others** ◯

Age: **(Years)**

Date of admission:

Transferred from another hospital/nursing home? Yes ◯ No ◯ Not known ◯

Current SARS-COV-2 RT PCR Status: Positive ◯ Negative ◯Not known ◯

Earlier (> 6 weeks) SARS-COV-2 RT PCR Status: Positive ◯ Negative ◯Not known ◯

Height: (cm)**Body weight at Hospitalization:** (kg)

Clinical history:

Hypertension ◯ Hypotension ◯ T2DM ◯ Cardiovascular disease ◯ Obesity ◯ Asthma ◯
COPD ◯ Tuberculosis ◯ Any undiagnosed respiratory difficulty ◯ Sickle cell disease ◯ Cancer ◯
Chronic kidney disease ◯ Autoimmune disease ◯ Dust allergy ◯ Pollen allergy ◯ Other allergy ◯

Life-style history:

Regular smoker ◯ Occasional smoker ◯ Regular drinker ◯ Occasional drinker ◯

Clinical Presentation before admission:

Fever ◯ Dry cough ◯ Productive cough ◯ Sore throat ◯ Difficulty in breathing ◯ Tiredness ◯
Nasal congestion ◯ Loss of smell ◯ Loss of taste ◯ Loose stool (> 3 times a day) ◯ Headache ◯
Body ache◯ Low appetite◯ Poor sleep ◯ Skin rash◯ Spasm ◯ Confusion◯ Oxygen support◯

Days since symptom onset: (days) **X-ray or CT scan performed?** Yes ◯ No ◯

Days since fever onset: (days) **Highest temperature recorded: °F**

Any known contact with COVID-19 patient? Yes ◯ No ◯

Days since suspected contact: (Days)

Day 2 at the hospital (date of admission considered as Day 1**): Clinical Presentation**

Fever ◯ Dry cough ◯ Productive cough ◯ Sore throat ◯ Difficulty in breathing ◯ Tiredness ◯
Nasal congestion ◯ Loss of smell ◯ Loss of taste ◯ Loose stool (> 3 times a day) ◯ Headache ◯
Body ache◯ Low appetite◯ Poor sleep ◯ Skin rash◯ Spasm ◯ Confusion◯ Oxygen support◯

Fasting blood sample collected? Yes ◯ No ◯

Fasting urine sample collected? Yes ◯ No ◯

Highest temperature recorded in hospital:°F

X-ray performed? Yes ◯ No ◯ **CT scan performed?** Yes ◯ No ◯

Oxygen support (mode, rate and total volume):

Medicines used (in detail with name, dose):

Any other observation/comment:

<u>Day 5 at the hospital: Clinical Presentation</u>

Fever ◯ Dry cough ◯ Productive cough ◯ Sore throat ◯ Difficulty in breathing ◯ Tiredness ◯
Nasal congestion ◯ Loss of smell ◯ Loss of taste ◯ Loose stool (> 3 times a day) ◯ Headache ◯
Body ache◯ Low appetite◯ Poor sleep ◯ Skin rash◯ Spasm ◯ Confusion◯ Oxygen support◯

Fasting blood sample collected? Yes ◯ No ◯

Fasting urine sample collected? Yes ◯ No ◯

Highest temperature recorded in hospital: °F

X-ray performed? Yes ◯ No ◯ **CT scan performed?** Yes ◯ No ◯

Oxygen support (mode, rate and total volume):

Medicines used (in detail with name, dose):

Any other observation/comment:

<u>Day 8 at the hospital: Clinical Presentation</u>

Fever ◯ Dry cough ◯ Productive cough ◯ Sore throat ◯ Difficulty in breathing ◯ Tiredness ◯
Nasal congestion ◯ Loss of smell ◯ Loss of taste ◯ Loose stool (> 3 times a day) ◯ Headache ◯
Body ache◯ Low appetite◯ Poor sleep ◯ Skin rash◯ Spasm ◯ Confusion◯ Oxygen support◯

Fasting blood sample collected? Yes ◯ No ◯

Fasting urine sample collected? Yes ◯ No ◯

Highest temperature recorded in hospital: °F

X-ray performed? Yes ◯ No ◯ **CT scan performed?** Yes ◯ No ◯

Oxygen support (mode, rate and total volume):

Medicines used (in detail with name, dose):

Any other observation/comment:

<u>Clinical presentation before discharge:</u>

Fever ◯ Dry cough ◯ Productive cough ◯ Sore throat ◯ Difficulty in breathing ◯ Chest discomfort/pain ◯ Tiredness ◯ Numbness ◯ Vertigo ◯ Nasal congestion ◯ Loss of smell ◯ Loss of taste ◯ Loose stool (> 3 times a day) ◯ Headache ◯ Body ache ◯ Joint pain ◯ Low appetite◯ Poor sleep ◯ Skin rash ◯ Confusion ◯ Irritability ◯ Anxiety ◯ Other (specify) ◯

Date of Discharge: **Body weight at discharge:** **(kg)**

Medicines prescribed on discharge:

Any other advice/comment:

Date of expiry:

Cause of death:

Authorized signatory