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ORIGINAL RESEARCH—CLINICAL

Fecal Leukocyte Esterase, an Alternative Biomarker to Fecal Calprotectin in Inflammatory Bowel Disease: A Pilot Series

Roberto Trasolini,[1,2] Kai Zhu,[3] Natasha Klemm,[3] Sophia Park,[4] and Baljinder Salh[3,5]

[1]Department of Gastroenterology, Center for Advanced Endoscopy, Beth Israel Deaconess Hospital and Harvard Medical School, Boston, Massachusetts, [2]Division of Gastroenterology, Beth Israel Deaconess Medical Center, Boston, Massachusetts, [3]Department of Medicine, University of British Columbia, Vancouver, British Columbia, Canada, [4]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada, and [5]Division of Gastroenterology, University of British Columbia, Vancouver, British Columbia, Canada

BACKGROUND AND AIMS: Fecal calprotectin (FC) is a noninvasive biomarker used in inflammatory bowel disease (IBD) management and risk stratification of nonspecific gastrointestinal symptoms. Leukocyte esterase is an inexpensive and widely available point-of-care inflammatory marker present on urinalysis test strips. We aim to assess the diagnostic accuracy of fecal leukocyte esterase (FLE) relative to FC and endoscopy and demonstrate its use as an alternative biomarker for IBD. **METHODS:** In this prospective cohort study, 70 patients who had FC ordered as part of standard clinical care also received FLE testing. FLE levels were compared with various FC cutoff values and endoscopy and pathology findings as the gold standard. **RESULTS:** As the FC cutoff increased from 50 to 500 μg/g, FLE sensitivity increased from 67% to 95% while the specificity decreased from 86% to 76%. The area under the receiver operating characteristic (AUROC) curve increased from 0.79 to 0.90. An FLE of $\geq$1+ had the best test characteristics. Among patients who underwent endoscopic evaluation, FLE demonstrated an identical sensitivity (75%) and specificity (86%) to FC in predicting endoscopic inflammation. AUROC was 0.80 for FLE and 0.85 for FC with an optimal cutoff of $\geq$2+ and 301 μg/g, respectively. When used to distinguish between patients with active IBD and no/inactive IBD, FLE had a sensitivity of 84% and specificity of 90%, comparable with the 84% and 83%, respectively, of FC. AUROC was 0.88 for FLE and 0.91 for FC with an optimal cutoff of $\geq$2+ and 145 μg/g, respectively. **CONCLUSION:** FLE demonstrates adequate correlation and comparable accuracy with FC in predicting endoscopic inflammation and distinguishing between patients with active vs inactive IBD.

Keywords: Inflammatory Bowel Disease; Fecal Calprotectin; Biomarker; Noninvasive.

Introduction

Inflammatory bowel disease (IBD) is a chronic condition with nonspecific symptoms that may resemble irritable bowel syndrome (IBS). Further complicating management is the increased rate of IBS in those with IBD.[1] In the absence of more concerning IBD symptoms, determining the extent of workup can be challenging and may result in diagnostic delay and unnecessary procedural risks. IBD has become a global disease with increasing prevalence in resource-limited settings; diagnosis and differentiation from IBS pose substantial social and economic burden on health care systems worldwide.[2–5]

Fecal calprotectin (FC) is a noninvasive biomarker used to risk stratify patients and distinguish between IBD and IBS. Despite its usefulness in IBD management, its availability is limited in primary care settings, many health authorities may take weeks to obtain results, and, dependent on the region, it may not be covered by health insurance.[6] An alternative marker that is widely available, rapid, and inexpensive would be advantageous in triaging patients with suspected IBD.

Leukocyte esterases (LEs) are cytoplasmic enzymes present in white blood cells and markers of inflammation.[7] LE colorimetric strip testing is ubiquitous in health care settings, providing rapid diagnosis of urinary tract infections, and recently has demonstrated utility in detecting cerebrospinal, pleural, peritoneal, and joint inflammation.[8–12] We present a study determining the sensitivity and specificity of fecal LE (FLE) in the detection of active IBD and endoscopic inflammation relative to FC with the goal of introducing FLE as an alternative biomarker for IBD.

Methods

Study Population

In this prospective cohort study, eligible patients were included between February 2020 and May 2021. All patients

Abbreviations used in this paper: **AUROC, area under the ROC curve; FC, fecal calprotectin; FLE, fecal leukocyte esterase; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; IQR, interquartile range; LE, leukocyte esterase; ROC, receiver operating characteristic.**

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who had an FC ordered as part of standard clinical care at the Vancouver General Hospital inpatient and outpatient laboratories were included in the study. This included patients from multiple hospitals and outpatient laboratories within the Vancouver Coastal Health Authority which centralize FC testing. Baseline patient and disease characteristics, as well as endoscopy and pathology reports, were obtained from the electronic medical record. The study was approved by the University of British Columbia Research Ethics Board.

Specimen Analysis

Samples were stored at 4 °C until analysis. For FLE analysis, stool specimens were processed with the Roche fecal sample preparation kit (Roche Diagnostic) as per the manufacturer's instructions. Hundred mg of stool and 5 mL of EliA Calprotectin Extraction Buffer (Thermo Fisher Scientific) were added to the sample tube and vortexed for 2 minutes. For firmer stool, the sample was left to stand with the buffer for up to 15 minutes before vortexing. The sample was then centrifuged at 1600–1800 relative centrifugal force for 10 minutes. One drop was transferred to the LE pad on the Multistix 10 SG reagent strip (Siemens) and manually read after 120 seconds. Samples were interpreted as negative, trace, 1+, 2+, or 3+ corresponding to 0, 15, 70, 125, and 500 leukocytes/μL, respectively. For FC analysis, stool specimens were processed with the LIAISON calprotectin stool extraction device (DiaSorin), and calprotectin was measured on the LIAISON platform (DiaSorin) as per the manufacturer's instructions.

The LIAISON calprotectin assay is an automated chemiluminescent sandwich immunoassay which uses solid-phase paramagnetic particles coated with monoclonal mouse antibodies against calprotectin and second, conjugated monoclonal mouse calprotectin detection antibodies labeled with an isoluminol derivative.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 8 (GraphPad Software Inc, La Jolla, CA). Results of numerical data are presented as means with standard deviation or medians with interquartile range (IQR) where appropriate. Receiver operating characteristic (ROC) curves and the area under the ROC curve (AUROC) were used to assess the diagnostic accuracy of FLE and FC for predefined outcomes. For each endpoint, the sensitivity, specificity, and positive and negative likelihood ratios were calculated relative to FC and, where available, endoscopic inflammation as the gold standard.

Results

Baseline Patient Demographics and Clinical Characteristics

Between February 2020 and May 2021, 70 patients received concurrent FC and FLE testing. The median age of the population was 49 years (range, 17–92 years) with a slight male predominance of 51% (Table 1). Among the cohort, 36 patients (51%) had IBD (18 with Crohn's disease and 18 with ulcerative colitis), 29 (41%) had no IBD, and 5 (7%) were diagnostically uncertain. A stool culture was performed in 41 patients (59%) with 5 patients testing positive. Only 27 patients (39%) underwent a colonoscopy after the initial workup.

Table 1. Baseline Patient Demographics and Clinical Characteristics

Number of patients	70
Age, y (range)	49 (17–92)
Male sex, N (%)	36 (51)
IBD status, N (%)	
IBD	36 (51)
Crohn's disease	18 (50)
Ulcerative colitis	18 (50)
No IBD	29 (41)
Diagnostically uncertain	5 (7)
Stool culture, N (%)	41 (59)
Positive culture	5 (12)
Colonoscopy, N (%)	27 (39)
Inflammation	20 (74)
No inflammation	7 (26)

Diagnostic Accuracy of FLE as a Predictor of FC

The accuracy of FLE to predict FC was assessed by plotting an ROC curve. Given the variability of optimal FC cutoff values for the prediction of IBD, FLE measurements were compared with different FC cutoff values ranging from 50 to 500 μg/g (Figure 1). At an FC cutoff of 50 μg/g, FLE had a sensitivity of 67%, a specificity of 86%, a positive likelihood ratio of 4.67, and a negative likelihood ratio of 0.39 (Table 2). FLE sensitivity increased to 74%, 88%, and 95% while specificity decreased to 83%, 78%, and 76% as the FC cutoff increased to 100, 250, and 500 μg/g, respectively. The positive likelihood ratio decreased, and the negative likelihood ratio increased with increasing FC cutoff values (Table 2). An FLE interpretation of 1+ or greater was selected as the optimal cutoff to optimize the sensitivity of the test such that a negative FLE can more accurately rule out a positive FC.

The diagnostic accuracy of FLE, as determined by the AUROC, increased with increasing FC cutoffs. The AUROC

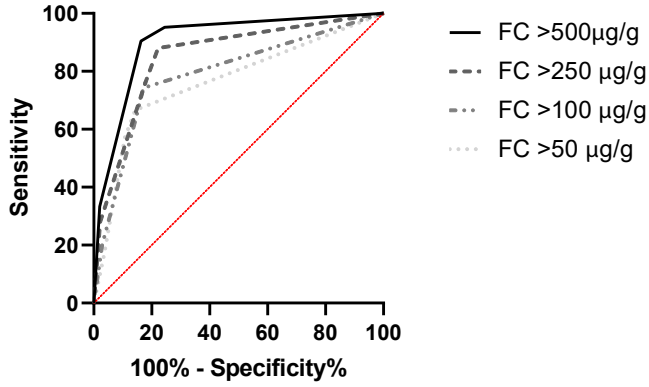

Figure 1. Receiver-operator characteristic curves for FLE relative to different FC cutoffs. FLE of 1+ or greater was the optimal cutoff value. N = 70 for all cutoffs.

Table 2. FLE Sensitivity and Specificity Relative to Different FC Cutoffs

Statistical parameter	FC ≥ 50 μg/g	FC ≥ 100 μg/g	FC ≥ 250 μg/g	FC ≥ 500 μg/g
Sensitivity (%)	67	74	88	95
Specificity (%)	86	83	78	76
Positive likelihood ratio	4.67	4.33	3.96	3.89
Negative likelihood ratio	0.39	0.31	0.15	0.06

N = 70 for all cutoffs. The ROC curve indicates that an FLE of 1+ or greater is the optimal cutoff value.

was 0.76, 0.79, 0.85, and 0.90 at FC cutoffs of 50, 100, 250, and 500 μg/g, respectively (Figure 1). FLE and FC also demonstrated a positive linear relationship with a moderate correlation coefficient R^2 of 0.49 (Figure 2).

Diagnostic Accuracy of FLE and FC as a Predictor of Endoscopic Inflammation

Data on endoscopic evaluation were available for 27 of the 70 patients (39%). Among the 27 patients, 20 (74%) were identified to have findings of endoscopic inflammation. The accuracy of FLE to predict endoscopic inflammation was assessed by plotting an ROC curve (Figure 3). AUROC for FLE was 0.80 with an optimal cutoff of 2+ or greater. FC had a slightly higher AUROC of 0.85 with an optimal cutoff of 301 μg/g. Both FLE and FC had a sensitivity of 75%, specificity of 86%, positive likelihood ratio of 5.25, and negative likelihood ratio of 0.29 in predicting endoscopic inflammation (Table 3).

Diagnostic Accuracy of FLE and FC as a Predictor of Active IBD

Based on the clinical history, investigations, and, where available, colonoscopy and biopsy results, 19 patients had active IBD and 40 had no or inactive IBD. The remaining 11 patients were excluded from the comparison as 5 had positive stool cultures and 6 were uncertain.

FLE and FC both demonstrated better accuracy in predicting active IBD status with an AUROC of 0.88 and 0.91, respectively. The optimal cutoff was 2+ or greater for FLE and 145 μg/g for FC. FLE had a sensitivity of 84% and specificity of 90% with a positive and negative likelihood ratio of 8.42 and 0.18, respectively. FC had a sensitivity of 84% and specificity of 83% with a positive and negative likelihood ratio of 4.81 and 0.19, respectively (Table 3).

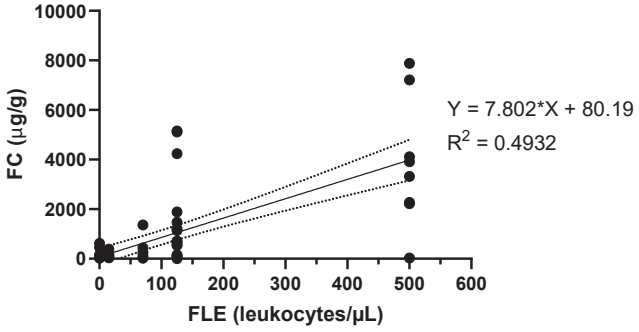

Figure 2. Linear regression comparison of FLE vs FC. N = 70. R^2 = 0.4915 with a *P*-value < .001.

Increasing FLE and FC With Severity of Histological Inflammation

As expected, the distributions of FLE and FC increased with severity of histological inflammation. Median FLE was 1+ (IQR, 0–2) in "mild", 2+ (IQR, 1–3) in "moderate", and 2+ (IQR 2–3) in "severe" inflammation. Similarly, median FC was 55 μg/g (IQR, 19–211 μg/g) in "mild", 1140 μg/g (IQR, 121–3320 μg/g) in "moderate", and 3910 μg/g (IQR, 1200–7210 μg/g) in "severe" inflammation (Figure 4).

Discussion

Our study is the first to demonstrate FLE as a reliable, quick, and inexpensive tool to differentiate IBD from noninflammatory gastrointestinal disorders and as an alternative bedside screening test for IBD, in the ambulatory, emergency room, and resource-limited setting. IBD is a chronic condition characterized by relapsing and remitting inflammation of the gastrointestinal tract. A challenge in management arises when symptoms are similar to IBS, which frequently occurs in those with IBD.[1] These similarities create a diagnostic challenge that may result in therapeutic delay and procedural risks.

Colonoscopy is the gold standard for distinguishing IBD from IBS; however, it is an invasive procedure with societal expense. Noninvasive fecal biomarkers have been increasingly utilized to risk stratify patients by identifying the inflammatory burden of stool samples. Fecal markers are particularly important for Crohn's disease, which can evade a sigmoidoscopy diagnosis, unlike ulcerative colitis.[13] Intestinal inflammation results in a 10-fold increase in neutrophil migration to the intestine, where the contents, including calprotectin, are sloughed into the lumen.[14] Calprotectin accounts for 60% of the neutrophil's cytosolic proteins and was first discovered at high levels in the feces of those with IBD by Roseth et al in 1992.[14] It was first described as an objective marker of IBD by Tibble et al in 2000.[13] Meta-analyses have demonstrated a sensitivity of 83%–95% and specificity of 84%–91% for differentiating IBD from non-IBD diagnoses in adults, owing to its

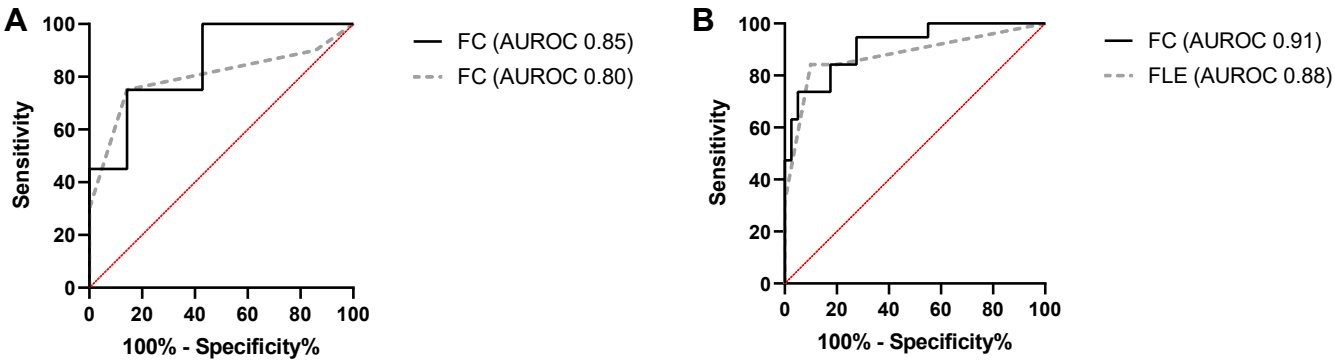

Figure 3. Receiver-operator characteristic curves for FLE and FC relative to the presence of (A) inflammation on colonoscopy and (B) active IBD. N = 27 (n = 20 inflammation vs 7 no inflammation) and 59 (n = 19 active IBD vs 40 no/inactive IBD), respectively. The ROC curve indicates that an FLE of 2+ or greater is the optimal cutoff for detecting both inflammation on colonoscopy and active IBD. FC of 301 μg/g is the optimal cutoff value for inflammation on colonoscopy and 145 μg/g for active IBD.

homogeneous and stable distribution in stool.[15–17] Additional studies demonstrate FC as a surrogate marker of mucosal healing and treatment monitoring.[18–22]

Gastroenterologists often use FC to differentiate between IBD and IBS—a common inpatient and outpatient referral—avoiding the risks, time, and societal cost of unnecessary colonoscopies.[18,23] However, the utility of FC is limited by its availability, processing time, and direct patient cost. Fecal lactoferrin (FL), an iron-binding protein and major component of neutrophil secondary granules, is a widely available alternative marker with comparable accuracy with FC.[18,20] Furthermore, FL is covered by Medicare and, frequently, by private insurance.[24] However, interpretation of FL requires enzyme-linked immunosorbent assays which take time and are not available in primary care settings. An ideal screening fecal biomarker of inflammation is one that is widely available, inexpensive, and rapid.

FLE was first proposed as a tool for IBD monitoring in 1993.[25] Similar to calprotectin and lactoferrin, LE activity reflects the presence of white blood cells and has been developed as a rapid, inexpensive, and widely available screening test for bacteriuria.[12,26] Furthermore, LE has potential utility for simple and rapid testing in meningitis and periprosthetic joint infections in adults and spontaneous bacterial peritonitis in adults and children,[7,9,10,27–36] offering inexpensive testing alternatives in resource-limited countries and preventing treatment delay. Recently, Dumoulin et al[26] described the application of LE strip testing in fecal extracts and found a reasonable correlation between FLE and FC. However, to our knowledge, there are no studies on the use of FLE strip testing in IBD.

Our study is the first to investigate the application of FLE strip testing in IBD. Our institutional analysis of FLE demonstrates an adequate correlation and comparable accuracy with FC using endoscopy as a gold standard. FLE had a moderate diagnostic accuracy for prediction of FC which improved with higher FC cutoff values. Interestingly, when used to predict endoscopic inflammation, at a cutoff of 2+, the accuracy of FLE markedly increased to levels identical to FC. The sensitivity and specificity of FLE further increased to 84% and 90%, respectively, when distinguishing between patients with active IBD and no/inactive IBD, comparable with the accuracy of FC in previous meta-analyses reported by Van Rheenen et al and Rokkas et al.[15,37] The results suggest that FLE is more accurate as a predictor of endoscopic inflammation and active IBD status than as a predictor of FC. This is not surprising as FC itself is an imperfect marker with limitations that may not apply to FLE. For instance, FC can be normal in isolated ileal Crohn's because of proteolysis, but FLE may evade breakdown.[26,38]

Table 3. FLE and FC Sensitivity and Specificity Relative to the Presence of Inflammation on Colonoscopy and Active IBD

	Inflammation on colonoscopy		Active IBD	
	FLE ≥ 2+	FC > 301 μg/g	FLE ≥ 2+	FC > 145 μg/g
Sensitivity (%)	75	75	84	84
Specificity (%)	86	86	90	83
Positive likelihood ratio	5.25	5.25	8.42	4.81
Negative likelihood ratio	0.29	0.29	0.18	0.19

N = 27 (n = 20 inflammation vs 7 no inflammation) and N = 59 (n = 19 active IBD vs 40 no/inactive IBD), respectively. The ROC curve indicates that an FLE of 2+ or greater and FC of 301 and 145 μg/g for the presence of inflammation and active IBD, respectively, are the optimal cutoff values.

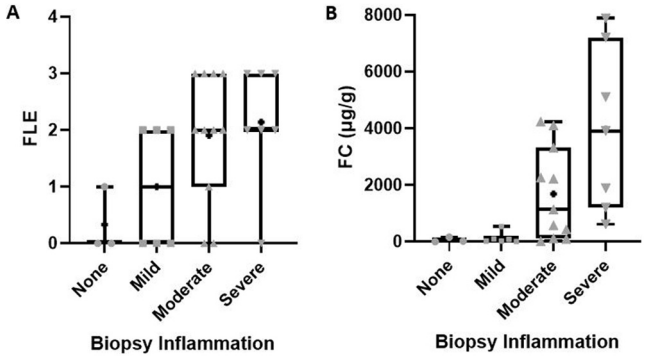

Figure 4. FLE (A) and FC (B) values with increasing biopsy inflammation severity. N = 3, 6, 11, and 7 for none, mild, moderate, and severe inflammation, respectively. The mean is represented by "+". The top of the box represents the 75th percentile, the bottom of the box represents the 25th percentile, and the line in the middle represents the 50th percentile. The whiskers represent the highest and lowest values that are not outliers or extreme values.

Although both FLE and FC are markers of inflammation, they have many differences. FLE strip testing is readily available at most primary care centers and hospitals and can be used at the bedside, whereas FC is only available at select sites with access to the FC stool extraction device and assay in the laboratory. Test time, not including the stool extraction step, of FLE is only 2 minutes compared with 10–35 minutes for FC depending on the assay used. The faster test result of FLE is especially useful in primary care settings such as the emergency department where patient turnover is high and time is limited. Furthermore, the low cost of the FLE test strips (<$1.00/strip) makes it an attractive screening tool before proceeding to the much more expensive FC test. However, future studies would be helpful to compare the accuracy of manual vs automated FLE interpretation as previous studies have demonstrated automated measurement of LE in urinalysis to be more accurate and consistent than manual.[39,40] Regulatory requirement of automated strip reading over the manual approach may limit the cost benefit of FLE testing.

FLE and FC are also affected differently by stool characteristics. The presence of blood in the stool may cause a mild FC elevation but rarely to the levels observed in patients with IBD.[41] In contrast, any highly colored substance such as blood or beet ingestion can cause a false positive result for FLE. A prospective study by Lasson et al[42] also found higher levels of FC to be significantly correlated with looser stool consistency although such parameters have not been studied for FLE.

There are limitations to our study. FC concentration depends on various physiological factors, such as age and clinical comorbidities, and may have considerable day-to-day variability.[18,19] It is not known whether FLE is similarly impacted by these factors and were not accounted for in this study. Furthermore, esterase activity originating from sources other than leukocytes, such as from the pancreas, could produce false positives.[43] Given this is a preliminary study, we did not assess the role of FLE compared with FC in specific settings of IBD management such as predicting clinical relapse, mucosal healing, treatment response, post-surgical Crohn's recurrence, and pouchitis. Finally, endoscopic disease activity assessment was not standardized as the procedure was performed by different gastroenterologists without the use of a central reading. Preliminary data by Dumoulin et al[26] demonstrated that FLE activity remains stable for 24 hours when the stool is kept at 4 °C, but further research is required for the optimal handling and processing of FLE.

In conclusion, our study demonstrated that FLE is a reliable, rapid, and inexpensive alternative screening test for IBD with comparable diagnostic accuracy with FC in predicting endoscopic inflammation and distinguishing between patients with active IBD and no/inactive IBD. However, research on FLE remains limited, and further studies are warranted to validate the diagnostic accuracy and explore the cost-effectiveness before it is introduced in a clinical setting. Furthermore, additional research is required to define the role of FLE in various settings of IBD management.

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Correspondence:
Address correspondence to: Baljinder Salh, MBChB, FRCP (UK), FRCPC, Division of Gastroenterology, Vancouver General Hospital, 2775 Laurel Street, Vancouver, British Columbia V5Z 1M9, Canada. e-mail: bill.salh@ubc.ca; fax: 604-875-5161.

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Authors' Contributions:
Roberto Trasolini and Baljinder Salh contributed to the conceptualization of the project. Roberto Trasolini and Sophia Park contributed toward the methodology, data curation, and project administration. Kai Zhu and Natasha Klemm contributed toward the writing, and the remaining authors assisted with the reviewing and editing of the manuscript.

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Ethical Statement:
The corresponding author, on behalf of all authors, jointly and severally, certifies that their institution has approved the protocol for any investigation involving humans or animals and that all experimentation was conducted in conformity with ethical and humane principles of research.

Data Transparency Statement:
Data, analytic methods, and study materials will be made available to other researchers on request. Please contact the authors.