Serial Measurements of Neutrophil Gelatinase-Associated Lipocalin levels for Assessment of Contrast Induced Nephropathy among Chronic Kidney Disease Patients Who Underwent Elective Coronary Angiography

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ABSTRACT **Background:** Among chronic kidney disease (CKD) patients, baseline neutrophil gelatinase-associated lipocalin (NGAL) may reflect the severity of renal impairment. No data exists on serial changes in serum NGAL levels in CKD patients before and after percutaneous coronary intervention (PCI).

Objectives: To evaluate serial serum NGAL levels relation to contrast induced acute kidney injury (CI-AKI) following PCI.

Methods: The study included 58 patients with CKD who underwent elective PCI. Plasma NGAL measurements were performed before (pre-NGAL) and 24 hours following (post-NGAL) PCI. Patients were followed for CI-AKI and changes in NGAL levels. Receiver operator characteristic identified the optimal sensitivity and specificity for pre-NGAL levels compared with post-NGAL for patients with CI-AKI.

Results: Overall CI-AKI incidence was 33%. Both pre-NGAL (172 vs. 119 ng/ml, $P < 0.001$) and post-NGAL (181 vs. 121 ng/ml, $P < 0.001$) levels were significantly higher in patients with CI-AKI, but no significant changes were detected. Pre-NGAL levels were similar to post-NGAL levels in predicting CI-AKI (area under the curve 0.753 vs. 0.745). Optimal cutoff value for pre-NGAL was 129 ng/ml (sensitivity of 73% and specificity of 72%, $P < 0.001$). Post-NGAL levels > 141 ng/ml were independently associated with CI-AKI (hazard ratio [HR] 4.86, 95% confidence interval [95%CI] 1.34–17.64, $P = 0.02$) with a strong trend for post-NGAL levels > 129 ng/ml (HR 3.46, 95%CI 1.23–12.81, $P = 0.06$).

Conclusions: In high-risk patients, pre-NGAL levels may predict CI-AKI. Further studies on larger populations are needed to validate the use of NGAL measurements in CKD patients.

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KEY WORDS: acute kidney injury (AKI), chronic kidney disease (CKD), neutrophil gelatinase-associated lipocalin (NGAL), ST-elevation myocardial infarction (STEMI)

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Neutrophil gelatinase-associated lipocalin (NGAL) is a glycoprotein released from granules of mature neutrophils present in the by renal tubular cells following injury [1-3]. Among patients with chronic kidney disease (CKD), previous studies have found that elevated NGAL levels in the serum may represent renal tubular damage underlying the chronic damage. [4-6]. This finding is especially true for patients with estimated glomerular filtration rate (eGFR) ≤ 60 ml/min, where eGFR is the strongest determinant of plasma NGAL [7]. By contrast, eGFR > 60 contributes little to plasma NGAL [8]. The presence of CKD is one of the most important risk factors for the development of contrast induced-acute kidney injury (CI-AKI), affecting approximately 50% of patients with CKD stage 3/4 [9,10]. Elevation of serum NGAL levels was present in serum of patients within hours after suspected renal insult, and predicted renal injury earlier, compared to serum creatinine (sCr) [11,12]. We have previously demonstrated that among CKD patients who underwent percutaneous coronary intervention (PCI) baseline NGAL levels performed better than eGFR in predicting the risk for CI-AKI [13]. To the best of our knowledge, no study to date has investigated serial changes in serum NGAL in CKD patients prior to and following application of contrast media.

Among hospitalized patients, especially those in intensive care units, NGAL levels may also reflect a response to an acute inflammatory state [14]. Accordingly, we performed serial measurement serum NGAL levels and evaluated their relation for the occurrence of CI-AKI among CKD patients who underwent elective PCI.

PATIENTS AND METHODS

We performed a prospective, observational, open label study at the Tel Aviv Medical Center. Based on the availability of NGAL kits, we screened 141 patients with CKD admitted to the cardiology ward following PCI between December 2018 and March

2022 in whom serial NGAL measurements were performed. The presence of CKD for all patients included was based on medical charts and laboratory results from the out-patient clinics prior to index elective PCI and during hospitalization. All patients included had at least one laboratory result of eGFR < 60 ml/min/1.73 m^2 for a period of > 3 month prior to index hospitalization. As plasma NGAL levels may reflect the response to an acute inflammatory state [15], we excluded 78 patients who underwent emergent PCI for acute myocardial infarction and 5 patients with chronic inflammatory syndromes. The final study population consisted of 58 patients who underwent elective PCI. Iodixanol was used as a contrast agent (Visipaque, GE healthcare, Ireland). The study protocol was approved by the local institutional ethics committee (#TLCV-16-224).

LABORATORY RESULTS

Blood samples for serum NGAL levels were collected at the catheterization laboratory, and prior to contrast media administration (pre-NGAL level) and 24 hours following PCI (post-NGAL level. Samples were centrifuged within 10 minutes using a cooled centrifuge, and plasma and serum were stored at -20°C. NGAL levels were analyzed using NGAL rapid turbidimetric immunoassay (Bioporto Diagnostics, Copenhagen, Denmark). Baseline venous blood samples were used for sCr and high sensitivity C-reactive protein (CRP) level measurements. sCr was also tested repeatedly for 48 hours following PCI. CRP levels were analyzed quantitatively using the Bayer wide-range assay. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [15]. CKD was defined as eGFR ≤ 60 ml/min/1.73 m^2 based on past laboratory reports, admission blood samples, and patient data. CI-AKI was defined using the KDIGO criteria as an increase in sCr by 0.3 mg/dl or more within 48 hours following PCI [16,17].

STATISTICS

Categorical variables were expressed as frequencies and percentages. Distribution of continuous variables was assessed using histogram and quantile-quantile plots. Normally distributed continuous variables were described using mean and standard deviations (SD) and non-normally distributed variables using median and interquartile range (IQR). Chi-square test was used to evaluate association between categorical variables. Continuous variables were compared using the independent sample *t*-test and the Mann–Whitney U test. Spearman's rank correlation was used to measure the correlation between the logarithmic transformations of NGAL and eGFR. Direct comparison between pre- and post-NGAL levels for patients with and without CI-AKI was performed using the paired *t*-test. Receiver operator characteristic (ROC) curve analysis was performed to identify the optimal cutoff point of serum pre- and post-NGAL (at which sensitivity and specificity would be maximal) for the prediction of CI-AKI using the Youden index. Area under the curve (AUC) was calculated as a measure of the accuracy of the tests. A multivariate logistic regression model was used to assess the association between serum NGAL levels and AKI as well as to control for potential confounders. A two-tailed *P*-value < 0.05 was considered significant for all analyses. Statistical analyses were performed using IBM Statistical Package for the Social Sciences statistics software, version 25 (SPSS, IBM Corp, Armonk, NY, USA).

RESULTS

The study included 58 CKD patients who underwent elective PCI (mean age 64 ± 13 years, 82% men), 19 of whom (33%) developed CI-AKI during hospitalization. Baseline characteristics and laboratory results for CKD patients with and without CI-AKI are presented in Table 1. We found no statistically significant differences in age, sex, and co-morbidity in these groups. Importantly, no difference was demonstrated between the groups regarding the amount of contrast media used during PCI. Serum NGAL was significantly and inversely correlated with eGFR (r = -0.58, $P < 0.001$). Table 2 presents the key laboratory findings between patients with and without CI-AKI. Both pre-NGAL (172 vs. 119 ng/ml, $P < 0.001$) and post-NGAL (81 vs. 121 ng/ml, $P < 0.001$) levels were significantly higher in patients with CI-AKI than in patients without CI-AKI. Figure 1 shows a direct comparison between pre- and post-NGAL levels within the two groups. We found no significant changes in the serial NGAL levels, both in those with and without CI-AKI [Figure 1].

According to the ROC curve, pre-NGAL levels demonstrated similar efficacy compared with post-NGAL levels in the prediction of CI-AKI (AUC 0.753 vs. 0.745) [Figure 2], with the

Figure 1. Comparison between pre- and post-NGAL levels for patients with and without CI-AKI

CI-AKI = contrast induced acute kidney injury, NGAL = neutrophil gelatinase-associated lipocalin

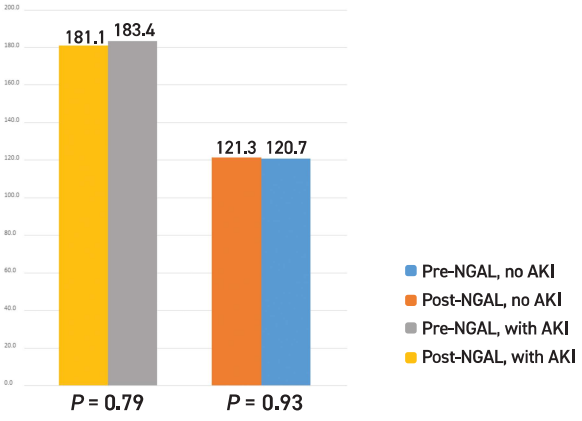


Table 1. Baseline characteristics of 58 chronic kidney disease patients who underwent elective percutaneous coronary intervention according to CI-AKI occurrence

	CI-AKI		*P*-value
	No	Yes	
Total N (%)	n=39	n=19	
Age in years, mean ± SD	78 ± 11	76 ± 9	0.426
Male sex, n (%)	28 (72)	8 (42)	0.374
Diabetes mellitus, n (%)	18 (46)	12 (63)	0.271
Body mass index, median (IQR)	26.4 (5.0)	25.3 (2.7)	0.087
Hyperlipidemia, n (%)	26 (67)	16 (84)	0.217
Family history of IHD, n (%)	6 (15)	4 (10)	1.000
History of smoking, n (%)	18 (46)	9(50)	1.000
Hypertension, n (%)	34 (87)	18(94)	0.653
History of myocardial infarction, n (%)	20 (51)	11 (57)	0.781
Contrast volume, mlm, mean ± SD	142 ± 47	134 ± 48	0.12
LV ejection fraction, mean ± SD	55 ± 8	48 ± 9	0.009

CAD = coronary artery disease, CI-AKI = contrast induced acute kidney injury SD = standard deviation, IHD = ischemic heart disease, LV = left ventricle, SD = standard deviation

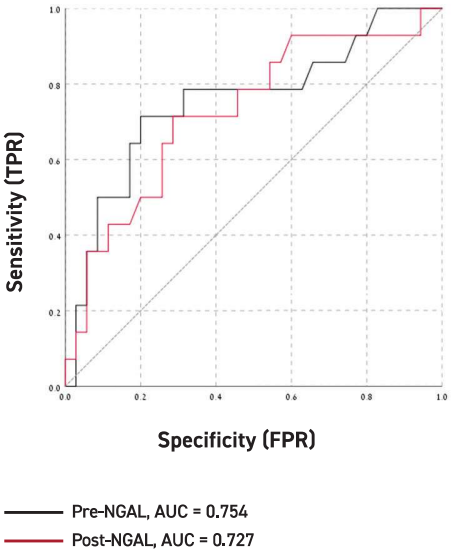
Table 2. Laboratory results of 58 CKD patients who underwent elective PCI according to CI-AKI occurrence

	CI-AKI		P-value
	No	Yes	
Total N (%)	n=39	n=19	
Baseline eGFR (ml/min/1.73 m^2), mean ± SD	45 ± 8	36 ± 9	0.02
Pre-NGAL (ng/ml), mean ± SD	119 ± 50	172 ± 80	0.001
Post-NGAL (ng/ml), mean ± SD	121± 53	182 ± 72	0.008
NGAL change NGAL (ng/ml), mean ± SD	5 ± 9	9 ± 20	0.378
Baseline C-reactive protein (mg/L), mean ± SD	3.9 ± 2.1	4.2 ± 2.3	0.725
White blood cells, mean ± SD	7.8 ± 3.6	8.2 ± 2.9	0.447
Total neutrophils, mean ± SD	5.5 ±3.2	6.1. ± 2.4	0.527

CAD = coronary artery disease, CI-AKI = contrast induced acute kidney injury SD = standard deviation eGFR = estimated glomerular filtration rate, IHD = ischemic heart disease, LV = left ventricle, NGAL = neutrophil gelatinase-associated lipocalin, SD = standard deviation

Figure 2. ROC curve analysis for the prediction of CI-AKI for pre- and post-NGAL levels

AUC = area under the curve, CI-AKI = contrast induced acute kidney injury, NGAL = neutrophil gelatinase-associated lipocalin, ROC = receiver operator characteristic



optimal cutoff value for pre-NGAL to predict CI-AKI being 129 ng/ml (sensitivity of 73% and specificity of 72%, $P < 0.001$) compared with 141 ng/ml (sensitivity of 73% and specificity of 71%, $P < 0.001$) for post-NGAL levels.

Multivariate binary logistic regression demonstrated that post-NGAL levels > 141 ng/ml were independently associated with CI-AKI (hazard ratio [HR] 4.86, 95% confidence interval [95%CI] 1.34–17.64; $P = 0.02$) with a strong trend for post-NGAL levels > 129 ng/ml (HR 3.46, 95%CI 1.23–12.81; $P = 0.06$).

DISCUSSION

We demonstrated for the first time that among CKD patients who underwent elective PCI no significant changes were demonstrated in serial NGAL measurements, even among patients who developed CI-AKI. Furthermore, baseline (pre-NGAL) levels performed similarly to post-NGAL in the prediction of CI-AKI. These findings may point to the unique role and dynamics of NGAL in CKD patients.

NGAL is expressed in granulocytes, epithelial cells, hepatocytes, and renal tubular cells and released during injury [18,19], and is now viewed as a multifunctional glycoprotein. Among hospitalized patients, plasma levels of NGAL reflect the sum contribution from several sources, mainly neutrophil leukocytes, representing an acute response to inflammatory process,

and renal tubular cells, in response to structural damage [20]. It seems that the prognostic/diagnostic value of NGAL is dependent on the specific clinical setting. Lindberg and colleagues demonstrated that in a large cohort of non-hospitalized patients, the cumulative neutrophil count and serum CRP level were the main determinants of NGAL in the plasma. In this population, eGFR was associated with plasma NGAL mainly in patients whose eGFR was <60 ml/kg [21]. Similarly, we previously demonstrated that among CKD patients admitted with myocardial infarction, baseline plasma levels were elevated compared with no-CKD patients [22], and that among CKD patients baseline plasma NGAL correlated with eGFR. Accordingly, it appears that among healthy humans, plasma NGAL mainly reflects inflammation, whereas the kidney only contributes to plasma NGAL in the setting of chronic kidney disease or AKI. In the current cohort of elective patients who underwent PCI baseline, CRP levels were within the normal range, thus negating the possible effect of ongoing, low-grade inflammation on serum NGAL level.

NGAL is synthesized in response to kidney damage followed by glomerular filtration and tubular uptake, and it could be produced locally by injured tubules.

Previous reports have suggested the possible role of serum NGAL for early diagnosis of CI-AKI in patients with normal renal function [23,24]. Following contrast exposure, the presence of CKD is one of the most important risk factors for renal injury, with up to 50% of CKD patients developing CI-AKI [9,10]. In these particularly vulnerable patients, early identification of those who may be at risk is important. Furthermore, as the treatment of CI-AKI treatment, once established, is limited, preventive measures could help limit renal damage and reduce mortality in these high-risk patients.

We demonstrated that among CKD patients who underwent primary PCI, baseline serum NGAL level performed better than eGFR for the prediction of CI-AKI [13]. In this cohort of patients hospitalized with an acute myocardial infarction, serum levels of NGAL could have also represented the acute inflammatory result (reflected also by the elevated CRP levels). Elective patients (reflected by the normal CRP levels) lacked significant changes in serum NGAL levels following PCI, which may point to the specific role of NGAL in CKD patients who underwent elective procedures. The fact that no significant changes were detected in serial measurements may point to different response of renal tubules to injury among CKD patients, representing lower synthetic reserve, glomerular filtration rate, reduced tubular uptake, or their combination. In CKD patients with elevated baseline NGAL levels, in addition to pre-PCI-hydration, we can delay the use of potentially nephrotoxic drugs such as nonsteroidal anti-inflammatory drugs and angiotensin-converting enzyme inhibitors/ angiotensin-II receptor blockers.

The current study has notable limitations. This single center study included a modest sample size. While we used serum NGAL measurements, the addition of urinary NGAL to the analysis would have strengthened our conclusions. Nevertheless, in CKD patients, urinary NGAL does not seem to have any predictive value for the identification of those who develop CI-AKI [25]. Based on this data, in CKD patients with plasma, NGAL may be a better marker of CI-AKI than urinary NGAL. Last, AKI diagnosis based on sCr may underestimate renal injury. The Acute Dialysis Quality Initiative reported a combination of kidney functional (sCr) and damage markers (new biomarkers including NGAL) to stratify patients with acute kidney damage. Subclinical AKI can be diagnosed using only damage markers, such as NGAL, even when no change in sCr is observed.

CONCLUSIONS

Among CKD patients who underwent elective PCI, we detected no significant changes in serial NGAL levels. In these high-risk patients, baseline (pre-NGAL) levels may be used with similar efficacy as post-NGAL levels to predict CI-AKI, as well as for the early diagnosis of acute renal dysfunction. Further studies on larger populations are needed to validate the potential utility of NGAL measurements in CKD patients.

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References

1. Marenzi G, Assanelli E, Campodonico J, et al. Acute kidney injury in ST-segment elevation acute myocardial infarction complicated by cardiogenic shock at admission. *Crit Care Med* 2010; 38 (2): 438-44.
2. Shacham Y, Leshem-Rubinow E, Steinvil A, et al. Renal impairment according to acute kidney injury network criteria among ST elevation myocardial infarction patients undergoing primary percutaneous intervention: a retrospective observational study. *Clin Res Cardiol* 2017; 103 (7): 525-32.
3. Corbacıoglu SK, Cevik Y, Akinci E, et al. Value of plasma neutrophil gelatinase-associated lipocalin (NGAL) in distinguishing between acute kidney injury (AKI) and chronic kidney disease (CKD). *Turk J Emerg Med* 2017; 17 (3): 85-8.
4. Bolignano D, Coppolino G, Campo S, et al. Urinary neutrophil gelatinase-associated lipocalin (NGAL) is associated with severity of renal disease in proteinuric patients. *Nephrol Dial Transplant* 2008; 23 (1): 414-16.
5. Bolignano D, Coppolino G, Campo S, et al. Neutrophil gelatinase-associated lipocalin in patients with autosomal-dominant polycystic kidney disease. *Am J Nephrol* 2007; 27 (4): 373-8.
6. Bolignano D, Lacquaniti A, Coppolino G, Campo S, Arena A, Buemi M. Neutrophil gelatinase-associated lipocalin reflects the severity of renal impairment in subjects affected by chronic kidney disease. *Kidney Blood Press Res* 2008; 31 (4): 255-8.
7. Lindberg S, Jensen JS, Hoffmann S, et al. Plasma neutrophil gelatinase-associated lipocalin reflects both inflammation and kidney function in patients with myocardial infarction. *Cardiorenal Med* 2016; 6 (3):180-90.
8. Lindberg S, Jensen JS, Hoffmann S, et al. Plasma neutrophil gelatinase-associated lipocalin (NGAL) in the general population: association with inflammation and prognosis. *Arterioscler Thromb Vasc Biol* 2014; 34: 2135-42.
9. Margolis G, Gal-Oz A, Letourneau-Shesaf S, Khoury S, Keren G, Shacham Y. Acute kidney injury based on the KDIGO criteria among ST elevation myocardial infarction patients treated by primary percutaneous intervention. *J Nephrol* 2018; 31 (3): 423-8.

10. Parikh CR, Coca SG, Wang Y, Masoudi FA, Krumholz HM. Long-term prognosis of acute kidney injury after acute myocardial infarction. *Arch Intern Med* 2008; 168 (9):987-95.
11. Haase M, Bellomo R, Devarajan P, Schlattmann P, Haase-Fielitz A. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. *Am J Kidney Dis* 2009; 54 (6):1012-24.
12. Haase-Fielitz A, Haase M, Devarajan P. Neutrophil gelatinase-associated lipocalin as a biomarker of acute kidney injury: a critical evaluation of current status. *Ann Clin Biochem* 2014; 51 (Pt 3): 335-51.
13. Lupu L, Abukatash H, Banai A, et al. Relation of baseline neutrophil gelatinase-associated lipocalin (ngal) levels and contrast-induced nephropathy following percutaneous coronary intervention among chronic kidney disease patients. *J Clin Med* 2021; 10 (22): 5403.
14. Abella V, Scotece M, Conde J, et al. The potential of lipocalin-2/NGAL as biomarker for inflammatory and metabolic diseases. *Biomarkers* 2015; 20 (8): 565-71
15. Parikh CR, Coca SG, Wang Y, Masoudi FA, Krumholz HM. Long-term prognosis of acute kidney injury after acute myocardial infarction. *Arch Intern Med* 2008; 168 (9): 987-95.
16. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; 150 (9): 604-12.
17. Kellum JA, Lameire N. Diagnosis, evaluation, and management of acute kidney injury: a KDIGO summary (Part 1). *Crit Care* 2013; 17 (1): 204.
18. Mori K, Lee H, Rapoport D, et al. Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. *J Clin Invest* 2005; 115: 610-21.
19. Xu S, Venge P. Lipocalins as biochemical markers of disease. *Biochim Biophys Acta* 2000; 1482: 298-307.
20. Devarajan P. Neutrophil gelatinase-associated lipocalin - an emerging troponin for kidney injury. *Nephrol Dial Transplant* 2008; 23: 3737-43.
21. Lindberg S, Jensen JS, Hoffmann S, et al. Plasma neutrophil gelatinase-associated lipocalin (NGAL) in the general population: association with inflammation and prognosis. *Arterioscler Thromb Vasc Biol* 2014; 34: 2135-42
22. Banai A, Rozenfeld KL, Levit D, et al. Neutrophil gelatinase-associated lipocalin (NGAL) for the prediction of acute kidney injury in chronic kidney disease patients treated with primary percutaneous coronary intervention. *Int J Cardiol Heart Vasc* 2020; 32: 100695.
23. Hirsch R, Dent C, Pfriem H, et al. NGAL is an early predictive biomarker of contrast-induced nephropathy in children. *Pediatr Nephrol* 2007; 22 (12): 2089-95.
24. Bachorzewska-Gajewska H, Malyszko J, Sitniewska E, et al. Could neutrophilgelatinase-associated lipocalin and cystatin C predict the development of contrast-induced nephropathy after percutaneous coronary interventions in patients with stable angina and normal serum creatinine values? *Kidney Blood Press Res* 2007, 30 (6):408-15.
25. Koyner JL, Vaidya VS, Bennett MR, et al. Urinary biomarkers in the clinical prognosis and early detection of acute kidney injury. *Clin J Am Soc Nephrol* 2010; 5 (12):2154-65.

Art is the elimination of the unnecessary.

Pablo Picasso (1881–1973), Spanish painter, sculptor, printmaker, ceramicist and theatre designer painter and sculptor

Genius is the gold in the mine, talent is the miner who works and brings it out.

Marguerite Gardiner (1789–1849), Countess of Blessington, Irish novelist, journalist, and literary hostess

Capsule

Gut microbial metabolism of 5-ASA diminishes its clinical efficacy in inflammatory bowel disease

For decades, variability in clinical efficacy of the widely used inflammatory bowel disease (IBD) drug 5-aminosalicylic acid (5-ASA) has been attributed, in part, to its acetylation and inactivation by gut microbes. Identification of the responsible microbes and enzyme(s), however, has proved elusive. To uncover the source of this metabolism, **Mehta** et al. developed a multi-omics workflow combining gut microbiome metagenomics, metatranscriptomics, and metabolomics from the longitudinal IBDMDB cohort of 132 controls and patients with IBD. This associated 12 previously uncharacterized microbial acetyltransferases with 5-ASA inactivation, belonging to two protein superfamilies: thiolases and acyl-CoA *N*-acyltransferases. In vitro characterization of representatives from both families confirmed the ability of these enzymes to acetylate 5-ASA. A cross-sectional analysis within the discovery cohort and subsequent prospective validation within the independent SPARC IBD cohort (n = 208) found three of these microbial thiolases and one acyl-CoA *N*-acyltransferase to be epidemiologically associated with an increased risk of treatment failure among 5-ASA users. Together, these data address a longstanding challenge in IBD management, outline a method for the discovery of previously uncharacterized gut microbial activities and advance the possibility of microbiome-based personalized medicine.

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