First Experience Addressing the Prognostic Utility of Novel Urinary Biomarkers in Patients with COVID-19

Udaya S. Tantry, Kevin P. Bliden, Alastair Cho, Naval Walia, Jeffrey R. Dahlen, Gordon Ens, Malina Traianova, Christophe Jerjian, Abira Usman, Paul A. Gurbel

1 Sinai Center for Thrombosis Research and Drug Development, Sinai Hospital of Baltimore, Lifebridge Health, Baltimore, MD, USA

2 Hikari Dx, Inc, San Diego, CA, USA

3 Inflammatory Markers Laboratory, Wichita, KS, USA

4 CEDEX Laboratory, Nashua, NH, USA

Send Correspondence to:
Paul A. Gurbel, M.D.
Director, Sinai Center for Thrombosis Research and Drug Development
Sinai Hospital of Baltimore, Baltimore, MD, USA, 21215
Phone: 410-367-2590; Fax: 410-367-2596
E-mail: pgurbel@lifebridgehealth.org

© The Author(s) 2021. Published by Oxford University Press on behalf of Infectious Diseases Society of America.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
ABSTRACT

Urine 11-dehydro-thromboxane B$_2$ (u11-dh-TxB$_2$), 8-hydroxy-2'-deoxyguanosine, and liver-type fatty acid binding protein levels (L-FABP) at the time of hospitalization were higher in COVID-19 patients with adverse events versus without events. Higher u11-dh-TxB$_2$ and L-FABP levels were associated with longer hospitalization, more thrombotic events, and greater mortality, providing evidence for potential utility as early prognostic biomarkers for COVID-19.

**Keywords:** COVID-19, urinary 11-dehydro-thromboxane B$_2$, urinary 8-hydroxy-2'-deoxyguanosine, liver-type fatty acid binding protein
INTRODUCTION
COVID-19, a global pandemic, can be associated with rapid and severe disease progression leading to death. Standard biomarkers of inflammation and hypercoagulability such as C-reactive protein, interleukin-6, procalcitonin, fibrinogen, and d-dimer are currently measured in blood samples as diagnostic and-prognostic markers (1). However, limited data exist on the use of urinary biomarkers and their relation to COVID-19 severity and outcomes. Early assessment of COVID-19 using urinary markers may facilitate rapid and effective intervention to prevent the progression of the disease to a severe state. Measurement of biomarkers in urine specimens may be advantageous compared to blood specimens due to the comparative ease of sample collection. Urine in some cases is also associated with improved analyte stability and reduced matrix-related assay interference.

METHODS
Patient Consent Statement
We report a sub-analysis of the evaluation of hemostasis in hospitalized COVID-19 patients (TARGET-COVID) study (URL: https://www.clinicaltrials.gov; Unique identifier: NCT04493307). The study was performed in accordance with standard ethical principles and the design of the study was approved by the local institutional review board of the LifeBridge Health. All patients provided written consent.

Patients and Laboratory Methods
We enrolled hospitalized patients who were diagnosed with COVID-19 by reverse transcription-polymerase chain reaction assay (n=123) and patients without COVID-19 but with pneumonia and an elevated D-dimer (COVID-19 negative, n=18). Blood and urine samples were collected within 48 hours of hospital admission and serial samples were collected on a subset of patients (n=22) at day 3 and day 5-8 from the baseline. Standard blood biomarkers were determined in the central hospital laboratory (Sinai Hospital, Baltimore, MD, USA). Urinary 11-dehydro- thromboxane B₂ (u11-dh-TxB₂) levels were determined using an enzyme-linked immunoassay and microalbumin levels were determined using the Dimension clinical chemistry system at Inflammatory Markers Laboratory (Wichita, KS) (2). Urinary 8-hydroxy-2’-deoxyguanosine (8-OHdG) levels were measured by enzyme-linked immunoassay at CEDx Labs (Nashua, NH, USA) and liver-type fatty acid binding protein (L-FABP) levels were determined using a rapid, point-of-care lateral flow
immunoassay (Timewell Medical, Tokyo, Japan) whose results were quantified using a CHR-631 Rapid Test Reader (Kaiwood Technology Co., Ltd., Tainan City, Taiwan) (3,4). In-hospital all-cause death, thrombotic events (venous thrombosis, pulmonary thromboembolism, myocardial infarction, and ischemic stroke), non-convalescent plasma transfusion and bleeding occurrences were recorded.

RESULTS
Sixty-seven percent of the patients were African Americans. There were no significant differences in age, sex, co-morbidities, Sequential Organ Failure Assessment Score (SOFA), or levels of urine biomarkers between COVID-19 positive and negative patients (p=ns for all measurements). Thirty-eight patients had an in-hospital clinical event including death (n=21), pulmonary embolism (n=6), stroke (n=3), and myocardial infarction (n=8). Compared to patients without events, patients with events were older (57±19 versus 67±14 years), had a higher prevalence of diabetes (40% versus 53%), higher SOFA score (2.3±1.7 versus 5.2±4.3), more days of hospitalization (9±6 versus 17±20), and higher d-dimer (1.9±2.7 versus 4.5±4.8 fibrinogen equivalent units), lactate dehydrogenase (402±281 versus 540±280), and procalcitonin levels (0.9±3.5 versus 3.6±11.8 ng/mL) (p<0.05 for all).

In our study, in patients who were on aspirin at the time of hospitalization, u11-dh TxB₂ levels were lower (p=0.001) compared to patients not on aspirin, but only 17% of patients had u11-dh TxB₂ levels lower than the cut-off value for aspirin therapeutic response (<1520 pg/mg creatinine) (2). At the time of hospitalization, u11-dh TxB₂ levels were higher in aspirin-treated patients with events versus without events (median [95CI%]), 7,603 [7,541 to 19,791] vs. 4,890 [5,049- to 8,290] pg/mg creatinine, respectively, p=0.002) (Table) and in non-aspirin treated patients with events versus without events (p=0.001) (Figure 1). In addition, u11-dh TxB₂ levels were higher in patients with prolonged (≥10 days) hospitalization (p=0.02), death (p=0.004), and mechanical ventilation requirement (p<0.001) (Table). Urinary 11-dh-TxB₂ levels were increased at day 3 (p=0.01) and returned to similar levels as baseline levels at day 5-8. In patients not treated with aspirin, there was a good correlation between u11-dh TxB₂ and d-dimer (AUC 0.60, p<0.001), but not between CRP or any other conventional markers tested.

Similarly, L-FABP levels were higher in patients with events versus without events, (9.9 [9.7 to 32.2] versus 3.9 [3.4 to 8.6] ng/mL, p=0.001); higher in patients with prolonged
hospitalization (p=0.05), death (p=0.03), and mechanical ventilation requirement (p=0.01) (Table). 8-OHdG levels were higher in patients with events versus without events (78 [78-227] versus 64 [53 to 116] ng/mg creatinine, p=0.04) (Table). Patients with L-FABP >10 ng/mL, an indicator of renal ischemia, compared to patients <10 ng/mL had greater u11-dh-TxB2 levels (13,070 [5,122 to 19,488] versus 5,203 [4,264 to 7,264] pg/mg creatinine, respectively p=0.02). Both 8-OHdG and L-FABP levels remained similar across all timepoints during hospitalization. Urinary microalbumin and urinary creatinine levels were numerically higher in patients with events (p=0.47 and p=0.45, respectively), whereas urinary creatinine levels were higher in patients with prolonged hospitalization (p=0.007) (Table).

**DISCUSSION**

Our results suggest the prognostic utility of 11-dh TxB2, L-FABP, and 8-OHdG measured in urine in patients with COVID-19 at the time of hospitalization. Urinary 11-dh TxB2 is a marker of whole-body inflammation and TxA2 biosynthesis contributed by platelets, leukocytes, and endothelial cells (5). The independent relation of urine u11-dh TxB2 to adverse outcomes in patients with cardiovascular disease and diabetes treated with aspirin has been demonstrated in major clinical trials (6-8). Among COVID-19 patients in our study, we found significantly elevated levels of u11-dh TxB2 in patients with events compared to patients without events and higher u11-dh TxB2 levels were associated with prolonged hospitalization, death, and mechanical ventilation requirement. These observations highlight the potential prognostic utility of u11-dh TxB2 in patients with COVID-19. This is in line with the observations of systemic cytokine storm, endothelial dysfunction, and elevated thrombotic-risk in COVID-19. In addition, it is also interesting to note that aspirin therapy at the time of hospitalization in most patients was pharmacodynamically inadequate to provide a therapeutic response based on measurements of u11-dh TxB2 (2).

L-FABP, expressed in proximal renal tubules, is excreted in urine following hypoxia and is a more accurate indicator of acute kidney injury than serum creatinine (9). L-FABP may assist in predicting the severity of COVID-19 at an early stage (10). Due to the high metabolic demand of the kidneys, combined with the sensitivity and specificity of urinary L-FABP for renal ischemia, it has been hypothesized that elevations of urinary L-FABP may be indicative
of renal ischemia associated with COVID-19. In our study, urine L-FABP levels were higher in COVID-19 patients with events. These observations indicate that patients with more severe COVID-19 have evidence of renal ischemia and that acute renal injury may be, in part, responsible for the adverse outcomes observed in patients with COVID-19. 8-OHdG is a proposed biomarker of oxidative damage of deoxyribonucleic acid (Figure 2). It has been shown to be associated with cardiovascular disease and endothelial dysfunction in patients with diabetes (11-13). Cytokine storm, and a higher prevalence of diabetes among patients with events appear linked to the higher levels of 8-OHdG observed in these patients.

These pilot data are hypothesis-generating, although this is the largest study to comprehensively study urine biomarkers in COVID-19. Nevertheless, the biomarkers we studied indicate that endothelial dysfunction and inflammation (11-dh TxB2), renal hypoxia and apoptosis (L-FABP), and oxidative stress (8-OHdG) are associated with the severity of the COVID-19. Most importantly, this study provides the first evidence for early prognostic information on COVID-19 severity, including death, using a simple, noninvasive urine sample. Evidence from larger studies are required to confirm the clinical validity and utility of these noninvasive markers.
References

1. Ishazli RM, Toraih EA, Elgaml A, et al. Diagnostic and prognostic value of hematological and immunological markers in COVID-19 infection: A meta-analysis of 6320 patients. *PLoS One*. 2020;15:e0238160.

2. Gurbel PA, Bliden KP, Tantry US. Defining platelet response to acetylsalicylic acid: the relation between inhibition of serum thromboxane B2 and agonist-induced platelet aggregation. *J Thromb Thrombolysis*. 2020 Nov 10. doi: 10.1007/s11239-020-02334-x. Epub ahead of print.

3. Endo K, Miyashita Y, Sasaki H, et al. Probucol and atorvastatin decrease urinary 8-hydroxy-2'-deoxyguanosine in patients with diabetes and hypercholesterolemia. *J Atheroscler Thromb*. 2006;13:68–75

4. Katagiri D, Doi K, Honda K, Negishi K, et al. Combination of two urinary biomarkers predicts acute kidney injury after adult cardiac surgery. *Ann Thorac Surg*. 2012;93:577-583.

5. Tantry US, Mahla E, Gurbel PA. Aspirin resistance. *Prog Cardiovasc Dis*. 2009;52:141-52.

6. Eikelboom JW, Hirsh J, Weitz JI, et al. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. *Circulation*. 2002;105:1650-5.

7. Eikelboom JW, Hankey GJ, Thom J, et al. Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management and Avoidance (CHARISMA) Investigators. Incomplete inhibition of thromboxane biosynthesis by acetylsalicylic acid: determinants and effect on cardiovascular risk. *Circulation*. 2008;118:1705-12.

8. B Rocca, G Buck, G Petrucci, et al. The ASCEND Study Collaborative Group, Thromboxane metabolite excretion is associated with serious vascular events in diabetes mellitus: a sub-study of the ASCEND trial, *Eur Heart J*, Volume 41, Issue Supplement-2, November 2020, ehaa946.2926.

9. Doi K, Negishi K, Ishizu T, et al. Evaluation of new acute kidney injury biomarkers in a mixed intensive care unit. *Crit Care Med* 2011;39:2464–2469.

10. Katagiri D, Ishikane M, Asai Y, et al. Evaluation of Coronavirus Disease 2019 Severity Using Urine Biomarkers. *Crit Care Explor*. 2020;2:e0170.

11. Di Minno A, Turnu L, Porro B, et al. 8-Hydroxy-2-deoxyguanosine levels and cardiovascular disease: a systematic review and meta-analysis of the literature. *Antioxid Redox Signal*. 2016;24:548–55.

12. Valavanidis A, Vlachogianni T, Fiotakis C, et al. “8-Hydroxy2’-deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis* J Environ Sci Health C Environ Carcinog Ecotoxicol Rev*. 2009;27:120-39.

13. Bigagli E, Lodovici M. Circulating Oxidative Stress Biomarkers in Clinical Studies on Type 2 diabetes and Its Complications. *Oxid Med Cell Longev*. 2019;2019:5953685.
SOURCE OF FUNDING

Platelet and Thrombosis Research, LLC, Baltimore, MD, USA.

POTENTIAL CONFLICT OF INTEREST

Dr. Gurbel reports grants and personal fees from Bayer HealthCare LLC, Otitopic Inc, Amgen, Janssen, and US WorldMeds LLC; grants from Instrumentation Laboratory, Haemonetics, Medicure Inc, Idorsia Pharmaceuticals, and Hikari Dx; personal fees from UpToDate; Dr Gurbel is a relator and expert witness in litigation involving clopidogrel; in addition, Dr. Gurbel has two patents, Detection of restenosis risk in patients issued and Assessment of cardiac health and thrombotic risk in a patient.

Dr. Tantry reports receiving honoraria from UptoDate and Aggredyne.

Other author reports no disclosures.

AUTHOR CONTRIBUTIONS

Udaya Tantry, Kevin Bliden, Malina Traianova, and Jeffrey Dahlen performed experiments. Naval Walia, Christophe Jerjian, Alastair Cho, Kevin Bliden and Abira Usman enrolled patients and collected patient information. Alastair Cho, Gordon End, Kevin Bliden and Udaya Tantry performed data Analysis. Udaya S Tantry Kevin Bliden and Paul Gurbel wrote the manuscript. Paul A Gurbel supervised the study.

All authors contributed to the final manuscript.
Figure Legends

**Figure 1.** Urinary 11-dehydro Thromboxane B\(_2\) in Aspirin and Non-Aspirin Treated Patients with and without Events

**Figure 2.** Mechanistic pathways of urinary biomarkers and relation to COVID-19

COVID-19 is associated with a hypercoagulable state and marked systemic inflammation with resultant tissue ischemia and necrosis. Early elevation in markers of oxidative stress (8-OHdG), proximal glomerular tubular ischemia (L-FABP), and platelet activation/heightened inflammation (11-dehydro TxB\(_2\)) found in urine may facilitate assessment of risk.
|                                | n  | Median (95% CI) | n  | Median (95% CI) | p-value |
|--------------------------------|----|----------------|----|----------------|---------|
| **Without Events**             |    |                |    |                |         |
| L-FABP (ng/mL)                 | 67 | 3.9 (3.4 to 8.6) | 24 | 9.9 (9.7 to 32.2) | <0.001  |
| 11-dh TxB2 (pg/mg creatinine)  | 47 | 4,890 (5,049 to 8,290) | 18 | 7,603 (7,541 to 19,791) | 0.002   |
| 8-OHdG (ng/mg creatinine)      | 37 | 64 (67 to 116)     | 15 | 78 (78-227)      | 0.04    |
| Microalbumin (mg/L)            | 36 | 22 (15 to 58)      | 9  | 66 (24 to 283)   | 0.47    |
| Creatinine (mg/dL)             | 95 | 0.8 (0.7 to 0.9)   | 38 | 0.99 (0.8 to 1.4) | 0.45    |
| **With Events**                |    |                |    |                |         |
| Creatinine (mg/dl)             | 57 | 0.79 (0.76 to 0.99) | 54 | 0.91 (1.09 to 2.3) | 0.007   |
| 11-dh TxB2 (pg/mg creatinine)  | 35 | 4,801 (3,817 to 9,196) | 30 | 8,614 (7,990 to 14,316) | 0.02    |
| L-FABP (ng/mL)                 | 57 | 4.4 (3.8 to 10.4)  | 34 | 6.2 (3.9 to 14.1) | 0.05    |
| **<10 days of Hospitalization**|    |                |    |                |         |
| 11-dh TxB2 (pg/mg creatinine)  | 48 | 5,360 (5,907 to 10,038) | 6  | 15,069 (1,915 to 42,007) | 0.004   |
| L-FABP (ng/mL)                 | 80 | 4.7 (3.7 to 5.3)   | 11 | 10.6 (4.1 to 32.6) | 0.03    |
| **≥10 days of Hospitalization**|    |                |    |                |         |
| 11-dh TxB2 (pg/mg creatinine)  | 56 | 5,137 (4,498 to 7,512) | 9  | 20,121 (5,364 to 41,015) | <0.001  |
| L-FABP (ng/mL)                 | 82 | 4.6 (3.6 to 5.2)   | 9  | 5.4 (5.2 to 43.4)  | 0.01    |

*Includes patients not on aspirin therapy

L-FABP = liver fatty acid binding protein, 11-dh TxB2 = 11-dehydro thromboxane B2, 8-OHdG = 8-hydroxy-2’ -deoxyguanosine
Figure 2

SARS-CoV-2

ACE2 (lungs, heart, and kidneys)

COVID induced Coagulopathy
(†fibrinogen, d-dimer, hypercoagulability)

Pro-Inflammatory Cytokine Release

Systemic Inflammation
(TII-6, CRP, WBC procalcitonin)

Monocyte/Neutrophil Infiltration
and Apoptosis
(epithelial and endothelial cells)

† Cyclooxygenase Activity
(inflammatory cells and platelets)

Acute Cardiac and Lung Injury

Hypoxia, Hypotension, and Thrombosis

Oxidative Stress
(free radical induced DNA damage)

† 8-OHdG
† L-FABP
† 11-dehydro TxB2

Renal Injury

Proximal Tubular Ischemia

Thromboxane Generation