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Performance of Rapid Antigen Tests and Inflammatory Markers in SARS-CoV-2: Lessons for Future Respiratory Pandemics

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ABSTRACT

OBJECTIVE: To evaluate the diagnostic performance of rapid antigen tests (RATs) and the utility of inflammatory biomarkers in symptomatic and asymptomatic SARS-CoV-2 cases among healthcare workers and the general population in Punjab, Pakistan.

METHODOLOGY: This cross-sectional study enrolled 1,500 participants (734 symptomatic; 766 asymptomatic), including healthcare workers (HCWs) and the general population (GP), across three cities in Punjab, Pakistan, between November 2021 and July 2022 at the Institute of Microbiology, Government College University, Faisalabad, Pakistan. Participants underwent parallel RT-PCR and Rapid antigen testing (RAT) from nasopharyngeal swabs. Serum/plasma biomarkers (LDH, D-dimer, CRP, IL-6) were quantified using standardized assays.

RESULTS: Symptomatic individuals showed significantly higher SARS-CoV-2 positivity than asymptomatic individuals by PCR (92.3% vs. 38.8%), RAT (89.8% vs. 4.0%), and IgG serology (82.2% vs. 59.7%) (all $p<0.0001$). Symptomatic cases exhibited markedly elevated biomarkers (e.g., LDH: 394 U/L vs. 195 U/L; CRP: 29 mg/L vs. 0.81 mg/L; all $p<0.001$). HCWs had higher PCR positivity (68.0% vs. 62.6%; $p=0.041$) and higher levels of LDH, CRP, and IL-6 ($p<0.001$) than GP. RAT sensitivity was high in symptomatic cases (97.3%) but low in asymptomatic individuals (10.2%).

CONCLUSION: Rapid antigen tests excel at detecting symptomatic SARS-CoV-2 infections but are unreliable for asymptomatic infections. Biomarkers robustly indicate systemic inflammation and clinical risk in symptomatic individuals and HCWs. Context-based strategies are essential. For example, using RATs to control the spread among people with symptoms while using biomarkers to assess disease severity in high-risk groups, especially in low-resource settings, can improve the pandemic response.

KEYWORDS: SARS-CoV-2, Inflammatory biomarkers, Healthcare workers, Diagnostic performance, Resource-limited settings, Pandemic preparedness.

INTRODUCTION

COVID-19, caused by the SARS-CoV-2 virus, began in late 2019. It quickly spread into a global pandemic, overwhelming healthcare systems and resulting in millions of deaths[1]. Rapid recognition of infected individuals, especially during presymptomatic or early symptomatic periods when viral loads are highest, reduces secondary transmission and disease severity by enabling timely isolation and interventions[1,2].

RATs offer significant pandemic-control benefits over RT-PCR, including point-of-care use, faster turnaround, lower cost, and the ability to support high-frequency testing with same-day isolation[3]. However, RATs are less sensitive (68–99%) than RT-PCR, particularly during reduced viral loads or during symptomatic or asymptomatic infection[4,5]. Thus, RATs excel as public health tools for curbing transmission, whereas PCR remains the diagnostic gold standard for individual diagnosis when sensitivity is the primary concern[4]. Inflammatory biomarkers are critical for predicting COVID-19 severity and addressing RAT limitations. Markers such as lactate dehydrogenase (LDH), D-dimer, C-reactive protein (CRP), and interleukin-6 (IL-6) rise days before clinically evident symptoms, signalling hyperinflammation (e.g., a cytokine storm) and increased risk of organ damage[6,7].

The diagnostic accuracy for SARS-CoV-2 tests is not universally generalizable, particularly in genetically and epidemiologically diverse populations. In Pakistan, there is a lack of integrated data on the combined evaluation of RAT performance and inflammatory biomarkers (LDH, CRP, D-dimer, IL-6) in both healthcare workers and the general population. This evidence gap limits the development of diagnostic and prognostic strategies tailored to local needs and may result in the misapplication of international data in future respiratory pandemics. Therefore, this study was undertaken to: (i) assess the diagnostic performance of RATs compared with RT-PCR in symptomatic and asymptomatic individuals; and (ii) evaluate the levels of key inflammatory biomarkers among SARS-CoV-2 cases, with subgroup analyses for healthcare workers and the general population.

The findings aim to generate context-specific evidence to guide pandemic response strategies in resource-limited settings.

METHODOLOGY

A cross-sectional molecular epidemiological study was conducted across three major cities in Punjab, Pakistan (Lahore, Faisalabad, Sargodha) between November 2021 and July 2022. The study enrolled 1,500 participants presenting with suspected SARS-CoV-2 infection, comprising 734 symptomatic and 766 asymptomatic individuals. The convenience sampling was used, and participant recruitment was conducted entirely in person. No online or social media-based survey methods were used. The sample distribution was as follows: Lahore contributed 471 participants (31.4%; 249 symptomatic, 222 asymptomatic), Faisalabad 538 (35.9%; 261 symptomatic, 277 asymptomatic), and Sargodha 491 (32.7%; 256 symptomatic, 235 asymptomatic) (**Figure I**). The sample size was determined using the Raosoft sample size calculator (Raosoft, Inc., USA), and all procedures adhered to the Punjab Health Care Commission guidelines[8].

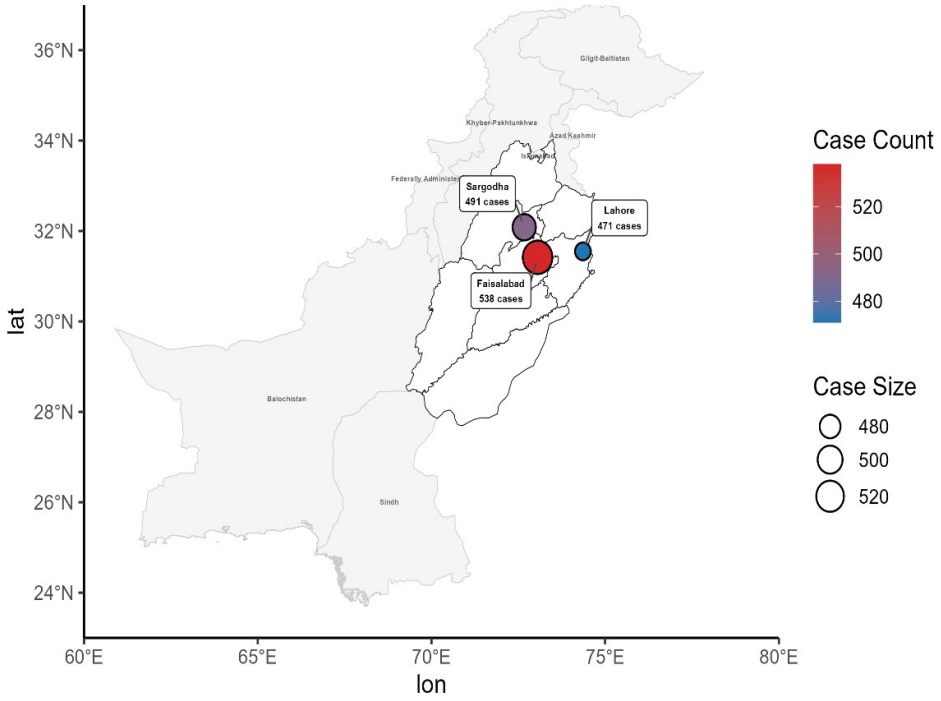

Figure I: Geographic Distribution of Study Participants Across Three Cities in Punjab, Pakistan

The map illustrates the number of enrolled participants from three major cities (Faisalabad, Sargodha, and Lahore). The relative participant counts are indicated by proportional markers and color intensity reflecting the distribution of the study population across the region.

Data and Sample Collection

Demographic, clinical, and occupational data, including age, gender, symptom status, vaccination history, profession, residence, and travel history, were recorded using standardized questionnaires. Paired biological samples were collected from each participant: nasopharyngeal swabs preserved in viral transport medium (VTM) for viral detection, and venous blood drawn into 3.2% Sodium Citrate tubes (for plasma) for D-Dimer measurement and serum separator tubes (for serum) for serological and biomarker analysis. Sample

temperatures were maintained between 2°C and 8°C throughout transport from collection sites to the laboratory using insulated cold boxes. Cold chain integrity was monitored using an HTC-2 digital thermometer to track temperature and humidity, ensuring sample stability for molecular detection. Upon arrival, samples were stored at 2–8°C in the laboratory before processing.

SARS-CoV-2 Detection

Nasopharyngeal swabs underwent parallel batch-wise testing via RT-PCR and rapid antigen test (RAT) upon receipt. For RT-PCR analysis, RNA extraction was performed using the HERO 32 Magnetic Bead System (Ascend Biotechnology, China). Amplification was followed using a TaqMan-based assay (ACON Biotech, China) targeting the N, E, and ORF1ab genes on an Applied Biosystems QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific, USA). The thermal cycling protocol comprised reverse transcription at 50°C for 20 minutes, followed by 45 cycles of denaturation at 94°C for 15 seconds and annealing/extension at 60°C for 30 seconds. Samples with cycle threshold (Ct) values < 36 were considered positive per manufacturer guidelines.

Concurrent RAT testing utilized the Abbott Panbio™ COVID-19 Ag Rapid Test Device, with results interpreted visually 15–20 minutes after sample application.

Serological and Biomarker Analysis

Serum samples were tested for SARS-CoV-2 IgG antibodies using the Abbott Panbio COVID-19 IgG Rapid Test Device, with results interpreted visually at 10–20 minutes. Biomarker quantification included: serum lactate dehydrogenase (LDH) measured via colorimetric enzymatic assay on a Roche Cobas c501 analyzer (reference range: 135–214 U/L); plasma D-dimer quantified by immunoturbidimetric assay on (Roche Cobas c501; reference range: 0–0.5 µg/mL); serum C-reactive protein (CRP) assessed via turbidimetric immunoassay (Roche Cobas c501; reference: <10 mg/L); and serum interleukin-6 (IL-6) measured using high-sensitivity ELISA (Monobind Inc., USA) with absorbance read at 450 nm (reference: <35 pg/mL).

Statistical Analysis

Data analysis utilized R (v4.4.1; RStudio 2023.12.1), Python (v3.13.5), and IBM SPSS Statistics (v23). Analytical approaches included chi-square/Fisher's exact tests for prevalence comparisons, Mann-Whitney U/Kruskal-Wallis tests for biomarker level comparisons, Spearman's rank correlation for association analyses, and multivariate regression to adjust for confounding variables.

RESULTS

Demographic Correlates of Symptomatic SARS-CoV-2 Infection

Descriptive analysis of participant characteristics revealed a significant association between gender and symptomatic status ($p < 0.0001$), with a higher proportion of symptomatic individuals being male (63.8%) compared to asymptomatic individuals (45.9%). However, no statistically significant differences were observed between the two groups with respect to age (mean: 34.1 ± 9.7 vs. 34.4 ± 10.0 years; $p = 0.507$), occupation, residence (urban vs. rural), or COVID-19 vaccination status (**Table I**). The age distribution differed slightly by symptom status, with symptomatic individuals generally older than asymptomatic participants, as shown in **Figure II**.

Table I: Demographic Characteristics of patients

Variable	Symptomatic (n=766)	Asymptomatic (n=734)	p-value (test)
Age (years)	34.1 ± 9.7	34.4 ± 10.0	0.507 (Independent t-test)
Gender			
Female	277 (36.2%)	397 (54.1%)	<0.0001 (X^2)
Male	489 (63.8%)	337 (45.9%)	<0.0001 (X^2)
Occupation			
Essential Worker	184 (24.0%)	160 (21.8%)	0.336 (X^2)
Frontline Worker	115 (15.0%)	110 (15.0%)	1.000 (X^2)
Healthcare Worker	213 (27.8%)	202 (27.5%)	0.947 (X^2)
Housewife	75 (9.8%)	70 (9.5%)	0.937 (X^2)
Other	18 (2.3%)	19 (2.6%)	0.895 (X^2)
Shopkeeper	62 (8.1%)	70 (9.5%)	0.371 (X^2)
Student	82 (10.7%)	88 (12.0%)	0.482 (X^2)
Unemployed	17 (2.2%)	15 (2.0%)	0.955 (X^2)
Residence			
Urban Residence	463 (60.4%)	449 (61.2%)	0.814 (X^2)
Rural Residence	303 (39.6%)	285 (38.8%)	0.814 (X^2)
Vaccination status			
Vaccinated	258 (33.7%)	240 (32.7%)	0.727 (X^2)
Unvaccinated	508 (66.3%)	494 (67.3%)	0.727 (X^2)

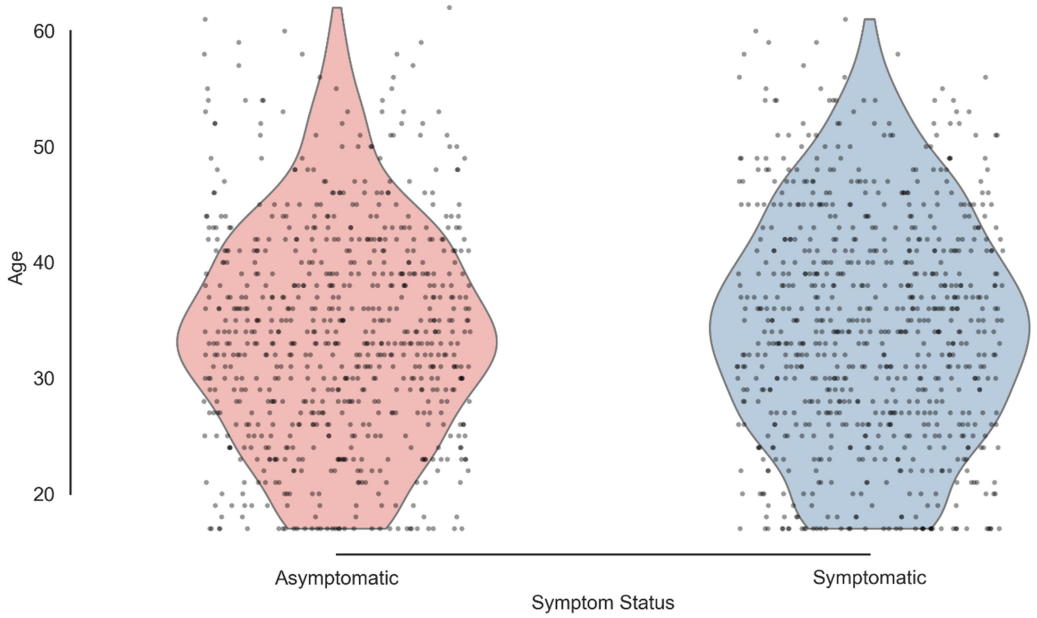

Figure II: Age Distribution by Symptom Status Among COVID-19 Participants

The violin plot depicts the distribution of age among asymptomatic and symptomatic individuals. Each violin shape illustrates the probability density of the age distribution at different values. The width of the violin represents the relative frequency of participants at a given age.

Diagnostic Test Positivity Among Symptomatic vs. Asymptomatic Individuals

A comparison of SARS-CoV-2 diagnostic test results between symptomatic and asymptomatic individuals revealed significant differences across all testing modalities (**Table II**). Among the 1500 participants, 66.1% tested positive by PCR, with a notably higher positivity rate in symptomatic individuals (92.3%) than in asymptomatic ones (38.8%), a statistically significant difference (X^2 = 476.08, $p < 0.0001$). Similarly, rapid antigen test (RAT) positivity was observed in 47.8% overall, with 89.8% in the symptomatic group and only 4.0% in the asymptomatic individuals (X^2 = 1104.16, $p < 0.0001$). The presence of IgG antibodies was also significantly more frequent in symptomatic participants (82.2%) than in asymptomatic ones (59.7%) (X^2 = 92.04, $p < 0.0001$).

Table II: Diagnostic Test Results by Symptom Status Among Randomly Sampled Individuals Screened for COVID-19

Test Result	Overall (n = 1500)	Symptomatic (n = 766)	Asymptomatic (n = 734)	Statistical Test Chi-square, p-value)
PCR Positive	992 (66.1%)	707 (92.3%)	285 (38.8%)	X^2 = 476.08, p = 0.0000
PCR Negative	508 (33.9%)	59 (7.7%)	449 (61.2%)	
RAT Positive	717 (47.8%)	688 (89.8%)	29 (4.0%)	X^2 = 1104.16, p = 0.0000
RAT Negative	783 (52.2%)	78 (10.2%)	705 (96.0%)	
IgG Positive	1068 (71.2%)	630 (82.2%)	438 (59.7%)	X^2 = 92.04, p = 0.0000
IgG Negative	432 (28.8%)	136 (17.8%)	296 (40.3%)	

Inflammatory and Coagulation Biomarker Profiles in SARS-CoV-2 Cases

A comparative analysis of inflammatory and coagulation biomarkers between symptomatic and asymptomatic individuals revealed statistically significant differences in all measured parameters ($p < 0.001$ for each) as shown in **Table III**. Median serum levels of LDH, CRP, D-dimer, and IL-6 were markedly elevated in symptomatic patients compared to their asymptomatic counterparts. Specifically, LDH levels were nearly twice as high in symptomatic individuals (median: 394 U/L vs. 195 U/L), while CRP levels showed an even greater disparity (29 mg/L vs. 0.81 mg/L). Similarly, symptomatic individuals exhibited significantly elevated D-dimer (1.3 µg/mL vs. 0.3 µg/mL) and IL-6 levels (50 pg/mL vs. 5 pg/mL). The distribution of inflammatory markers (CRP, D-dimer, IL-6) stratified by symptom status among COVID-19 patients is shown in **Figure III**. Chi-square test results confirm that differences in biomarker elevations between groups are statistically significant, suggesting a strong association between symptom manifestation and systemic inflammatory response (**Table IV**).

Table III: Comparison of Biomarker Levels in Symptomatic and Asymptomatic Individuals

Biomarker	Group	Median	IQR	Min–Max	p-value (Mann–Whitney U)
LDH (U/L)	Symptomatic	394	56.0	149 – 547	< 0.001
	Asymptomatic	195	25.6	135 – 268	
CRP (mg/L)	Symptomatic	29	13.8	1.3 – 59.3	< 0.001
	Asymptomatic	0.81	0.2	0.42 – 17.6	
D-Dimer (µg/mL)	Symptomatic	1.3	0.6	0.0 – 3.1	< 0.001
	Asymptomatic	0.3	0.2	0.0 – 0.6	
IL-6 (pg/mL)	Symptomatic	50	14.3	19 – 80.2	< 0.001
	Asymptomatic	5	1.0	2.5 – 72	

Table IV: Frequency and Percentage of Elevated Biomarkers Among Symptomatic and Asymptomatic Individuals

Biomarker	Threshold	Symptomatic (n=766)	Asymptomatic (n=734)	p-value (X^2 test)
LDH Elevated	> 214 U/L	741 (96.7%)	94 (12.8%)	< 0.001
D-Dimer Elevated	> 0.5 µg/mL	692 (90.3%)	45 (6.1%)	< 0.001
CRP Elevated	> 10 mg/L	686 (89.6%)	31 (4.2%)	< 0.001
IL-6 Elevated	> 35 pg/mL	710 (92.7%)	32 (4.4%)	< 0.001

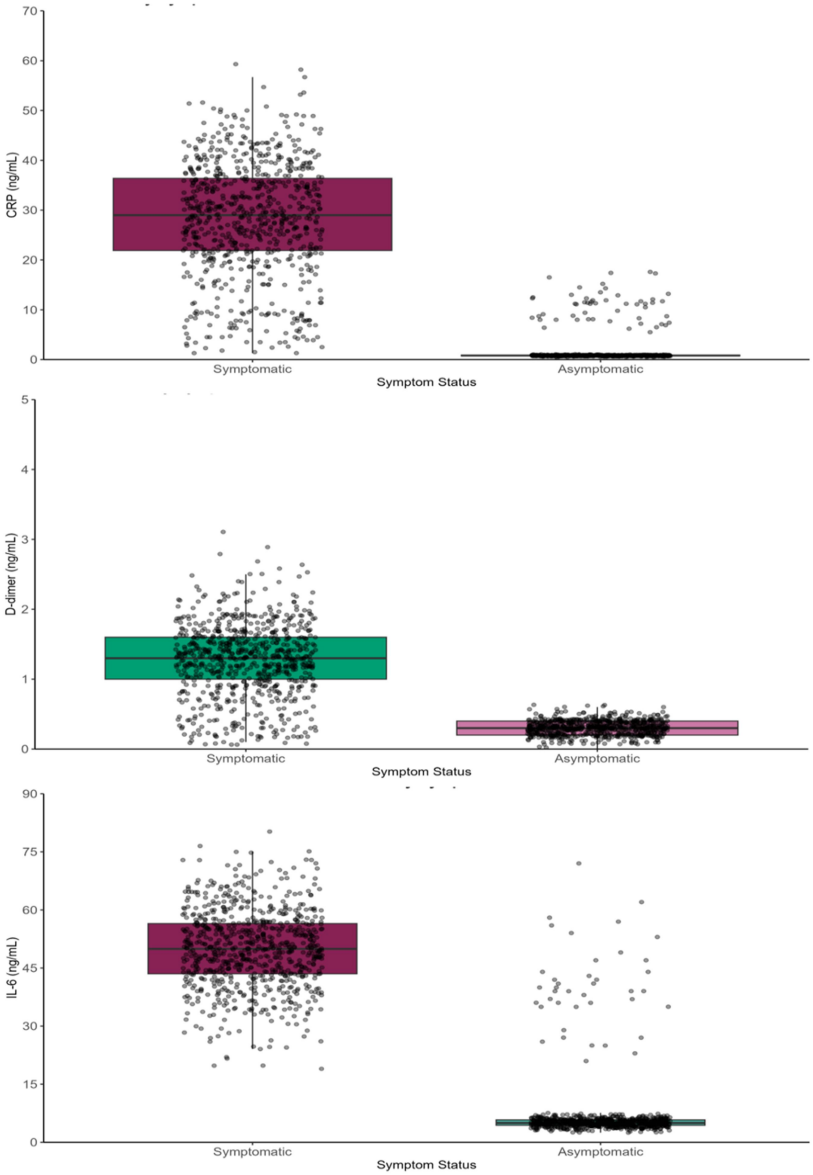

Figure III: Inflammatory Laboratory Markers Stratified by Symptom Status in COVID-19 Patients

Boxplots show the distributions of CRP, D-dimer, and IL-6 levels in asymptomatic and symptomatic individuals. Individual data points are overlaid on boxplots to illustrate variability and density within groups. Median values are indicated by horizontal bars within each box. Outliers and wider interquartile ranges are more prominent among symptomatic individuals, suggesting greater heterogeneity in inflammatory response.

Correlation analysis was conducted to assess the relationships between inflammatory and coagulation markers (D-dimer, CRP, IL-6) and patient age in COVID-19 cases. As shown in both Pearson and Spearman correlation heatmaps (**Figure IV**), a strong positive correlation was observed among the three biomarkers. The highest correlation was noted between IL-6 and CRP (Pearson: r = 0.78; Spearman: r = 0.73), followed by IL-6 and D-dimer (Pearson: r = 0.74; Spearman: r = 0.67). In contrast, age demonstrated a very weak correlation with all laboratory parameters (Pearson r range: 0.05–0.11; Spearman r range: 0.04–0.09).

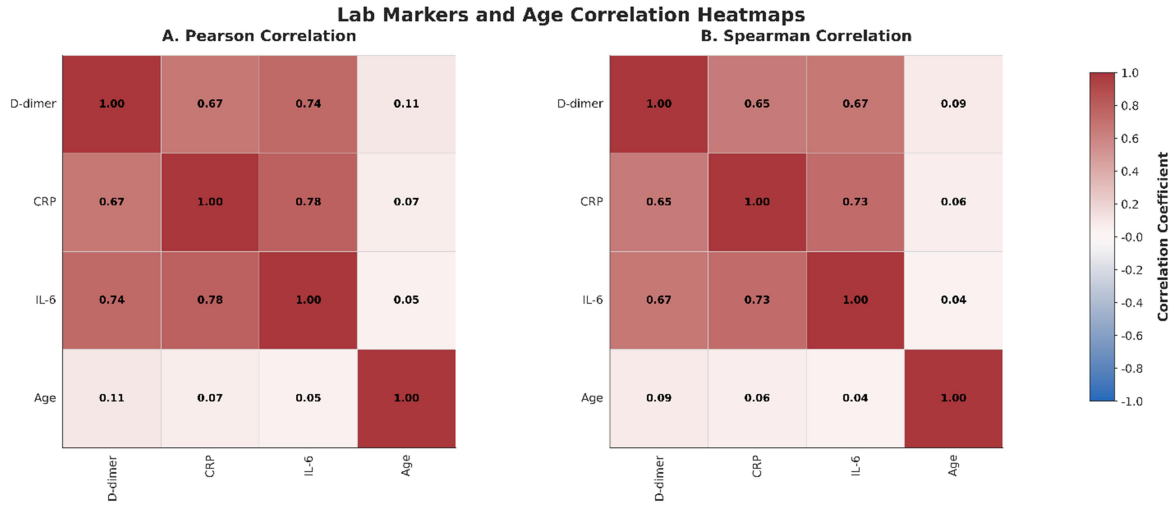

Figure IV: Correlation heatmaps of laboratory markers and age in COVID-19 patients

(A) Pearson correlation and (B) Spearman correlation matrices illustrate the relationships among D-dimer, CRP, IL-6, and age. Each cell displays the corresponding correlation coefficient, with a color gradient ranging from light (negative correlation) to dark (positive correlation). Strong positive correlations were observed among D-dimer, CRP, and IL-6 across both correlation methods, while age showed weak or negligible correlations with all laboratory parameters.

Regional Comparison of COVID-19 Diagnostic and Inflammatory Profiles

Table V summarizes the comparison of clinical and laboratory features among COVID-19 patients from three major cities in Punjab. No statistically significant differences were observed across the cities in symptom status, test positivity (PCR, RAT, IgG), or biomarker levels (LDH, D-dimer, CRP, IL-6).

Table V: Distribution of diagnostic test positivity and inflammatory markers among individuals screened for COVID-19 in three major cities of Punjab, Pakistan

City	n	Asymptomatic (n, %)	Symptomatic (n, %)	PCR+ (n, %)	RAT+ (n, %)	IgG Positive (n, %)	Median LDH	Median D-Dimer	Median CRP	Median IL-6
Lahore	471	222 (47.1%)	249 (52.9%)	316 (67.1%)	232 (49.3%)	340 (72.1%)	252	0.4	6.35	36.9
Faisalabad	538	277 (51.5%)	261 (48.5%)	337 (62.6%)	248 (46.1%)	388 (72.2%)	223	0.5	11.4	23.3
Sargodha	491	235 (47.9%)	256 (52.1%)	339 (69.0%)	237 (48.3%)	340 (69.2%)	254	0.5	8.3	36.7
p-value	–	0.3706*	0.3706*	1.0*	1.0*	1.0*	0.7166†	0.5731†	0.5731†	0.7166†

** p-values calculated using the Chi-square test for categorical variables*

† p-values calculated using the Kruskal–Wallis test for non-normally distributed continuous variables

Comparison of SARS-CoV-2 Positivity and Biomarker Levels Between Healthcare Workers and the General Population

Healthcare workers had significantly higher PCR positivity (68.0% vs. 62.6%; p = 0.041) and elevated LDH, CRP, and IL-6 levels (all p < 0.001) compared to the general population. Symptom prevalence was similar (52.0% vs. 49.2%; p = 0.328), and no difference was observed in D-dimer levels (p = 1.000). (**Table VI)**

Table VI: Comparison of Symptom Status, PCR Positivity, and Inflammatory Biomarkers between Healthcare Workers and General Population

Variable	Healthcare Workers (n = 984)	General Population (n = 516)	p-value (Test)
Symptomatic (%)	512 (52.0%)	254 (49.2%)	0.328 (Chi-square test)
PCR Positive (%)	669 (68.0%)	323 (62.6%)	0.041 (Chi-square test)
Median LDH (U/L)	254	219	<0.001* (Mann–Whitney U test)
Median D-Dimer (µg/mL)	0.4	0.4	1.000 (Mann–Whitney U test)
Median CRP (mg/L)	9.1	7.45	<0.001* (Mann–Whitney U test)
Median IL-6 (pg/mL)	36.25	27.85	<0.001* (Mann–Whitney U test)

Evaluation of RAT Sensitivity and Specificity

RAT showed high sensitivity (97.3%) and specificity (100%) in symptomatic individuals, with PPV and NPV of 100% and 75.6%, respectively. In asymptomatic cases, sensitivity dropped to 10.2%, while specificity and PPV remained at 100%. (**Table VII**)

Table VII: Performance of the Rapid Antigen Test (RAT) in Asymptomatic and Symptomatic Individuals

RAT Metric	Asymptomatic (%)	Symptomatic (%)	Total (%)
Sensitivity	10.2	97.3	72.3
Specificity	100.0	100.0	100.0
Positive Predictive Value (PPV)	100.0	100.0	100.0
Negative Predictive Value (NPV)	63.7	75.6	64.9

DISCUSSION

This analysis provides an overview of demographic, diagnostic, and inflammatory markers in symptomatic SARS-CoV-2 infection within a large Punjab cohort. Symptomatic subjects exhibited higher viral detection, antibody prevalence, and inflammatory markers than asymptomatic individuals. Participant characteristics revealed a significant association between gender and symptomatic status ($p < 0.0001$), with males comprising 63.8% of symptomatic versus 45.9% asymptomatic cases, aligning with global COVID-19 severity disparities[9]. This likely reflects inherent immunological advantages in females, including X-chromosome-mediated immune gene expression, estrogen-driven antiviral responses, and reduced cardiometabolic comorbidities[9].

Among 1,500 participants, 66.1% tested PCR-positive, with significantly higher positivity in symptomatic (92.3%) versus asymptomatic individuals (38.8%; $X^2 = 476.08$, $p < 0.0001$). This aligns with evidence of prolonged viral shedding in symptomatic cases, though asymptomatic cases remain transmissible[10,11].

The rapid antigen test (RAT) shows a markedly higher positivity rate in symptomatic individuals (89.8%) than in asymptomatic (4.0%) groups in this study. These findings are consistent with prior studies. Symptomatic patients tend to have a higher viral load, especially in the early phases of the disease when symptoms appear. This elevated viral load increases the likelihood of RAT, which targets viral proteins; this is consistent with multiple studies reporting RAT sensitivity up to 90% in symptomatic subjects[12,13]. RATs are less sensitive than RT-PCR tests, particularly in populations with low viral loads, such as asymptomatic cases. Even among asymptomatic individuals, RAT sensitivity varies by timing and viral load; for example, the RAT shows sensitivity of ~80-90% sensitivity in presymptomatic/early asymptomatic phases but much lower in other phases[14]. However, the lower sensitivity in asymptomatic individuals requires cautious interpretation and may warrant confirmatory RT-PCR testing, particularly for negative RAT results in asymptomatic contacts or in screening programs[15].

The significantly higher frequency of IgG antibodies in symptomatic participants (82.2%) compared to asymptomatic ones (59.7%) suggests a more robust or detectable adaptive immune response following clinically apparent infection. Most symptomatic patients develop strong neutralizing and anti-SARS-CoV-2 IgG antibody responses, often with higher titers than asymptomatic or mild cases. For example, pneumonia patients showed 100% neutralizing antibody production, mild symptoms in about 93.9%, but only about 80% in asymptomatic groups, with lower antibody titers in asymptomatic cases[16]. Some studies found that asymptomatic patients have a faster but sometimes less durable spike-directed IgG response than mildly symptomatic cases[17,18]. Longitudinal studies show antibody responses, including IgG, persist for months in both groups but are usually stronger and sustained longer in symptomatic patients[19]. Some population studies found exceptions with higher antibody positivity in asymptomatic individuals in certain age groups, reflecting heterogeneity in immune responses across different demographics and severities[20]. These findings emphasize that symptomatic COVID-19 cases tend to trigger a stronger, more easily detectable humoral immune response, whereas asymptomatic infections induce a variable, generally lower-magnitude IgG response, yet still contribute to immunity and epidemiological dynamics.

The study data show a statistically significant elevation of inflammatory and coagulation biomarkers (LDH, CRP, D-dimer, and IL-6) in symptomatic COVID-19 patients compared to asymptomatic patients. This aligns well with extensive evidence in the literature on COVID-19 pathophysiology and severity markers. Elevated LDH levels correlate with tissue damage and are commonly higher in symptomatic and severe COVID-19 cases, reflecting cellular injury in the lungs and other organs. Higher median LDH in symptomatic (roughly double

that of the asymptomatic individuals) is consistent with disease-related tissue damage seen in the literature[21,22]. CRP is a widely recognized acute-phase inflammatory marker strongly associated with COVID-19 severity. The observed apparent differences (29 mg/L vs. 0.81 mg/L) in this study are typical of this inflammatory response gradient. Multiple studies report substantially elevated CRP levels in symptomatic and severe cases compared with asymptomatic or mild infections[21,23,24]. D-dimer indicates coagulation activation and fibrinolysis; elevated levels predict worse prognosis and more severe disease. Studies show significantly higher D-dimer levels in symptomatic and hospitalized patients than in asymptomatic individuals, reflecting COVID-19-associated coagulopathy and increased thrombosis risk[21,22]. IL-6 is a central cytokine in the COVID-19 cytokine storm and correlates with poor outcomes. Median levels, as seen in our study (i.e., 50 pg/mL in symptomatic versus 5 pg/mL in asymptomatic), mirror this pronounced inflammatory activation. It is frequently reported as markedly elevated in symptomatic and severe patients versus asymptomatic ones[21,22,24]. Collectively, these findings highlight distinct inflammatory and prothrombotic profiles between symptomatic and asymptomatic groups, substantiating the role of these biomarkers in predicting disease progression and informing clinical management.

The comparative analysis of COVID-19 clinical and laboratory features among patients from Lahore, Faisalabad, and Sargodha revealed no statistically significant differences in key parameters, including the prevalence of symptoms, RT-PCR positivity, RAT positivity, and IgG seropositivity. This uniformity suggests a broadly consistent epidemiological and immunological pattern of COVID-19 infection across these major cities of Punjab. Similarly, inflammatory and biochemical biomarkers, including LDH, D-dimer, CRP, and IL-6, did not show significant variation among these regions. These findings imply comparable disease processes and immune-inflammatory responses regardless of geographic location within the province. For inflammatory and coagulation biomarkers such as LDH, CRP, D-dimer, and IL-6, systematic reviews and cohort studies report these markers are consistently elevated in symptomatic and severe COVID-19 patients[25-27]. The similarity in symptom proportions and PCR/RAT positivity across cities in Punjab aligns with the finding that geographic differences may be secondary to other patient-level factors[25,27].

Our comparative analysis revealed that healthcare workers had a significantly higher SARS-CoV-2 PCR positivity rate than the general population, reinforcing their classification as a high-risk group for COVID-19 infection due to occupational exposure[28,29]. Despite similar proportions of symptomatic individuals in both groups, healthcare workers exhibited significantly elevated levels of inflammatory biomarkers LDH, CRP, and IL-6, suggesting a more pronounced systemic inflammatory response[30,31]. These findings may be attributable to greater viral exposure among healthcare workers, potentially resulting in higher viral loads or repeated antigenic stimulation that amplifies immune activation[32,33]. Our results align with existing literature underscoring the vulnerability of healthcare workers to SARS-CoV-2 infection and highlight the need for continued protective measures and immunological monitoring within this key occupational population[28-30].

CONCLUSION

This extensive cohort study from Punjab, Pakistan, provides critical insights and offers valuable lessons for future respiratory pandemics. Key findings demonstrate that rapid antigen tests (RATs) exhibit markedly high positivity (89.8%) and utility in symptomatic individuals. This underscores the primary role of RATs in early symptomatic case detection while highlighting the necessity for confirmatory PCR testing in asymptomatic screening contexts due to the risk of false negatives. Furthermore, symptomatic infection was characterized by a significantly more robust humoral immune response and a pronounced elevation of key inflammatory and coagulation biomarkers (LDH, CRP, D-dimer, IL-6) compared with asymptomatic infection. Collectively, these findings advocate for tailored public health strategies in future pandemics: prioritizing RATs for symptomatic individuals while recognizing their limitations for controlling asymptomatic spread, using inflammatory markers for prognosis, and implementing enhanced protective measures for high-risk occupational groups such as healthcare workers.

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AUTHOR CONTRIBUTION

Mustafa AU: Conceptualization, methodology, investigation, data curation, formal analysis, writing original draft.
Rasool MH: Conceptualization, supervision, validation, interpretation, writing, review and editing.
Aslam B: Methodology, resources, critical revision of the manuscript, writing, review and editing.
Shafique M: Data curation, formal analysis, visualization, writing, review and editing.
All authors read and approved the final version of the manuscript.

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