Urinary fatty acid binding protein 3 (uFABP3) is a potential biomarker for peripheral arterial disease

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Plasma levels of fatty acid binding protein 3 (pFABP3) are elevated in patients with peripheral artery disease (PAD). Since the kidney filters FABP3 from circulation, we investigated whether urinary fatty acid binding protein 3 (uFABP3) is associated with PAD, and also explored its potential as a diagnostic biomarker for this disease state. A total of 130 patients were recruited from outpatient clinics at St. Michael’s Hospital, comprising of 65 patients with PAD and 65 patients without PAD (non-PAD). Levels of uFABP3 normalized for urine creatinine (uFABP3/uCr) were 1.7-folds higher in patients with PAD [median (IQR) 4.41 (2.79–8.08)] compared with non-PAD controls [median (IQR) 2.49 (1.78–3.12), p-value = 0.001]. Subgroup analysis demonstrated no significant effect of cardiovascular risk factors (age, sex, hypertension, hypercholesteremia, diabetes and smoking) on uFABP3/uCr in both PAD and non-PAD patients. Spearman correlation studies demonstrated a significant negative correlation between uFABP3/uCr and ABI (ρ = −0.436; p-value = 0.001). Regression analysis demonstrated that uFABP3/Cr levels were associated with PAD independently of age, sex, hypercholesterolemia, smoking, prior history of coronary arterial disease and Estimated Glomerular Filtration rate (eGFR) [odds ratio: 2.34 (95% confidence interval: 1.47–3.75) p-value < 0.001]. Lastly, receiver operator curve (ROC) analysis demonstrated unadjusted area under the curve (AUC) for uFABP3/Cr of 0.79, which improved to 0.86 after adjusting for eGFR, age, hypercholesteremia, smoking and diabetes. In conclusion, our results demonstrate a strong association between uFABP3/Cr and PAD and suggest the potential of uFABP3/Cr in identifying patients with PAD.

Peripheral arterial disease (PAD) is a chronic atherosclerotic condition that affects over 200 million people globally1–3. Despite its prevalence, a large proportion of patients with PAD receive delayed treatment, and consequently, face a high risk of lower-extremity amputations and mortality4–7.

Currently, the ankle brachial index (ABI)—the ratio of the brachial artery blood pressure to the ankle blood pressure—is the only validated screening tool for PAD8. However, the ABI has limitations; it is often erroneously interpreted in primary care, and can be unreliable in patients with diabetes9–11.

We have previously demonstrated an association between elevated plasma levels of fatty acid binding protein 3 (pFABP3) and PAD12. FABP3 is a small intracellular protein that is normally absent from plasma but is released into the circulation following myocardial or skeletal injury13,14.

Because the kidneys play an important role in clearing and filtering pFABP3 from circulation15,16, we have now investigated whether urine fatty acid binding protein (uFABP3) levels are elevated in patients with PAD and could potentially serve as a diagnostic biomarker for this disease state.

Materials and methods

Ethics approval. This study received approval from the research ethics board at St. Michael’s Hospital-University of Toronto in Ontario, Canada. Informed verbal and written consent were obtained from all participants, and all methods were carried out in accordance with the relevant guidelines and regulations.
Baseline measurements and sample collection. We recorded baseline demographics, history of cardiovascular diseases, cardiovascular risk factors, and smoking status. Each patient also underwent lower limb arterial imaging with an ultrasound (US), ABI or toe brachial index (TBI). Patients were defined as having hypercholesterolemia if they had total cholesterol > 5.2 mmol/L, triglyceride > 1.7 mmol/L, or were taking lipid lowering therapy; as having hypertension if they had a systolic blood pressure ≥ 130 mmHg, diastolic pressure ≥ 80 mmHg, or were taking blood pressure lowering therapy; and as having diabetes if they had a glycosylated hemoglobin A1c ≥ 6.5% or were taking anti-diabetic medication. Patients were defined as having renal disease if they had an estimated glomerular filtration rate of less than 60 mL/min/1.73 m². The Estimated Glomerular Filtration rate (eGFR) was calculated for each patient as previously demonstrated by Levey et al. A history within the past 12 months of acute coronary syndrome (ACS), acute congestive heart failure (CHF), uncontrolled arrhythmia or elevated troponins were excluded.

Measurement of urinary creatinine and normalization of uFABP3. Urine creatinine (uCr) levels were measured at the Core laboratory at St. Michael's Hospital using the Beckman Coulter AU680 laboratory analyzer (Beckman Coulter; Pasadena, California). The uFABP3 concentration were normalized to uCr to adjust for urinary concentration errors and differences in hydration status, while relying on single-spot urine samples to achieve normalized uFABP3/uCr (μg/g).

Statistical methods. Data are presented as median and interquartile ranges (IQR). Normality was assessed using the Shapiro–Wilk test. Since the continuous variables were not normally distributed, the Mann–Whitney U or Kruskal–Wallis test was used to evaluate differences between groups. A post-hoc test was used for pairwise comparisons after multiple group testing. Fisher's exact test or chi-square test was used for categorical variables. In an attempt to compare uFABP3/uCr levels among PAD and non-PAD patients, patients with PAD where compared after multiple group testing. Fisher's exact test or chi-square test was used for categorical variables. U or Kruskal–Wallis test was used to evaluate differences between groups. A post-hoc test was used for pairwise comparisons after multiple group testing. Normality was assessed using the Shapiro–Wilk test. Since the continuous variables were not normally distributed, the Mann–Whitney U or Kruskal–Wallis test was used to evaluate differences between groups. A post-hoc test was used for pairwise comparisons after multiple group testing. The ability to achieve normalized uFABP3 (μg/g).

Results

Cohort description and levels of uFABP3/uCr in controls and patients with PAD. A total of 130 patients were enrolled in the study. Median age of the overall study participants was 66, and all collected patient demographics were significantly different between the PAD and non-PAD groups except for sex and eGFR (Table 1). Among the 65 patients with PAD, there were 4 patients (6%) with chronic limb threatening ischemia (CLTI) that had an average ABI of 0.54 and median age of 67 years. Relative to patients with PAD, the CLTI patients did not have any significant difference in measured demographic variables or cardiovascular risk factors. The uFABP3/uCr levels in the PAD group [median (IQR) 4.41 (2.79–8.08)] were 1.7 folds higher than levels in the non-PAD group [median (IQR) 2.49 (1.78–3.12)]. In additional analysis, we investigated the levels of the non-normalized uFABP3 in the PAD group. Our results demonstrated that the non-normalized uFABP3
in the PAD group [median (IQR) 3.48 (2.51–5.45), μg/g] were also significantly higher than in non-PAD controls [median (IQR) 1.89 (0.93–3.40), μg/g].

Correlation between uFABP3/uCr and PAD. To understand the association between uFABP3/uCr and the measured cardiovascular risk factors, subgroup analysis were conducted. Our analysis demonstrated that risk factors had no significant effect on uFABP3/uCr in both PAD and non-PAD patients (Table 2). We also investigated the association between uFABP3/uCr and PAD severity (based on the ABI) using spearman test. Our test demonstrated a significant negative correlation between uFABP3/uCr and ABI (ρ = −0.436; p-value = 0.001; supplemental Fig. 1). Next, we investigated levels of uFABP3/uCr after stratifying PAD patients based on their ABI as per the ESVM guidelines21. The stratified groups were: mild PAD (ABI 0.75–0.9, n = 11), moderate PAD (ABI 0.74–0.50, n = 29) and severe PAD (ABI < 0.50, n = 25) . Our data shows a significant difference in median uFABP3/uCr levels between the moderate and severe PAD subgroups when compared to the non-PAD group (p-value < 0.001, for both) (Fig. 1). Although we noted a trend in increasing levels of uFABP3/uCr as the ABI worsens, no statistical difference was observed when comparing PAD subgroups specifically (p-value = 0.231).

Influence of confounding factors on uFABP3/uCr levels in patients with PAD. To investigate the independent association between uFABP3/uCr levels and PAD, multiple stepwise regression analysis was conducted. Our analysis demonstrated that uFABP3/uCr levels were strongly associated with PAD, even after adjusting for age and sex (model 1; OR, 2.02, 95% confidence interval (95% CI), 1.41–2.90, p-value = 0.001). Table 3 displays the results of several other regression models that demonstrate a significant association between uFABP3/uCr levels and PAD after adjusting for age, sex, prior history of CAD, smoking, eGFR and hypercholesteremia.

Diagnostic potential of uFABP3/uCr for PAD. Lastly, a ROC analysis was performed to measure the diagnostic accuracy of uFABP3/uCr in diagnosing patients with PAD. Prior to adjusting for confounding factors,
the AUC for uFABP3/uCr was 0.79 (95%: 0.71–0.87). After adjusting for age, eGFR, hypercholesteremia, smoking and diabetes, the ROC analysis for uFABP3/uCr demonstrated an improved AUC of 0.86 (95%: 0.80–0.92) (Fig. 2).
Several studies have demonstrated the utility of uFABPs as potential biomarkers for diseased states. For instance, urinary FABP1 levels were noted to be higher in patients with septic shock when compared to patients with severe sepsis but without shock. Tanaka et al. demonstrated that uFABP3 is elevated in patients with acute myocardial infarction. Similarly, Nayashida et al. studied the influence of renal function on uFABP3 levels in patients undergoing a primary coronary artery bypass, and suggested, alongside others, that uFABP3 may be an early and sensitive biochemical marker for the diagnosis of myocardial injury in patients undergoing cardiac surgery. Studies have also suggested that after myocardial injury, FABP3 filtration by the kidney can be impaired when creatinine clearance is decreased, resulting in lower levels of uFABP3. Thus, uFABP3 may be utilized as a potential diagnostic biomarker for PAD in the absence of reduced creatinine clearance. Urinary FABPs may also serve as potential biomarkers for the earlier diagnosis of acute renal failure.

The present study is not without limitations. First, there was a relatively small sample size. Second, due to the small sample size, we were not able to adjust for all potential confounding factors. Thirdly, the cross-section study design of this pilot trial did not allow for patient follow-up. Lastly, patients with acute myocardial infarction and renal failure were not included due to their confounding effect on uFABP3.

### Discussion

In this study, an association was demonstrated between PAD and uFABP3/uCr. Our data shows uFABP3/uCr is elevated in patients with PAD when compared to non-PAD controls, even after adjusting for potential confounding factors. Furthermore, the ROC analysis suggests potential for uFABP3/uCr in distinguishing PAD patients from non-PAD patients. Collectively, this data serves as a strong foundation for future studies investigating urine FABP3 as a biomarker for PAD.

Normally, biomarkers are used along with a clinical exam and radiographical investigations to enhance diagnosis. For instance, a diagnosis of acute myocardial infarction is reached after using a combination of several biomarkers (creatinine kinase and troponin) in addition to an electrocardiogram and clinical symptoms. With regards to PAD, our research group previously identified that pFABP3 is potentially associated with PAD, and through this study, have been able to demonstrate that uFABP3/uCr can serve as a potential diagnostic biomarker for PAD as well.

Fatty acid binding proteins (FABPs) play an important role in the trafficking of intracellular fatty acids. Several studies have demonstrated the utility of uFABPs as potential biomarkers for diseased states. For instance, urinary FABP1 levels were noted to be higher in patients with septic shock when compared to patients with severe sepsis but without shock. Tanaka et al. demonstrated that uFABP3 is elevated in patients with acute myocardial infarction. Similarly, Nayashida et al. studied the influence of renal function on uFABP3 levels in patients undergoing a primary coronary artery bypass, and suggested, alongside others, that uFABP3 may be an early and sensitive biochemical marker for the diagnosis of myocardial injury in patients undergoing cardiac surgery. Studies have also suggested that after myocardial injury, FABP3 filtration by the kidney can be impaired when creatinine clearance is decreased, resulting in lower levels of uFABP3. Thus, uFABP3 may be utilized as a potential diagnostic biomarker for PAD in the absence of reduced creatinine clearance. Urinary FABPs may also serve as potential biomarkers for the earlier diagnosis of acute renal failure.

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### Conclusions

In summary, our study revealed for the first time, that uFABP3/uCr is significantly elevated in patients with PAD. Our data demonstrated an independent association between uFABP3 and PAD, and ROC analysis demonstrated strong ability to discriminate, raising its potential as a tool for PAD diagnosis. Therefore, uFABP3 is a potential diagnostic biomarker for PAD; however, larger clinical trials are needed to confirm these findings.

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**Table 3.** Influence of individual factors on the odds ratios for PAD per one unit increase in normalized urinary fatty acid-binding protein 3. CAD coronary arterial disease, eGFR estimated glomerular filtration rate, CI confidence interval. *Binary logistic regression models for PAD per one unit increase in uFABP3/uCr.

| Regression models | Odds ratio (95% CI) | p-value |
|-------------------|-------------------|---------|
| Unadjusted model   | 1.93 (1.45–2.56)  | 0.001   |
| Model 1 (adjusted for age and sex) | 2.02 (1.41–2.90) | 0.001   |
| Model 1 + CAD      | 2.03 (1.38–2.97)  | 0.001   |
| Model 1 + CAD + smoking | 2.11 (1.40–3.19) | 0.001   |
| Model 1 + CAD + smoking + hypercholesteremia | 2.15 (1.39–3.34) | 0.001   |
| Model 1 + CAD + smoking + hypercholesteremia + eGFR | 2.34 (1.47–3.75) | 0.001   |

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Author contributions
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