Evaluation of Urine L-FABP Point of Care Kit in the Philippines as Predictive Marker of Clinical Severity of COVID-19 (EPOCH COVID study)

Alberto Chua, MD,[1] Lynn Gomez, MD,[2] Rontgene Solante, MD,[3] Kay Beltran, MD,[1] Eric Chua, MD,[4] Renz Pasilan, MD,[2] Erika Chan, MD,[1] Nica Lee, MD,[4] Ann Celestyn Uichanco, MD,[3] Micaela San Diego, MD,[4] and Abegail Danasen, MD[4]

Abstract

Background: The search for simple clinical and laboratory markers to help predict the clinical severity of patients presenting with COVID-19 has prompted this study to look at the predictive value of urine L-FABP (Liver Type-Fatty Acid Binding Protein) point-of-care test kit at the initial presentation of COVID-19 patients to the hospital.

Methods: The validation study prospectively included 109 consecutive patients with mild to moderate COVID-19, mean age of 52.2 years (range 19-84) presenting at the Emergency Rooms of 4 participating Metro-Manila hospitals from February to April 2021, with available data for analysis for 103 patients. Urine L-FABP POC (Point-of-Care) test and other clinical parameters and the level of severity of COVID-19 were determined at Day 0, Day 4 and Day 7. Computations for Sensitivity, Specificity, Positive and Negative Predictive values and Likelihood ratios were performed

Results: Twenty-three patients tested positive for urine L-FABP, out of the 103 patients analyzed, while 80 tested negative. Of the 23 patients who tested positive for urine L-FABP, 6 has progressed in severity, while 17 did not progressed. Of the 80 patients who tested negative for urine L-FABP, 13 progressed, while 67 did not progressed in severity. Giving a Sensitivity of 31.58%, Specificity of 79.76%, Positive predictive value of 26.09%, Negative predictive value of 83.75%. Combining urine L-FABP and initial clinical parameters like SIRS (Systemic Inflammatory Response Syndrome) criteria to predict progression of severity yielded a higher Specificity of 91.67 % and Negative Predictive value of 84.62%.

Conclusions: The study shows the utility of initial urine L-FABP POC test as a negative screening test in triaging adult patients presenting to the ER with mild to moderate COVID-19. Patients at the ER with a negative urine L-FABP test, will most likely not progressed to severe COVID-19. Combining clinical parameters like SIRS Criteria with the urine L-FABP result can increase the negative predictive value.

Keywords: COVID-19, Urine L-FABP POC (Point-of-Care) test, SIRS Criteria

Introduction

COVID-19, was declared as a global pandemic on March 11, 2020 by the World Health Organization.[1] Although most of the infected individuals exhibit mild illness (>80%), 14% of COVID patients present with serious illness while 5% have critical illness. Approximately 10% will require hospital admission due to pneumonia, of which, approximately 10% will require ICU care. The older population and those with comorbidities like hypertension, diabetes, chronic lung disease, cardiovascular disease have a higher risk of mortality once afflicted. However, the young population also appear to have a risk for developing critical illness and death according to a study by Cunningham.[2]

There have been several researches describing several patient factors, including demographics, clinical, immunologic, biochemical and radiographic findings, that may be useful to the clinicians to predict clinical course and outcome of COVID 19 patients. Blood and urine are frequent biometrics for discovery of biomarkers of human diseases because of their accessibility and noninvasiveness. According to the study of Tjendra et al., hematologic parameters including lymphopenia, leukocytosis with increased neutrophil count, increased neutrophil to lymphocyte ratio (NLR), and

[1] University of the East Ramon Magsaysay Memorial Medical Center
[2] University of the Philippines, Philippine General Hospital
[3] San Lazaro Hospital
[4] Chinese General Hospital and Medical Center
Corresponding Author: Alberto Chua, MD eMail: albertchuamd@yahoo.com.ph

thrombocytopenia were the most common findings positively correlated with the disease severity.[3] Urine chemical tests have the characteristics of being quick, convenient and economical. A study of Liu et al, compared the biochemical properties of urine samples from healthy individuals and patients with moderate, severe, and critical COVID patients.[4] The study states that urine pH, specific gravity, protein, and blood can be indicators for the auxiliary differentiation of COVID-19 patients from healthy individuals.

Urine biomarkers are derived from the injured kidney and reflect a molecular process intimately connected with tissue injury (5). These biomarkers detect renal injury at an earlier stage than conventional tests like creatinine and blood urea nitrogen and they can detect subclinical kidney injury. Liver-type fatty acid-binding protein (LFABP) is a protein expressed in the liver, intestine, stomach, lungs and kidneys. In the kidneys, it is predominantly, located in the proximal tubules and is excreted into the tubular lumen due to renal tubular ischemia and oxidative stress on renal tubules. L-FABP is used clinically to detect acute kidney inquiry more accurately than serum creatinine, but can also be used to predict severity and mortality in ICUs. Urine L-FABP as a predictor of mortality in adult patients with sepsis showed a high AUC-ROC of 0.993.[6]

A study by Katagiri et al, showed that L-FABP levels were significantly higher among patients with severe COVID-19, even if they did not satisfy the AKI criteria of KDIGO.[7] They stated that a higher urine L-FABP within 10 days of onset indicates a high risk of developing severe disease.

The urine L-FABP point-of-care kit is FDA approved and commercially available, and is a simple diagnostic test for early detection of Acute Kidney injury. For this study, RENISCHEM® L-FABP POC Kit, which is an immunochromatographic test for detection and semi-quantitative determination of L-FABP was used. Urinary L-FABP levels can be visualized within 15 minutes after the urine specimen is added to the sample pad, it allows for semi-quantitative measurement by comparing the color density of Test Line with that on reference card which will be recorded as Negative, +1 or +2.

General Objective. To determine whether testing for urine LFABP levels using the Point-of Care kit during the initial presentation of Covid-19 patients can help predict the progression of disease severity

Specific Objective. To determine the Sensitivity, Specificity, Positive and Negative Predictive values and Likelihood ratios of urine LFABP test result in predicting progression of disease severity in Covid-19 patients

Methodology

Study Design and Subjects. This is a prospective, multicenter study that included 111 adult patients tagged as COVID-19 suspect/probable/positive based on existing local guidelines, who presented at the emergency rooms of the four participating tertiary hospitals: Chinese General Hospital and Medical Center (CGHMC), UP-Philippine General Hospital (UP-PGH), University of the East Ramon Magsaysay Memorial Medical Center, Incorporated (UERMMMCI), and San Lazaro Hospital (SLH). Two patients were excluded; one due to a negative RT-PCR test for COVID-19 and the other one was transferred to another health care facility.

Inclusion criteria. Ability to consent, Age >18 years old (both sexes), Mild to Moderate COVID-19 infection Confirmed by RT-PCR, and confined in the hospital

Exclusion criteria. Pregnancy, eGFR < 30ml/min (based on initial serum Creatinine and using CKD-EPI equation), patients clinically classified as severe COVID-19 at presentation (requiring mechanical ventilation or ICU care), anuric patients (urine output less than 150 ml/day), and patients who cannot adequately provide consent (e.g., cognitively impaired patients)

Sample Size Computation. 103 subjects were needed for this study. This is based on the sensitivity and specificity values of LFABP from the study by Katagiri et al. (2020), a 7% margin of error, 80% power, and a 5% level of significance. The sample size is computed using the following formula for diagnostic studies:

$$n = \frac{Z^2_{\frac{\alpha}{2}} \hat{P}(1 \quad \hat{P})}{d^2}$$

α: level of significance
P: sensitivity/specificity
d: margin of error

Data Collection: Demographic and clinical data, including vital signs, oxygen saturation, respiratory support and laboratory test results were obtained. Urinalysis, serum creatinine and Urine L-FABP using Point of Care (POC) kit were done on the initial day of consult.

Clinical classification of disease severity in the study by Katagiri et al, was used to classify patients as:

- Mild - does not require oxygen supplementation
- Moderate - requiring oxygen supplementation but not mechanical ventilation
- Severe - requiring ICU or mechanical ventilation

Patients were managed by their attending physicians or the residents in charge. Clinical course was monitored over one week by bedside visits or chart reviews. Repeat tests for urine L-FABP and serum creatinine were done on Day 4 and Day 7.

The following information were collected during the study:

1 Background of patient:
   a. Demographic information: age, birthday, gender
   b. Clinical history, onset of symptoms and past medical history
2 Physical finding
   a. Vital signs: body temperature, blood pressure, pulse rate, respiratory rate
   b. Degree of oxygen saturation, need for oxygen and respiratory support
3 Laboratory examinations:
   a. Urinalysis at Day 0 (Day of Admission)

b. Urine L-FABP test at Day 0, Day 4 and Day 7
c. Serum creatinine, eGFR using the CKD-EPI equation at Day 0, Day 4 and Day 7

4 Other available laboratory results like RT-PCR test for Covid, CBC platelet count and Chest Xray

5 Clinical status of the patient on Day 0, Day 4 and Day 7

The need for oxygen supplementation, use of mechanical ventilators and need for ICU care were noted. Any progression of clinical status from Mild to Moderate, Mild to Severe, or Moderate to Severe were noted.

The rating of the clinical severity was performed by the co-investigators from each Institution based on objective parameters like oxygen supplementation, use of ventilators, and/or need for ICU care.

Statistical Analysis. The Sensitivity, Specificity, Positive and Negative Predictive Values, Positive and Negative Likelihood Ratios of Urine L-FABP on predicting progression of severity of COVID-19 infection were computed by principal investigators. Sensitivity, Specificity, Positive and Negative Predictive Values, Positive and Negative Likelihood ratios of urine L-FABP on predicting the development of Acute Kidney Injury were computed.

Urine l-FABP Positive were reported as +1 and +2 results.

The primary outcome of interest was the progression of disease severity, which includes progression from Mild to Moderate, Mild to Severe and Moderate to Severe.

Positive predictive value (PPV) is the probability of the disease in a person who has a positive test result. Therefore, PPV relates to the predictive ability of a test to identify disease in individuals with positive results. Negative predictive value (NPV) defines the probability of the absence of the disease in a person who has a negative test result. Therefore, NPV relates to the predictive ability of a test to identify the absence of the disease in individuals with negative test results.

Likelihood ratio of a positive test (LR+) result tells us how many times it is more likely to observe a positive test result in a diseased than in a healthy individual. Likelihood ratio of a negative test result (LR-) tells us how many times less likely it is to observe a negative test result in a diseased than in a healthy individual.

Specimen Handling. The urine L-FABP POC urine test was performed by co-investigators, after undergoing a training session conducted by ULIV laboratory technician.

The urine samples were discarded according to standard laboratory protocol of each hospital. No urine specimen was stored. Blood and urine tests and radiographic tests were done in the hospital where patient was admitted.

Research Ethics Board Approval. The study was submitted to and approved by the Research Ethics Board of Chinese General Hospital, UERM Hospital and the Single Joint Research Ethics Board for UP-PGH and San Lazaro Hospital.

Results

There was a total of 111 patients recruited from the 4 participating hospitals. Two patients were excluded, one due to a negative Rt-PCR test for COVID-19 and one was transferred to another health care facility. The STARD Flowchart of participants is shown in *Figure 1*.

From the remaining 109 patients who were eligible based on the inclusion and exclusion criteria, 6 patients were discharged before Day 4 with no outcome data and thus considered not evaluable and not included in the data analysis. The baseline characteristics of the 109 patients are in *Table I*.

The distribution of participants based on clinical severity over time is in *Table II*. Among the 103 evaluable patients included in the analysis, 2 patients died within the 7 days follow-up period, one with positive and the other with negative urine L-FABP test.

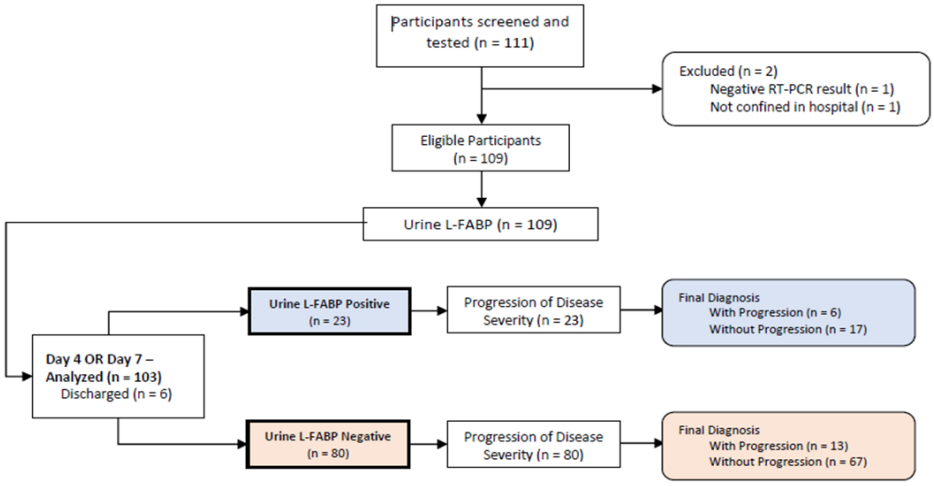

Figure 1. STARD Flowchart of Participants based on the Result of Urine L-FABP and the Assessment of Disease Severity at Day 4 or Day 7

Table I. Baseline characteristics of the 109 patients

Characteristic	Mean (Range) or Percentage
Age	52.22 years (19 - 84)
Male	54.13%
With Co-morbidities	71.56% (37.6% with > 1 co-morbidity)
Hypertension	61.54%
DM	43.59%
Respiratory disease	15.38%
Cardiovascular disease	12.82%
Clinical Manifestations	85.32% with multiple manifestations
Cough	77.98% (mean 5.58 days)
Fever	60.55% (mean 5.97 days)
Difficulty of breathing	38.53 % (mean 3.36 days)
Body weakness	31.19 % (mean 5.44 days)
Systolic Blood Pressure	124.93 mmHg (90 to 170)
Diastolic Blood Pressure	77.67 mmHg (58 to 105)
Temperature	36.69°C (35.3 to 39.1)
Heart Rate	93.83 bpm (60 to 122)
Respiratory Rate	22.7 cpm (16 to 48)
Abnormal Chest Xray	65.14%
Hemoglobin	138.41 gm/L (105 to 195)
White blood cell count	7.56 (2.2 to 36)
Neutrophils	70.41 % (20 to 93)
Lymphocytes	22.34 % (3 to 77)
Platelet count	229.81 (42 to 595)
Proteinuria on urinalysis	51.38%
Microscopic hematuria	16.51%
Pyuria	12.84%
Serum creatinine	71.95 mmol/L (33 to 158)
eGFR	94.71 ml/min (37.1 to 139)

Table II. Distribution of the Participants based on Clinical Severity of Covid-19 over Time

Disease Severity	Day 0 (n = 109)	Day 4 (n = 103)	Day 7 (n = 89)
Mild, n (%)	55 (50.46%)	54 (52.43%)	57 (64.04%)
Moderate, n (%)	54 (49.54%)	41 (39.81%)	25 (28.09%)
Severe, n (%)	0 (0%)	8 (7.77%)	7 (7.87%)

Table III. Urine L-FABP result on presentation vs progression of severity at Days 4 or 7

	Progression of Clinical Severity Day 4 or Day 7	
Urine L-FABP	Progression	No Progression
Positive	6	17
Negative	13	67

Table IV. Computed Sensitivity, Specificity, Positive and Negative Predictive values and Likelihood ratios for Urine L-FABP in predicting progression of disease severity in COVID-19 patients

PARAMETER	VALUE (95% CI)
Sensitivity	31.58% (10.68% to 52.48%)
Specificity	79.76% (71.17% to 88.35%)
Positive Predictive Value	26.09% (8.14% to 44.03%)
Negative Predictive Value	83.75% (75.67% to 91.83%)
Positive Likelihood Ratio	1.56 (0.71 to 3.43)
Negative Likelihood Ratio	0.86 (0.62 to 1.19)

Table V. Combination of Urine L-FABP result and SIRS criteria on presentation vs Progression of Severity at Day 4 or Day 7

	Progression of Clinical Severity Day 4 or Day 7	
Urine L-FABP and SIRS	Progression	No Progression
Positive	5	7
Negative	14	77

Table VI. Computed Sensitivity, Specificity, Positive and negative Predictive values and Likelihood ratios for the combination of Urine L-FABP and SIRS criteria in predicting progression of disease severity in COVID-19 patients

PARAMETER	VALUE (95% CI)
Sensitivity	26.32% (6.52% to 46.12%)
Specificity	91.67% (85.76% to 97.58%)
Positive Predictive Value	41.67% (13.77% to 69.56%)
Negative Predictive Value	84.62% (77.2% to 92.03%)
Positive Likelihood Ratio	3.16 (1.12 to 8.88)
Negative Likelihood Ratio	0.8 (0.61 to 1.06)

Twenty-three patients tested positive for urine L-FABP, out of the 103 patients analyzed, while 80 tested negative. Of the 23 patients who tested positive for urine L-FABP, 6 has progressed in severity, while 17 did not progressed. Of the 80 patients who tested negative for urine L-FABP, 13 progressed, while 67 did not progress in severity.

Table III shows the urine L-FABP test results and the progression of clinical severity on Days 4 or 7.

The result of the study showed that the initial urine L-FABP Point-of-care test in mild to moderate COVID-19 patients has a specificity of 79.76% and a NPV of 83.75% (*Table IV*) in predicting progression of disease severity. To improve the predictive value of the urine L-FABP POC test, the use of SIRS (Systemic Inflammatory Response Syndrome) clinical criteria was later added to the analysis. SIRS is present if 2 out of the following 4 criteria are present: Heart rate > 90/min; Respiratory rate > 20/min; Body Temperature >38°C or <36°C; WBC count > 12,000 or < 4000. *Table V* shows the combination of SIRS criteria and urine L-FABP test vs the progression of clinical severity in Day 4 or Day 7 outcome for the 103 patients.

Using the combination of urine L-FABP and initial clinical parameters like SIRS criteria to predict progression of severity yielded a higher specificity of 91.67 % and NPV of 84.62% (*Table VI*).

The results of urine L-FABP on Day 4 and Day 7 did not correlate with the clinical severity and no further analysis were made. Only one test subject developed Acute Kidney Injury (AKI) thus the predictive value of urine L-FABP for AKI was not analyzed.

Discussion

The COVID-19 pandemic has shown the vulnerability of the healthcare system and its finite resources to cope with the great influx of patients in a pandemic. It has become critical for a triage system to be able to predict who are the patients who will most likely progress to more severe COVID-19 and thus have to be confined in a hospital setting, and who are the patients who will most likely not progress in severity and can be observed in a community care quarantine facility.[8]

Holten et al., in their study of various clinical parameters at the emergency room setting among COVID-19 patients found that the AUROC (Area under the receiver operating characteristic curve) of the different scoring systems in predicting severe COVID-19 are: qSOFA (Quick Sequential Failure Assessment) = 0.70; SIRS = 0.70; CURB-65 = 0.75; PSI (Pneumonia Severity Index) = 0.75; and NEWS2 (National Early Warning Score 2) = 0.80.[9]

Katagiri et al., in Japan, studied urine biomarkers like L-FABP and Beta-2 microglobulin among COVID-19 patients and found that higher levels of these biomarkers may allow recognition of patients likely to become critically ill and requiring careful observation and early intervention.[5] They were able to show a specificity of 84.6% for Urine L-FABP in predicting severity of COVID-19, which is consistent with this study results showing specificity of 79.76% and the NPV of 83.75%.

During the analysis of the data for this study, we looked into the potential utility of combining the presence of clinical parameter like SIRS criteria and the urine L-FABP point-of-care test, to see if we could further increase the predictive value. The combination of clinical parameters like SIRS criteria and the urine L-FABP point-of-care test during the initial presentation, was used to predict progression in severity of COVID-19 with specificity of 91.67 % (CI 85.76% to 97.58%) and NPV of 84.62 % (95% CI 77.2% to 92.03%) with narrow confidence intervals for the combination.

To address potential source of bias, the co-investigators from each institution underwent a training session on the use of the urine L-FABP point-of-care test kit.

Limitations of the study includes the relative few numbers of patients that progressed in severity, with only one patient developing AKI. We also did not analyze other inflammatory markers like Ferritin, Hs-CRP, and D-dimer. The urine L-FABP test was not quantitative and not performed by a centralized laboratory and the co-investigators were also not blinded to the result of the Urine L-FABP test.

Progression of the disease severity may be missed after the study period (7 days) since there was a short duration of patient follow up. The strength of the study is its simplicity and applicability in the actual clinical setting, especially in triaging mild to moderate COVID-19 patients.

Conclusion

A negative urine L-FABP and absence of SIRS criteria at presentation in a mild to moderate COVID-19 patient may help predict that the patient will most likely not progress in clinical severity over the next 4 - 7 days. This is useful in triaging such patients to community care quarantine facility or to home care and has the potential for better hospital resource allocation.

Conflict of Interest: This research is funded by the government of Japan through its National Center for Global Medicine. The Clinical investigators and Research Assistants received honoraria for their work and participation. The LFABP point-of-care test kits were provided by the Japanese manufacturer thru its local distributor ULIV, a division of Unilab®.

Acknowledgement: Dr. Eisei Noiri – Director General of National Center Biobank Network at National Center for Global Health and Medicine (NCGM), Tokyo, Japan and Professor Takeshi Sugaya – CMIC, Japan

References

1 World Health Organization. WHO Coronavirus (COVID-19) Dashboard. Retrieved from https://covid19.who.int/

2 Cunningham JW, Vanuganathan M, Clagett BL, *et al.* Clinical Outcomes in Young US Adults Hospitalized with Covid-19. *JAMA Internal Medicine.*2021;181(3):379-381. doi:10.1001/jamainternmed.2020.5313

3 Tjendra Y, Al Mana AF, Espejo AP, et al. Predicting Disease Severity and Outcome in COVID-19 Patients: A Review of Multiple Biomarkers [published online ahead of print, 2020 Aug 20]. *Arch Pathol Lab Med*. 2020;10.5858/arpa.2020-0471-SA. doi:10.5858/arpa.2020-0471-SA

4 Liu, R., Ma, Q., Han, H., Su, H., Liu, F., Wu, K., Wang, W., & Zhu, C. (2020). The Value of Urine Biochemical Parameters in the Prediction of the Severity of Coronavirus Disease 2019, *Clinical Chemistry and Laboratory Medicine (CCLM)*, *58*(7), 1121-1124. doi: https://doi.org/10.1515/cclm-2020-0220

5 Schrezenmeier EV, Barasch J, Budde K, Westhoff T, Schmidt-Ott KM. Biomarkers in Acute Kidney Injury - Pathophysiological Basis and Clinical Performance. *Acta Physiol (Oxf)*. 2017 ;219(3):554-572. doi :10.1111/apha.12764

6 Sato E, Kamijo-Ikemori A, Oikawa T, Okuda A, Sugaya T, *et al.* Urinary Excretion of Liver-type Fatty Acid-Binding Protein Reflects the Severity of Sepsis. *Renal Replacement Therapy*. 2017; 3:26. doi:10.1186/s41100-017-0107-x

7 Katagiri D, Ishikane M, Asai Y, *et al*. Evaluation of Coronavirus Disease 2019 Severity Using Urine Biomarkers. *Crit Care Explor*. 2020;2(8): e0170. Published 2020 Jul 31. doi:10.1097/CCE.0000000000000170

8 Department of Health. Algorithm for COVID-19 Referral and Triage. Interim Management Guidance for COVID-19 Version 3.1 as of July 20, 2020

9 Holten A, Nore K, et al. Predicting Severe COVIC-19 in the Emergency Department. *Resuscitation Plus* 4 (2 0 2 0) 1 0 0 0 4 2. doi.org/10.1016/j.resplu.2020.100042