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Early biomarkers in hospitalized patients as predictors of post-acute sequelae of SARS-CoV-2 infection: a one-year cohort study

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Abstract

Background Post-acute sequelae of SARS-CoV-2 infection (PASC) represent a significant challenge in patient care, with symptoms persisting beyond three month's post-recovery. This study aimed to evaluate the incidence of PASC at one year post-COVID-19 and identify predictive biomarkers and comorbidities for effective risk stratification.

Methods A cohort of 120 adult patients, including 50 intensive care and 70 non-intensive care patients, was followed up at two weeks, six weeks, and one-year post-discharge using structured questionnaires. The study integrated comorbidities and laboratory biomarkers to forecast the risk for PASC.

Results The median age of participants was 56 years, with 40% having moderate to severe comorbidities. A year post-recovery, 32.8% exhibited post COVID-19 conditions. The most common symptoms were constitutional (16%), respiratory (8.4%), and neuropsychiatric (2.5%). Bayesian network analysis indicated significant correlations between constitutional symptoms, rehospitalisation, and biomarkers including C-reactive protein, lactate-dehydrogenase, ferritin, and albumin.

Conclusion This study highlights the prolonged impact of PASC, one-year post infection. It highlights the role of specific biomarkers such as C-reactive protein, lactate-dehydrogenase, ferritin, and albumin in tailoring individual patient care by advancing understanding in post-COVID-19 symptoms prediction. Our findings support the need for further research to refine these insights, which are pivotal for the ongoing care of patients in the aftermath of COVID-19.

Keywords SARS-CoV-2, Post-acute sequelae, Bayesian network

Background

SARS-CoV-2 infection is associated with persistent, relapsing, or new symptoms or other health effects occurring after acute infection, termed post-acute sequelae of SARS-CoV-2 infection (PASC), also known as long COVID [1]. Following recovery from COVID-19, patients may continue to experience a range of recurrent or persistent symptoms affecting various organ systems, collectively referred to as PASC [2]. Given that PASC presents significant challenges in

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patient management and necessitates individualized care plans, there is an imperative need to enhance the understanding of this condition among healthcare professionals [3]. Although PASC is defined as the persistence of symptoms beyond three months [4], various studies have observed symptom persistence among survivors for periods up to a year. The observed rate of PASC at the end of one year ranges from 30 to 47% among adults and up to 11% in children [5, 6].

PASC is hypothesized to be caused by a complex interplay of multiple pathophysiological pathways, including autoimmunity, persistent endothelial dysfunction and coagulopathy, gut dysbiosis, hormonal and metabolic dysregulation, mitochondrial dysfunction, and autonomic nervous system dysfunction. While the exact cause of PASC remains elusive, these factors are believed to interact in a complex manner [7]. Clinical manifestations, including laboratory parameters and biomarkers, may not exhibit a direct correlation with PASC, further complicating the prediction of the condition due to the absence of specific tests. Consequently, identifying risk factors and characteristics at the individual patient level becomes imperative to accurately identify those at high risk of developing PASC and to establish diagnostic criteria [8]. Therefore, prioritizing the development and validation of laboratory biomarkers for the accurate diagnosis and prediction of PASC is critical [9]. The role of vaccination in mitigating the prolonged symptoms of COVID-19 is significant. Compared to vaccinated individuals, those unvaccinated against COVID-19 may be more susceptible to developing prolonged symptoms [10].

The functional impairment caused by long-term COVID-19 significantly impacts economic recovery. Prolonged health complications may prevent individuals from returning to work for extended periods, leading to decreased labour force participation and productivity [11]. This, in turn, places strain on initiatives aimed at revitalizing the economy. Thus, managing and assisting individuals with functional impairments resulting from PASC is not only a public health imperative but also a crucial element in ensuring a robust and sustained economic recovery.

In our prior prospective study involving the same cohort, we observed a 60% prevalence of PASC at the end of six weeks [12]. However, that study did not employ laboratory parameter analysis to investigate predictive risk factors for PASC. The objective of the present study is to determine the incidence of PASC at the end of a year and to utilize laboratory biomarkers and comorbidities to forecast the risk for PASC.

Methods

Study design and setting

This study was conducted as a single-centre, hospital-based, prospective observational study at a 1350-bed academic tertiary care referral centre in South India from February 2021 to July 2021. The hospital had an exclusive isolation facility and catered to the regional needs of COVID-19 patients, ranging from mild to severe cases.

The study was approved by the Institutional Ethics Committee and informed signed consent was obtained from all subjects before enrolling.

Study population

All the adult patients who were admitted to the COVID-19 isolation ward or the COVID-19 isolation ICU with positive SARS-CoV-2 (Reverse Transcription-Polymerase Chain Reaction (RT-PCR)/Antigen) test at the time of admission during the specified period were screened for eligibility, and those meeting eligibility criteria were recruited after informed written consent. Only patients where COVID-19 was the primary reason for hospitalization were included in the study. Patients younger than 18 years of age, those with a life condition triggering palliative intent, those with COVID-19 and a surgical diagnosis (intended or actual), or those transferred outside continued COVID-19 locations for non-COVID-19 care not directly triggered by the COVID-19 infection were excluded. The participants who have not consented to long-term follow-up post-discharge have also been excluded from the study. The patient disposition diagram is shown in Fig. 1.

COVID-19 cases were categorized based on the severity for treatment purpose into Mild (Respiratory Rate < 24/min, SpO2 $> 94\%$ on room air), Moderate (Respiratory rate between 24–29, SpO2 between 91%-94% on room air) and Severe (Respiratory Rate ≥ 30, SpO2 $< 90\%$) based on the Kerala state government guidelines which was in circulation during the study period [13].

In-hospital data collection

Baseline variables about the demographic characteristics and measurements during the hospital stay were collected from the electronic medical records obtained from the hospital information system (HIS). The data collection included demographic characteristics like age, sex, social history, and co-morbidities. The study also recorded COVID-19 specific parameters, including the severity of COVID-19 infection, level of clinical care received (ICU or non-ICU locations), length of hospital stay (LOS), vaccination status, laboratory parameters, disease symptoms, and treatment details, including antiviral, anti-coagulation, and steroid

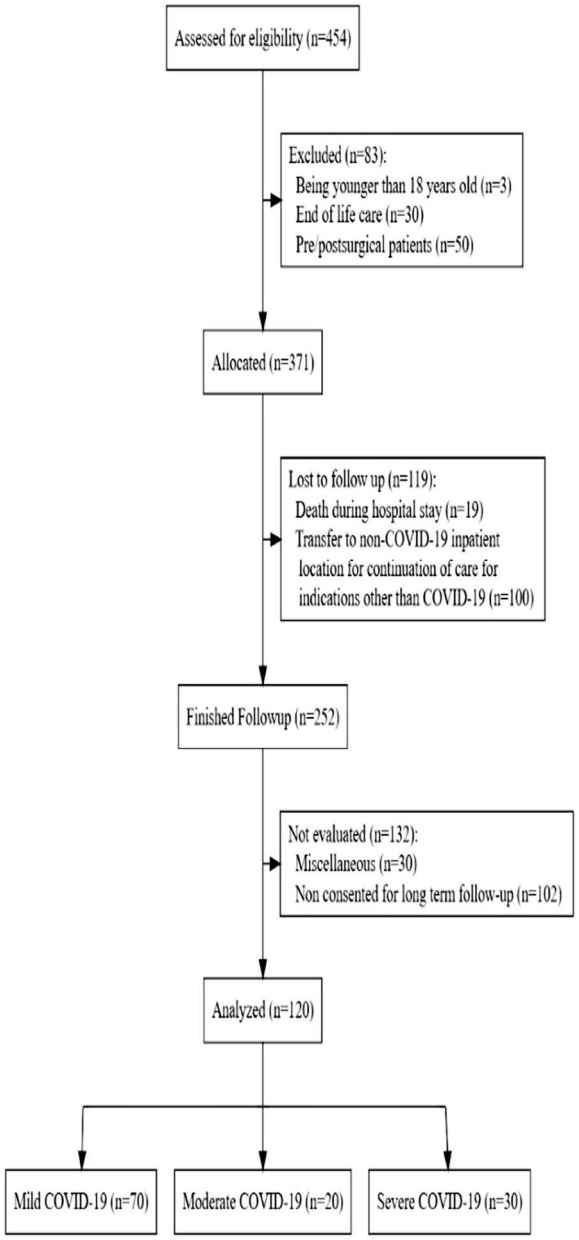

Fig. 1 Patient selection for this prospective cohort study

administration in COVID-19 patients. The age of the patients was separated into three groups: less than 40, 40 to 60, and greater than 60. The comorbidities were assessed using the Charlson Comorbidity Index (CCI) and categorized into into three groups: mild (less than or equal to 2), moderate (between 3 and 4), and severe (above or equals to 5) [14]. CCI was calculated using the MDCalc online calculator [15].

Follow-up after discharge

Information about PASC, reinfection, and rehospitalisation was assessed using a standardized questionnaire at two weeks, six weeks, and one year after discharge. The two-week follow-up was performed during review visits that all patients attended. The six weeks and one-year follow-ups were conducted by telephone. The symptoms were grouped into constitutional symptoms, including fatigue, fever, sore throat, loss of appetite, and weight loss; respiratory symptoms, including dyspnea, cough, and runny nose; neuropsychiatric symptoms, including headache, anxiety/depression, sleep disturbances, loss of smell, loss of taste, and blurred vision; musculoskeletal symptoms, including myalgia and arthralgia; gastrointestinal symptoms, including loose stools and nausea/vomiting; cardiovascular symptoms, including chest pain and palpitation; dermatological symptoms, including skin rashes; and symptoms suggestive of infections, including lower respiratory tract, urinary tract, and gastrointestinal infections.

Statistical methods

Continuous baseline characteristics were summarized by median and inter-quartile ranges (IQR). Categorical baseline characteristics were described using contingency tables. Categorical baseline characteristics were also stratified based on whether the patient was in the ICU or ward. The significance of the differences between the ICU and ward patients was assessed using the chi-square test for independence. The frequencies of the individual PASC after two weeks, six weeks, and one year were summarized by counts and percentages. A forest plot was used to visualize the risk of PASC at different time points, depending on the baseline characteristics. The forest plot shows the odds ratio 95% confidence intervals from univariable logistic regression models. Percentages of vaccination over time was visualized using a cumulative distribution plot.

A Bayesian network was used to analyse the interaction between the lab measurements taken during the hospital stay, post-COVID-19 symptom groups, and rehospitalisation after discharge. The network was created in two steps. In the first step, we fitted a discrete Bayesian network classifier [16] for the rehospitalisation depending on the symptom groups constitutional, neuropsychiatric, respiratory, and others, which include musculoskeletal, gastrointestinal, dermatological, and other infections. In the next step, Bayesian network classifiers for the symptom groups depending on the lab measurements and potential modifiers were fitted. Potential modifiers included age, sex, CCI, Chronic Kidney Disease (CKD), Chronic Liver Disease (CLD), and C-Reactive Protein

(CRP). To count for the repeated measurements of the lab parameters, mixed effects regression models with random intercepts for every person were used for these Bayesian network classifiers [17]. The Bayesian network classifier that is described for the prediction is the Tree Augmented Network (TAN) to predict the probability of the above biomarkers in rehospitalisation, with predictor variables as symptom groups shown in Fig. 3. The overall accuracy of the network was assessed using 10 times five-fold cross-validation. The structure of the resulting classifier was visualized in a network diagram. Relationships between connected discrete variables in the diagram were summarized by odds ratios and Fisher's exact tests. Estimated mean differences and *p*-values from the mixed effect regression were used to describe the relationship between lab measurements and symptom groups in the diagram. If such a relationship additionally depended on a modifier, then the relationship was summarized by the estimated mean difference in the lab measurements in each category of the effect modifier. The significance of the effect modification itself was assessed using type III ANOVA for mixed-effects regression via Satterthwaite's method. The bnclassify package in R was used for structure learning using Chow-Liu's algorithm maximizing Akaike Information Criterion (AIC) in a tree-augmented naive Bayes [18]. A significant level of alpha = 0.05 was used throughout the analysis. All the statistical analysis was done in R software, version 4.3.2.

Results

Baseline demographics

Figure 1 shows the patient's selection process. In total, 120 patients were included in the study. The median age of patients was 56 years (IQR 43 to 68). The median length of hospitalization was 10 days (IQR 9 to 29). Table 1 shows the distribution of the baseline characteristics grouped into ward and ICU patients. According to the CCI, 40% of patients belonged to the moderate or severe category. The most common comorbidities were diabetes mellitus and hypertension. There was a high incidence of new-onset diabetes, affecting 5% of patients. Compared to patients in the ward, the patients in ICU were significantly older and more likely to be male.

Symptom profile

Table 2 shows the prevalence of the PASC at different time points. The symptom prevalence decreased during the one-year follow-up period across the spectrum.

Table 1 Baseline characteristics of COVID-19 patients during the hospital stay stratified by ICU admission

Characteristics	Overall		ICU		Ward		p
	n	%	n	%	n	%	
Age less than 40	26	21.7	5	10	21	30.0	0.02
40 to 60	48	40.0	23	46	25	35.7	0.34
greater than 60	46	38.3	22	44	24	34.3	0.37
Female	54	45.0	14	28	40	57.1	<0.01
Alcohol consumption	13	10.8	7	14	6	8.6	0.52
Smoking	14	11.7	7	14	7	10.0	0.70
CCI mild	31	25.8	13	26	18	25.7	1.00
moderate	31	25.8	17	34	14	20.0	0.13
severe	17	14.2	7	14	10	14.3	1.00
New onset of diabetes	6	5.0	2	4	4	5.7	1.00
Oxygen requirement	49	40.8	48	96	1	1.4	<0.01
Anticoagulants during hospital stay	79	65.8	42	84	37	52.9	<0.01
Anticoagulants during discharge	44	36.7	29	58	15	21.4	<0.01
Antivirals during hospital stay	78	65.0	44	88	34	48.6	<0.01
Antivirals during discharge	44	36.7	29	58	15	21.4	<0.01
Steroids during hospital stay	60	50.0	48	96	12	17.1	<0.01
Steroids during discharge	24	20.0	19	38	5	7.1	<0.01

Throughout the one-year follow-up period, the most common PASCs were constitutional, followed by respiratory and neuropsychiatric symptoms. At the end of the year, the prevalence of PASC in the cohort was 32.8%, with the prevalence of constitutional symptoms at 16%, respiratory symptoms at 8.4%, and neuropsychiatric and musculoskeletal symptoms at 2.5% each. Among constitutional symptoms, fatigue, loss of appetite, and weight loss were common. It should be noted that the prevalence of fatigue sharply decreased over time, from 67.5% at two weeks to 7.6% after one year. On the other hand, loss of appetite and weight loss didn't show such a sharp decline and persisted for up to one year. With respect to the respiratory symptoms, both dyspnea and cough were common, and while they decreased over time, 5.9% of patients still experienced dyspnea at the end of one year. With respect to neuropsychiatric symptoms, sleep disturbance was the most common, and while it decreased somewhat over time, 5% of patients still experienced it after one year.

Table 2 Frequency of post covid conditions in symptom groups at two weeks, six weeks and one year after hospitalization

	Two weeks		Six weeks		One year	
	n	%	n	%	n	%
All symptoms						
Total	94	78.3	73	60.8	39	32.8
Constitutional symptoms						
Fatigue	81	67.5	67	55.8	9	7.6
Fever	2	1.7	0	0.0	0	0.0
Loss of appetite	20	16.7	13	10.8	11	9.2
Weight loss	23	19.2	20	16.7	11	9.2
Total	85	70.8	70	58.3	19	16.0
Respiratory symptoms						
Dyspnea	36	30.0	24	20.0	7	5.9
Cough	25	20.8	12	10.0	4	3.4
Total	50	41.7	29	24.2	10	8.4
Musculoskeletal symptoms						
Myalgia	12	10.0	6	5.0	2	1.7
Arthralgia	11	9.2	4	3.3	2	1.7
Total	22	18.3	9	7.5	3	2.5
Gastrointestinal symptoms						
Loose stools	11	9.2	4	3.3	0	0.0
Cardiovascular symptoms						
Chest pain	2	1.7	1	0.8	2	1.7
Dermatological symptoms						
Skin rashes	1	0.8	0	0.0	1	0.8
Infection symptoms						
LRTI	2	1.7	1	0.8	0	0.0
URTI	0	0.0	0	0.0	0	0.0
GI Infections	1	0.8	1	0.8	0	0.0
UTI	2	1.7	0	0.0	0	0.0
Total	5	4.2	2	1.7	0	0.0
Neuropsychatric symptoms						
Headache	10	8.3	3	2.5	0	0.0
Anxiety depression	10	8.3	6	5.0	2	1.7
Sleep disturbances	21	17.5	11	9.2	6	5.0
Blurred vision	1	0.8	1	0.8	1	0.8
Loss of smell	7	5.8	4	3.3	0	0.0
Loss of taste	9	7.5	4	3.3	0	0.0
Total	47	39.2	24	20.0	3	2.5

Baseline predictors for PASC

Figure 2 shows the risk of developing PASC at different time points depending on baseline characteristics. The figure also shows the risks of being readmitted to the hospital after discharge due to PASC. Not only do PASCs become less frequent over time (Table 2), but predicting them from baseline characteristics becomes more difficult the further away from the baseline the prediction is made. In fact, none of the demographic variables are a significant predictor of PASC after two weeks. While drug use during the hospital stay or given at discharge is a strong and significant predictor, the simple explanation is that patients with severe COVID-19 conditions are more likely to be given drugs and are also more likely to develop PASC. For this reason, drug use might be a good predictor but cannot be considered a risk factor for PASC. COVID vaccinations reduced the risk of persistence at one year and the risk of rehospitalisation. However, since 95% of the participants were vaccinated with at least two doses, the confidence interval for the difference between vaccinated and unvaccinated participants is rather wide (OR 0.2, 95% CI 0.03–4.8).

Biomarker predictors for PASC

Figure 3 shows the diagram of the Bayesian network. The estimated accuracy from cross-validation is 91%. All patients who were re-hospitalized had constitutional symptoms. Among patients with constitutional symptoms, the odds ratio of being re-hospitalized was 2.34 for respiratory symptoms, 1.77 for neuropsychiatric symptoms, and 2.95 for other symptoms (cardiovascular, gastrointestinal, infections, and dermatological). The relationships between symptom groups and the risk of rehospitalisation were all significant according to Fisher's exact tests ($p < 0.01$).

In the Bayesian network model, all symptom groups and their interactions with the constitutional symptoms were highly significant ($p < 0.01$) predictors for the risk of rehospitalisation. The relationships between the biomarkers and the symptom groups are summarized in Tables 3 and 4.Table 3 lists the biomarkers in the network

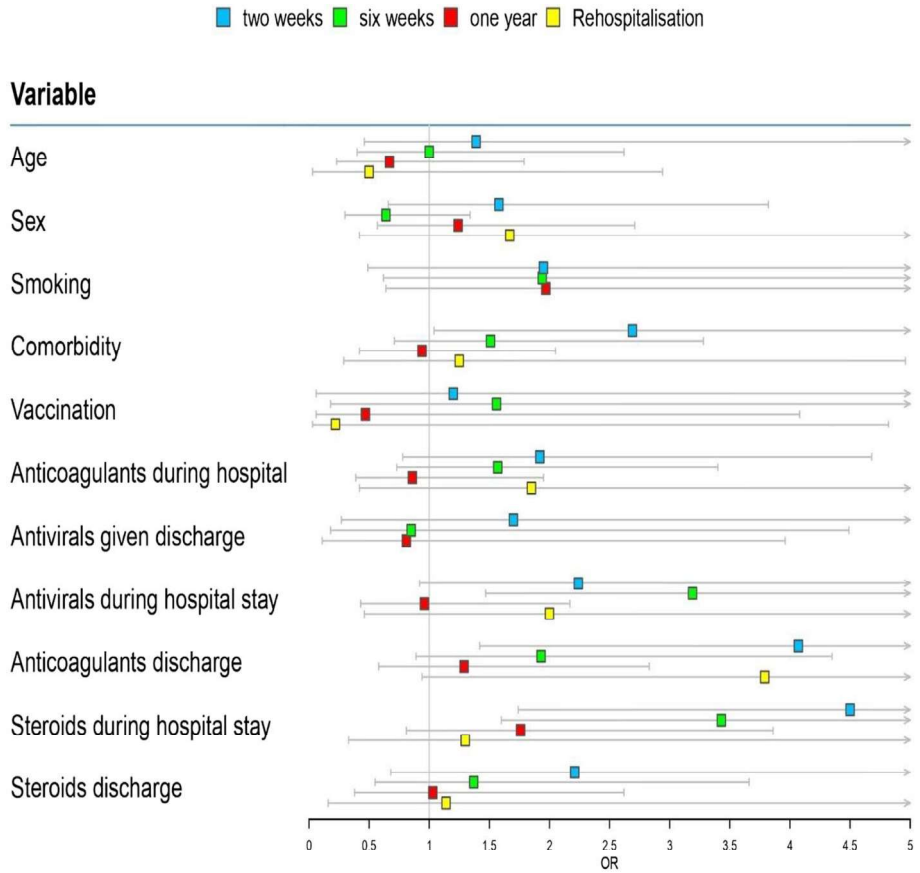

Fig. 2 Odds ratio and 95% confidence intervals for developing PASC at different time points depending on the baseline characteristics. Odds ratio for age is for patients older than 69 years compared to patients older than 69 years. Odds ratio for sex indicates the comparison of male patients with the female patients

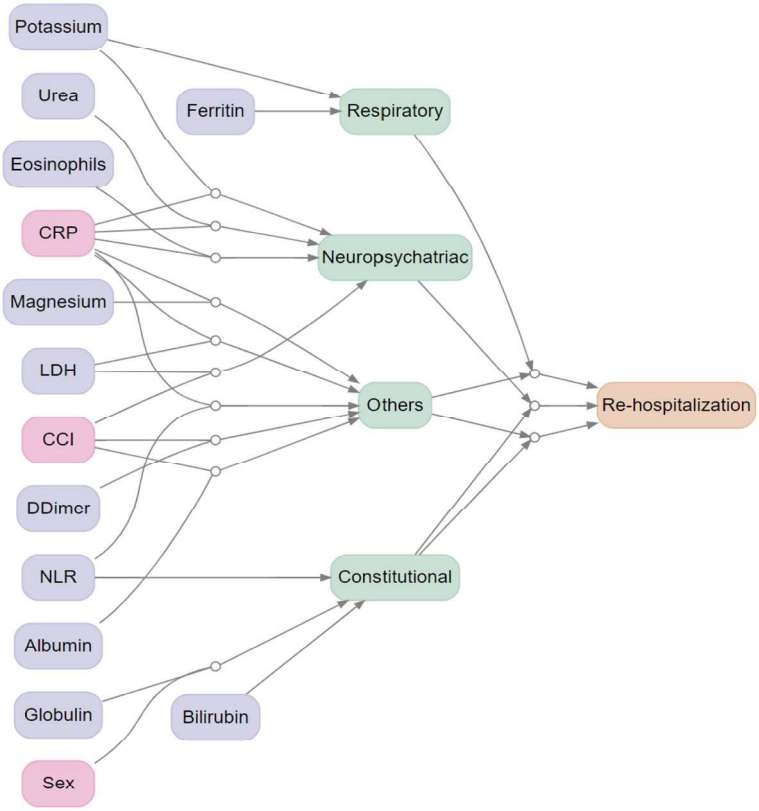

Fig. 3 Bayesian network diagram for the interaction between the lab measurements, effect modifiers, the symptom groups, and rehospitalisation in one year. Lab measurements (purple), effect modifiers (pink), symptom groups (green), rehospitalisation in one year (orange). (NLR = Neutrophil-to-lymphocyte ratio, LDH = Lactate dehydrogenase, CCI = Charlson comorbidity index, CRP = C-reactive protein.)

that have a direct effect on the risk of the development of symptom groups in the Bayesian network. Similarly, Table 4 lists the modified effects of the biomarkers on the symptom groups in the network. CRP is the most common effect modifier, modifying the effects of eosinophils, urea, and potassium on neuropsychiatric symptoms and the effects of magnesium and Lactate Dehydrogenase (LDH) on other symptoms. The two other effect modifiers in the network are the CCI and sex: with CCI modifying the effect of LDH on neuropsychiatric symptoms and the effect of serum albumin and D-Dimer on other symptoms, and sex modifying the effect of serum globulin on constitutional symptoms.

Figure 4 illustrates the effect of serum globulin and how this effect is modified by sex. Figure 4a and b show that neither serum globulin nor sex can predict the outcome independently. Figure 4c shows that serum globulin is higher in females than in men on average. However, stratifying by sex in Fig. 4d, we see that serum globulin is significantly different between the 4 groups and that for men, higher globulin levels are associated with constitutional symptoms. This influence of serum globulin is illustrated in Fig. 4e, where globulin levels are now discretized into two groups, less than 2 g/dL, and more than 2 g/dL, and the two globulin groups are predictive for men.

Table 3 Estimated effects (mean differences) in the lab measurements depending on the presence/absence of a particular symptom group. Additionally, the mean and the standard deviation of each lab measurement in the study sample is shown

Lab measurement	mean	sd	Symptom group	Effect	p
Direct Bilrubin	0.34	0.68	Constitutional	-0.29 (-0.51, -0.07)	0.01
Neutrophils	67.80	17.06	Constitutional	6.42 (0.01, 12.83)	0.05
Potassium	4.07	0.59	Respiratory	0.21 (0.05, 0.36)	0.01
Ferritin	579.90	980.58	Respiratory	331.22 (30.13, 631.80)	0.03

Table 4 Estimated effects (mean differences) of lab measurements on the presence/absence of a particular symptom group in each category of an effect modifier. The interaction effect measures the difference between the low-risk and highrisk of the effect modifier. Additionally, the mean and the standard deviation of each lab measurement in the study sample are shown. (NLR = Neutrophil-to-lymphocyte ratio, LDH = Lactate dehydrogenase, CCI = Charlson comorbidity index, CRP = C-reactive protein)

Lab measurement	Modifier	Symptom group	Overall		Low risk group		High risk group		
			mean	sd	Effect	p	Effect	Interaction Effect	p
Eosinophils	CRP > 10	Neuropsychatric	1.30	2.22	-1.74 (-2.76, -0.71)	<0.01	-0.25	1.49 (0.05, 2.92)	0.05
Urea	CRP > 10	Neuropsychatric	47.70	52.20	18.40 (0.45, 36.40)	0.05	-7.38	-25.80 (-50.9, -0.67)	0.05
Potassium	CRP > 10	Neuropsychatric	4.07	0.59	0.30 (0.07, 0.53)	0.01	-0.15	-0.45 (-0.76, -0.13)	0.01
NLR	CRP > 10	Other symptoms	21.50	13.50	-7.15 (-13.2, -1.09)	0.02	3.28	10.40 (1.51, 19.40)	0.02
Magnesium	CRP > 10	Other symptoms	2.03	0.36	0.64 (0.11, 0.57)	0.01	-0.03	-0.37 (-0.67, -0.07)	0.02
LDH	CRP > 10	Other symptoms	287.80	128.90	139.80 (55.10, 224.30)	<0.01	-2.13	-141.90 (-254.90, -28.90)	0.02
Serum Globulin	Sex = Male	Constitutional	2.97	0.55	-0.34 (-0.65, -0.02)	0.04	0.20	0.54 (0.12, 0.96)	0.01
D Dimer	CCI > mild	Other symptoms	1.07	2.01	2.10 (0.95, 3.26)	<0.01	0.30	-1.80 (-3.34, -0.26)	0.02
LDH	CCI > mild	Neuropsychatric	287.80	128.90	81.80 (6.27, 157.10)	0.04	-32.98	-114.70 (-219.80, -9.41)	0.04
Serum Albumin	CCI > mild	Other symptoms	3.69	0.66	-0.63 (-0.99, -0.27)	<0.01	-0.14	0.49 (0.02, 0.96)	0.04

Table 5 shows the subgroup analysis of all the estimates of the effects of the biomarkers in the Bayesian Network, if biomarkers are restricted to the normal ranges. Only serum albumin reached statistical significance.

Vaccination

Figure 5 shows the cumulative distribution plot depict the cumulative percentage of individuals receiving their first and second doses of a COVID-19 vaccine over a period of one year following a positive SARS-CoV-2 test. The percentage of individuals receiving their first dose increases rapidly in the first few months after the positive test where 97.5% of the patients had first dose. This suggests a strong initial response to the vaccine rollout. The curve for the second dose rises more gradually compared to the first dose. 96% of the patients had undergone second dose of vaccination. This suggests that a significant portion of the population eventually received both doses of the vaccine.

Discussion

In our prospective study of 120 patients, our primary objective was to determine the prevalence and potential predictors of PASC over a year's span. The study showed a 32.8% prevalence of PASC by the end of a one-year follow-up, with the most frequent being constitutional symptoms like loss of appetite and weight loss (both 9.2%), followed by fatigue (7.6%), dyspnoea (5.9%), and insomnia (5%). Distinctively, the primary organ systems impacted were the constitutional, respiratory, and neuropsychiatric domains.

Our study findings were in line with other prospective long COVID studies with a one-year follow-up, in which the prevalence of PASCs ranged from 11 to 50%, with one study reporting 47% of 366 subjects experiencing persistent symptoms and another noting a prevalence of 34% in adults and 11% in children out of 1013 participants [5, 6].

Our exploratory analysis aimed at generating hypotheses regarding lab parameters that could forecast PASC development, persistence, and rehospitalisation. Among the lab measurements examined for their direct association with PASC, ferritin and direct bilirubin were notable as potential predictive biomarkers. Ferritin correlates with the development of respiratory symptoms and neutrophilia to the development of neuropsychiatric symptoms.

While some lab parameters showed slight deviations in their average values, they remained within normal ranges, making them weak predictors for PASC. This is also shown in the subgroup analysis, where only one marker, i.e. Albumin, reached statistical significance in predicting PASC within the normal range and an average albumin value of 4 or more was found to be associated with lesser risk of PASC.

This observation can be attributed to albumin's role as a negative acute-phase reactant; its levels decline in response to systemic inflammation. Severe COVID-19 is associated with heightened inflammatory responses, resulting in lower albumin levels. Therefore, a normal albumin level (e.g., ≥ 4 g/dL) may indicate a reduced systemic inflammatory state. Higher levels of inflammation are likely to correspond to an increased risk of PACS.

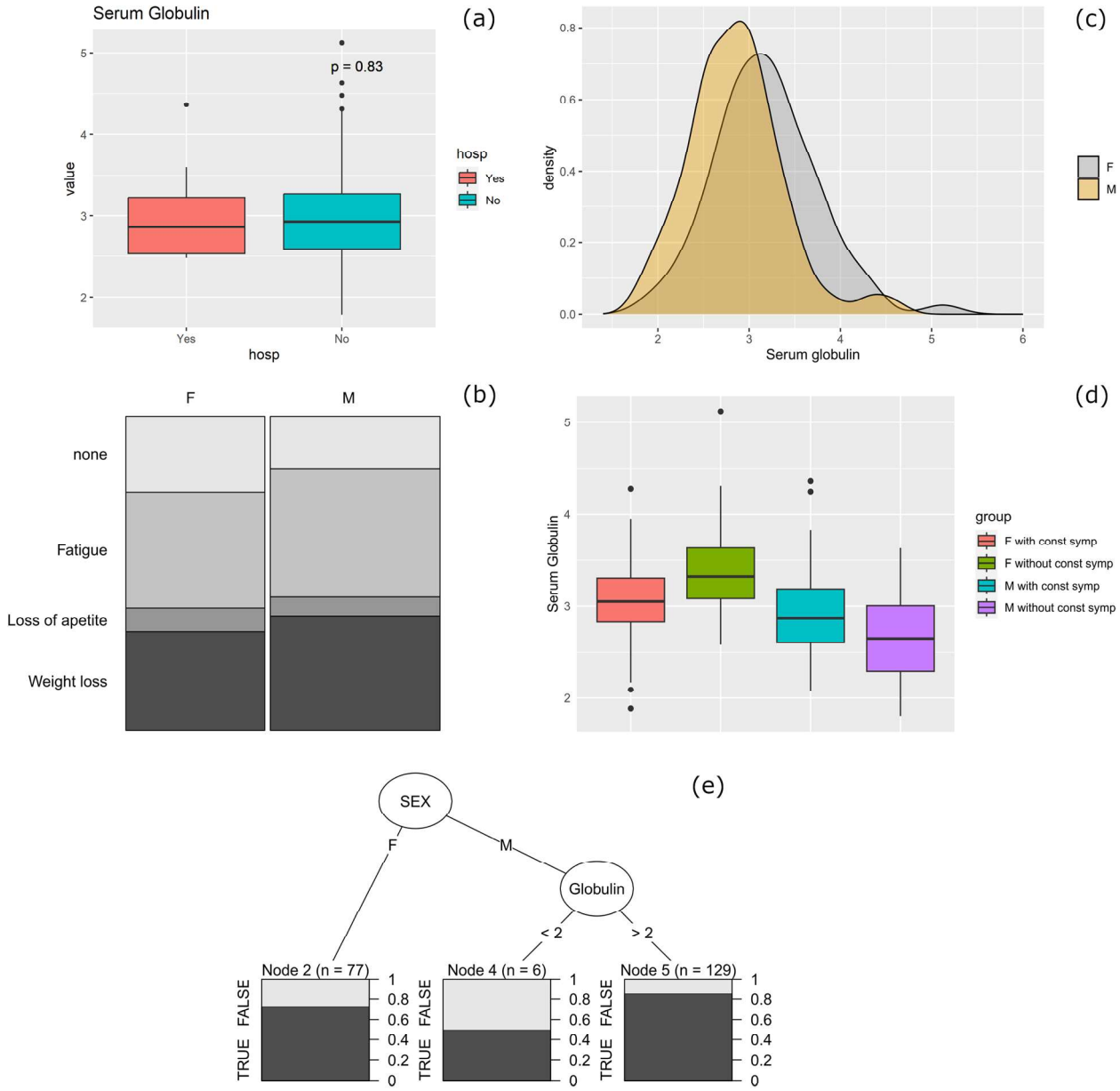

Fig. 4 **a** Boxplots of serum globulin values for patients who were re-hospitalised and patients who were not in 12-month period after discharge. **b** Frequencies of constitutional symptoms after 12 months for men and female. **c** Density plots of serum globulin values for men and female. **d** Boxplots of serum globulin values depending on sex and the presence of constitutional symptoms. **e** Decision tree for predicting rehospitalisation from sex and serum globulin

This can be explained by the fact that the albumin is a negative acute phase reactant and with increase in the inflammatory response the albumin level will come down. The severe the COVID-19, the severe the inflammatory response and lesser the value of Albumin. Hence, normal albumin value of 4 indicates that the systemic inflammatory response is normal.

Though there are numerous studies that have focused on ferritin and other biomarkers in predicting the severity of the COVID-19 disease [19, 20]. The studies that explored the role of acute phase biomarkers in predicting the PASC are minimal; in a recently published systematic review the role of acute phase reactants including CRP, Ferritin, Fibrinogen, Albumin, and C5b-9, has been explored and suggested as a potential predictor of PASC [21]. Various biomarkers, such as

Table 5 Subgroup analysis for biomarkers within the normal ranges, showing the estimated effects (mean differences) in the lab measurements depending on the presence/absence of a particular symptom group

Lab Measurement	Symptom group	Mean	SD	Effect	*p*-value
Neutrophils	Constituitional	70.63	5.63	1.76 (-0.35, 3.87)	0.11
Neutrophils	Other symptoms	70.63	5.63	0.51 (-1.28, 2.31)	0.58
Potassium	Neuropsychatric	4.13	0.41	0.04 (-0.07, 0.14)	0.49
Ferritin	Respiratory	150.1	89.21	16.71 (-26.86, 60.21)	0.45
Direct Bilrubin	Constituitional	0.12	0.05	0 (-0.02, 0.02)	0.85
Lymphocytes	Other symptoms	28.74	5.75	-1.17 (-3.53, 1.19)	0.34
Eosinophils	Neuropsychatric	0.15	0.22	-0.05 (-0.11, 0.01)	0.12
UREA	Neuropsychatric	31.72	9.84	1.11 (-2.34, 4.58))	0.53
Magnesium	Other symptoms	1.99	0.15	-0.06 (-0.14, 0.03)	0.19
LDH	Other symptoms	195.29	32.59	-7.77 (-31.43, 15.9)	0.52
Serum Globulin	Constituitional	3.06	0.36	-0.06 (-0.23, 0.11)	0.46
Serum Albumin	Other symptoms	4.09	0.39	-0.23 (-0.40, -0.06)	0.01
D Dimer	Other symptoms	0.33	0.09	0 (-0.04, 0.05)	0.84

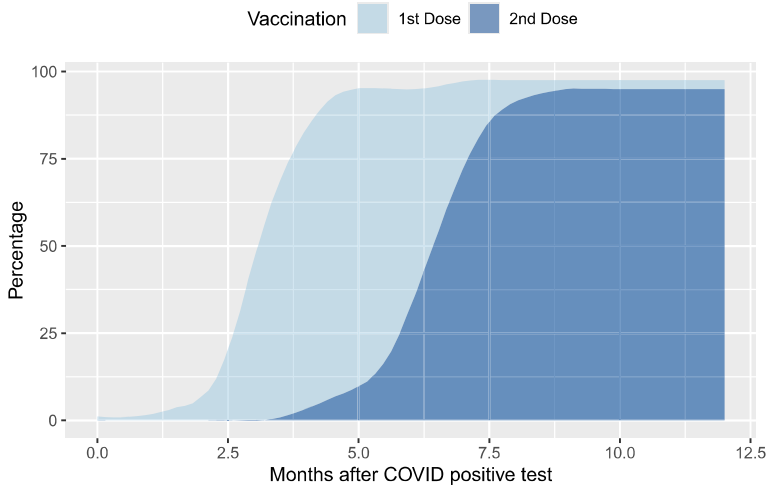

Fig. 5 A cumulative distribution plot for depicting the cumulative percentage of individuals receiving their first and second doses of a COVID-19 vaccine

D-Dimer levels, creatinine, and ferritin titers, are considered potential predictors for the manifestation of PASC [22–24].

Our network analysis indicates that CRP, an inflammatory marker, markedly impacts the influence of other laboratory parameters on PASC progression. Additionally, as comorbidities represented by the CCI score increase, interactions of other inflammatory markers such as LDH, ferritin, and the negative acute phase reactant albumin change, subsequently modifying the risk of PASC onset. Our study results are consistent with those of other prospective PASC studies which showed the association between the development of PASC and CCI, the comorbidities like type II diabetes mellitus, hypertension, dyslipidemia, coronary artery disease, asthma, and cancer, was in turn associated with increased risk of long COVID [25–27]. It is pertinent to perceive these laboratory parameters as elements of a biological continuum triggered by COVID-19, reflecting the sustained inflammatory environment and its role in PASC emergence. Adopting precision medicine approaches that risk-stratify patients into distinct phenotypes based on risk-modifying factors like CCI and CRP levels can enhance our ability to predict PASC with the aforementioned inflammatory markers.

This study contributes to the ongoing effort to identify biomarker predictors of PASC and the risk of rehospitalization after a year. Given that nearly one in three patients

manifest PASC within this time frame, identifying these predictors is invaluable for patient counselling, education, and rehabilitation. The study suggests that physicians should be aware of lab markers that could modify the risk of developing PASC. As indicated by our findings, physicians must consider the CRP levels before interpreting the PASC predictive ability of other biomarkers.

Our analysis revealed a rapid initial uptake in first-dose COVID-19 vaccinations following a positive test, followed by a slightly slower increase in second-dose coverage after three months. This pattern suggests a strong initial response to the vaccination campaign, potentially driven by the immediate health concerns associated with a positive test.

The PASC symptoms showed a declining trend during the study period, with a marked decline in the prevalence of fatigue from 55.8% at six week's post-infection to 7.6% at the one-year mark. However, PASC's persistence and impact are evident, with 19% of patients still not feeling normal at work, underscoring its profound public health ramifications. It's crucial to grasp this prolonged influence, not merely for individual patient care but also in informing wider healthcare policies and public health initiatives, emphasizing the necessity for policymakers to acknowledge and tackle this enduring issue.

Limitations

This study, while aiming to identify biomarkers for PASC development, carries certain limitations. The modest cohort size may affect generalizability, and self-reported outcomes could introduce bias. It represents the pre-vaccine COVID-19 cohort and doesn't address post-vaccination PASC or newer strains. The findings of the study lean more toward hypothesis generation than definitive conclusions. Future studies should explore more objective endpoints in larger cohorts.

Conclusion

In conclusion, our study highlights the continued prevalence and persistence of PASC one-year post-infection, with certain laboratory parameters (ferritin, LDH, and albumin) emerging as potential predictors. While our findings align with prior studies on PASC prevalence, they distinctively reveal the interconnected roles of inflammatory markers, especially CRP, and comorbidities in the progression of PASC. As we advance in the post-COVID-19 era, a deeper understanding of these biomarkers and their interplay will be crucial for tailoring patient care, and the prevalence of PASC can guide public health strategies. The insights from this study, while hypothesis-generating, pave the way for broader research.

Abbreviations

PASC	Post-acute sequelae of SARS-CoV-2 infection
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
ICU	Intensive Care Unit
LOS	Length of Stay
CCI	Charlson Comorbidity Index
IQR	Interquartile Ranges
CKD	Chronic Kidney Disease
CLD	Chronic Liver Disease
CRP	C-Reactive Protein
TAN	Tree Augmented Network
AIC	Akaike Information Criterion
LDH	Lactate Dehydrogenase

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Authors' contributions

D.T.S, M.M and K.G.K involved in conceptualization, manuscript writing, review and editing. C.V.N involved in data collection and follow-up. C.V.N, and M.K involved in writing manuscript, literature search and references. C.V.N, D.T.S, and M.M were responsible for project implementation. G.G and M.K done the data analysis and involved in review and editing of manuscript. G.G, M.K and C.V.N prepared all the figures in the manuscript. All authors reviewed the manuscript.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by Institutional Ethics Committee (Approval No: IEC-AIMS-2021-PHARM-095) of Amrita Institute of Medical Science and Research Centre, Kochi. Informed signed consent was obtained from all subjects before enrolling. The study was conducted in accordance to the regulations and guidelines of Amrita Institute of Medical Science and Research Centre, Kochi.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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