

2023

Oxidation-reduction potential as an indicator of disease activity in pediatric patients with inflammatory bowel disease

<https://hdl.handle.net/2144/47080>

"Downloaded from OpenBU. Boston University's institutional repository."

BOSTON UNIVERSITY

ARAM V. CHOBANIAN & EDWARD AVEDISIAN SCHOOL OF MEDICINE

Thesis

**OXIDATION-REDUCTION POTENTIAL AS AN INDICATOR OF DISEASE
ACTIVITY IN PEDIATRIC PATIENTS WITH INFLAMMATORY BOWEL
DISEASE**

by

GIULIO F. CATALDO

B.S., Worcester Polytechnic Institute, 2020

Submitted in partial fulfillment of the
requirements for the degree of
Master of Science

2023

© 2023 by
Giulio F. Cataldo
All rights reserved

Approved by

First Reader

Carol T. Walsh, Ph.D.
Professor of Pharmacology & Experimental Therapeutics

Second Reader

Paul A. Rufo, MD, MMSc
Assistant Professor of Pediatrics
Harvard Medical School

DEDICATION

This is dedicated to all of the patients whose data appear on these pages.

ACKNOWLEDGMENTS

To Dr. Rufo, thank you for teaching me so much about the practice of clinical medicine, and for the stellar movie recommendations along the way. I hope to be half the physician you are someday.

To Dr. Walsh, your thorough feedback has sharpened my skills and pushed me to improve my skills as a researcher, and thank you for showing me the joys of pharmacology.

To Nathan, Aravindh, Justin, and especially Bernadette, this thesis would be nothing without your previous work on this project.

To Afi, Subear, and Allie, thank you for being the best lab partners I could dream of having.

To the nurses who collected samples for us, this project would have been impossible without your help.

To my parents, who always exemplify love of their fellow man through expert patient care. You both inspire me in ways I can't put into words.

To my sisters, it would feel weird to not include you here, so I hope you all feel acknowledged.

To all of my friends who reminded me that there are fun things to do when school is not in session.

To Cleopatra, you're the best pet gecko a boy could ask for.

**OXIDATION-REDUCTION POTENTIAL AS AN INDICATOR OF DISEASE
ACTIVITY IN PEDIATRIC PATIENTS WITH INFLAMMATORY BOWEL
DISEASE**

GIULIO F. CATALDO

ABSTRACT

INTRODUCTION: Inflammatory bowel disease (IBD) is a complex, chronic, autoimmune disease of the gastrointestinal tract. Reactive oxygen species (ROS), a product of active leukocytes, have been implicated in the pathogenesis of IBD. The ability to reliably measure ROS in blood, urine, and stool samples could represent a new approach to assessing disease activity and response to therapy in pediatric patients with IBD.

OBJECTIVES: To assess the relationship between redox measurements and clinical disease activity in pediatric patients with IBD.

METHODS: Biological specimens, including stool, urine, blood plasma, and intestinal aspirates, were collected from patients at Boston Children's Hospital. Each sample's oxidation-reduction potential was measured by two oxidation-reduction potential probes (an Arrowdox probe and a Mettler Toledo probe). Probes were directly immersed into the sample, returning a millivolt measurement of oxidation-reduction potential. Linear regression was performed to explore the relationship between patient-reported outcome measures (PROMs) and redox measurements of biological specimens. Patients were also stratified by disease severity, and ANOVA testing was performed to test for differences

in oxidation-reduction potential observed in patients with remittent, mild, moderate, and severe disease activity.

RESULTS: Redox values in stool, urine, plasma, and intestinal aspirate did not significantly correlate with PROMs or differ significantly among groups categorized by disease severity.

CONCLUSIONS: Measurements of oxidation-reduction potential from stool, urine, plasma, and intestinal aspirate do not appear to be useful for assessing disease severity in pediatric patients with inflammatory bowel disease.

TABLE OF CONTENTS

DEDICATION	iv
ACKNOWLEDGMENTS	v
ABSTRACT	vi
TABLE OF CONTENTS.....	viii
LIST OF TABLES	x
LIST OF FIGURES	xiv
LIST OF ABBREVIATIONS.....	xv
INTRODUCTION	1
Inflammatory Bowel Disease.....	1
Measuring Disease Activity.....	5
PROMs.....	5
Laboratory Biomarkers	9
Oxidation-Reduction Potential	9
AIMS	12
MATERIALS AND METHODS.....	13
Patient Identification and Recruitment	13
Probe Measurement	14
Sample Processing and Testing	15
Subdivision of Patients	17
Statistical Analysis.....	17
RESULTS	19

Enrollment and Demographics	19
Probe Measurement Correlation	22
Localization of Oxidation-Reduction Potential	23
Oxidation-Reduction Potential and Disease Severity Assessed by PROMs	25
Redox and Other Laboratory Values	37
DISCUSSION	40
CONCLUSION	45
BIBLIOGRAPHY	47
CURRICULUM VITAE.....	56

LIST OF TABLES

Table 1: Montreal Classification of Crohn’s Disease (Silverberg, 2005).....	4
Table 2. PUCAI Scoring Criteria (Turner, 2009)	7
Table 3: Disease severity cutoffs for PUCAI and PCDAI (Dotson, 2016; Turner, 2009)	21
Table 4: The frequency of each disease severity category (as assigned by PUCAI and PCDAI). This shows severity for each disease separately and the frequency of each severity category in the overall study population.	22
Table 5: Pearson correlation data between the Arrowdox and Mettler Toledo probes for a given biological sample.	22
Table 6: Pearson correlation coefficients for the redox values of different biological samples from all enrolled patients as measured with the Arrowdox probe	24
Table 7: Pearson correlation coefficients for the redox values of different biological samples from all enrolled patients as measured with the Mettler Toledo probe	24
Table 8: A summary of Pearson correlation coefficients for patient-reported disease severity (as indicated by PUCAI or PCDAI) vs. oxidation-reduction potential in mV of a given sample type measured with the Arrowdox Probe.	26
Table 9: A summary of Pearson correlation coefficients for patient-reported disease severity (as indicated by PUCAI or PCDAI) vs. oxidation-reduction potential in mV of a given sample type measured with the Mettler Toledo Probe.....	27
Table 10: A summary of normality tests used to see if a sample oxidation-reduction potential is normally distributed within a severity category. If any severity categories for a given sample have a significant p-value ($p < 0.05$), then the category is considered to not be normally distributed, and an independent-samples Kruskal-Wallis test is needed. ORP values were measured with the Arrowdox probe.....	28
Table 11: A summary of normality tests used to see if a sample oxidation-reduction potential is normally distributed within a severity category. If any severity categories for a given sample have a significant p-value ($p < 0.05$), then the category is considered to not be normally distributed, and an independent-samples Kruskal-Wallis test is needed. ORP values were measured with the Mettler Toledo probe. .	29
Table 12: Summary of one-way ANOVA performed for samples from all IBD patients whose severity categories are normally distributed. No significant differences in	

sample redox values were found based on severity categories. The redox values were measured with the Arrowdox probe.	29
Table 13: Summary of one-way ANOVA performed for samples from all IBD patients whose severity categories are normally distributed. No significant differences in sample redox values were found based on severity categories. The redox values were measured with the Mettler Toledo probe.	30
Table 14: Summary of Independent-Samples Kruskal-Wallis Tests performed for each sample. No significant differences in sample oxidation-reduction potential were found based on severity categories. The redox values were measured with the Arrowdox probe.	30
Table 15: Summary of Independent-Samples Kruskal-Wallis Tests performed for each sample. No significant differences in sample oxidation-reduction potential were found based on severity categories. The redox values were measured with the Mettler Toledo probe.	30
Table 16: A summary of normality tests used to see if a sample oxidation-reduction potential is normally distributed within a severity category for UC patients only. If any severity categories for a given sample have a significant p-value ($p < 0.05$), then the category is considered to not be normally distributed, and an independent-samples Kruskal-Wallis test is needed. ORP values were measured with the Arrowdox probe.	32
Table 17: A summary of normality tests used to see if a sample oxidation-reduction potential is normally distributed within a severity category for UC patients only. If any severity categories for a given sample have a significant p-value ($p < 0.05$), then the category is considered to not be normally distributed, and an independent-samples Kruskal-Wallis test is needed. ORP values were measured with the Mettler Toledo probe.	33
Table 18: A summary of normality tests used to see if a sample oxidation-reduction potential is normally distributed within a severity category for CD patients only. If any severity categories for a given sample have a significant p-value ($p < 0.05$) then the category is considered to not be normally distributed, and an independent-samples Kruskal-Wallis test is needed. ORP values were measured with the Arrowdox probe.	34
Table 19: A summary of normality tests used to see if a sample oxidation-reduction potential is normally distributed within a severity category for CD patients only. If any severity categories for a given sample have a significant p-value ($p < 0.05$), then the category is considered to not be normally distributed, and an independent-	

samples Kruskal-Wallis test is needed. ORP values were measured with the Mettler Toledo probe.	35
Table 20: Summary of one-way ANOVA performed for samples from UC patients whose severity categories are normally distributed. No significant differences in sample redox values were found based on severity categories. The redox values were measured with the Arrowdox probe.....	35
Table 21: Summary of one-way ANOVA performed for samples from UC patients whose severity categories are normally distributed. No significant differences in sample redox values were found based on severity categories. The redox values were measured with the Mettler Toledo probe.	36
Table 22: Summary of one-way ANOVA performed for samples from CD patients whose severity categories are normally distributed. No significant differences in sample redox values were found based on severity categories. The redox values were measured with the Arrowdox probe.....	36
Table 23: Summary of one-way ANOVA performed for samples from CD patients whose severity categories are normally distributed. No significant differences in sample redox values were found based on severity categories. The redox values were measured with the Mettler Toledo probe.	36
Table 24: Summary of Independent-Samples Kruskal-Wallis Tests performed for non-normally distributed samples for UC patients only. No significant differences in sample oxidation-reduction potential were found based on severity categories. The redox values were measured with the Arrowdox probe.....	36
Table 25: Summary of Independent-Samples Kruskal-Wallis Tests performed for non-normally distributed samples for UC patients only. No significant differences in sample oxidation-reduction potential were found based on severity categories. The redox values were measured with the Mettler Toledo probe.	37
Table 26: Summary of Independent-Samples Kruskal-Wallis Tests performed for non-normally distributed samples for CD patients only. No significant differences in sample oxidation-reduction potential were found based on severity categories. The redox values were measured with the Arrowdox probe.....	37
Table 27: Summary of Independent-Samples Kruskal-Wallis Tests performed for non-normally distributed samples for CD patients only. No significant differences in sample oxidation-reduction potential were found based on severity categories. The redox values were measured with the Mettler Toledo probe.	37

Table 28: A summary of Pearson correlation coefficients for laboratory biomarkers vs. oxidation-reduction potential of a given sample type measured with the Arrowdox probe.	38
Table 29: A summary of Pearson correlation coefficients for laboratory biomarkers vs. oxidation-reduction potential of a given sample type measured with the Mettler Toledo probe.	39

LIST OF FIGURES

Figure 1: Comparing and contrasting common features of ulcerative colitis and Crohn's disease.	3
Figure 2: The Nernst equation, as used to calculate oxidation-reduction potential of a sample (Bier, 2009).....	11
Figure 3: Enrollment flow diagram.....	20
Figure 4: A visual representation of the relationship between PUCAI and stool oxidation-reduction potential score (n=56).	25
Figure 5: A box plot of stool redox values, in mV, measured with the Arrowdox probe for control and IBD disease severity categories. The horizontal black line is the median and the blue-shaded region is the interquartile range (25 th percentile to 75 th percentile). Open circles and asterisks are outlier data points.	31

LIST OF ABBREVIATIONS

ANCA	Antineutrophil Cytoplasmic Autoantibodies
ASCA	Anti-Saccharomyces Cerevisiae Antibodies
Ax	Arrowdox ORP Probe
BCH	Boston Children's Hospital
CD	Crohn's Disease
GI	Gastrointestinal
IBD	Inflammatory Bowel Disease
IC	Indeterminate Colitis
IPAA	Ileal Pouch-Anal Anastomosis
MT	Mettler Toledo ORP Probe
mV	Millivolt
ORP	Oxidation-Reduction Potential
PCDAI	Pediatric Crohn's Disease Activity Index
PROM	Patient Reported Outcome Measure
PUCAI	Pediatric Ulcerative Colitis Activity Index
UC	Ulcerative Colitis

INTRODUCTION

Inflammatory Bowel Disease

Inflammatory Bowel Disease (IBD) describes chronic inflammatory disorders of the gastrointestinal tract that manifest as periods of quiescent (remittance) and active (relapse) disease (Nakase, 2021). IBD can be subcategorized into two main diseases: Crohn's disease (CD) and ulcerative colitis (UC). The gold standard for diagnosing IBD is a colonoscopy and/or endoscopy (Mitchell, 2007).

The incidence of IBD is increasing in both the developed and developing world (Kaplan, 2021). The global rise in rates makes it imperative that research is done to better understand the etiology of the disease, which would afford clinicians more opportunities to better prevent, predict, and treat remitting disease. The effects of IBD fall disproportionately on children, who may suffer from severe pain, malnutrition, anemia, delays in linear growth and/or physical maturity, and psychosocial maladjustment.

The inflammation in patients with ulcerative colitis is limited to the colon, typically begins in the rectum, and extends in a contiguous fashion to involve part or all (pancolitis) of the colon (Mitchell, 2007). The inflammation involves only the mucosal lining, with deeper layers of the colon unaffected (Spiceland, 2018). The disease appears to be mediated primarily by the recruitment and activity of neutrophils (Xavier, 2007). Strictures, fistulas, and perianal involvement are uncommon in patients with classic UC (Choi, 2018).

In contrast, inflammation in patients with Crohn's Disease can be found anywhere in the gastrointestinal (GI) tract, from mouth to anus (Mitchell, 2007). Unlike the

contiguous pattern found in patients with UC, the inflammation in patients with Crohn's disease is often patchy, with involved segments separated by otherwise normal-appearing mucosa. The most common location for CD is in the ileocecal region, where the small and large intestines meet. The penetrating nature of Crohn's disease can cause full-thickness inflammation involving the entire bowel wall (Nikolaus, 2007). Similarly, transmural inflammation can result in fistulae to other bowel segments, hollow organs, or the skin (especially perianally). Another unique characteristic of CD is its ability to form strictures, which can cause recurrent bowel obstructions in some patients. Histologically, Crohn's disease and ulcerative colitis can be differentiated by the presence of non-necrotizing granulomas (Nikolaus, 2007; Xavier, 2007; Choi, 2018).

Indeterminate Colitis (IC) is a term used to refer to patients with clinical, endoscopic, and histologic findings that could be consistent with either UC or CD. Nonetheless, accurately classifying a patient's disease can be extremely important to understand the best long-term medical and surgical course (Nikolaus, 2007). For example, a patient with medically refractory UC may be an excellent candidate for colectomy and subsequent ileal pouch-anal anastomosis (IPAA) as their disease is confined to the colon. However, the diffuse and unpredictable geographic involvement in patients with Crohn's disease relegates surgery to palliation and not cure (Frizelle, 2001). Diagnostic tools that would help clinicians to better understand and differentiate patients with UC, CD, and IC would be valuable.

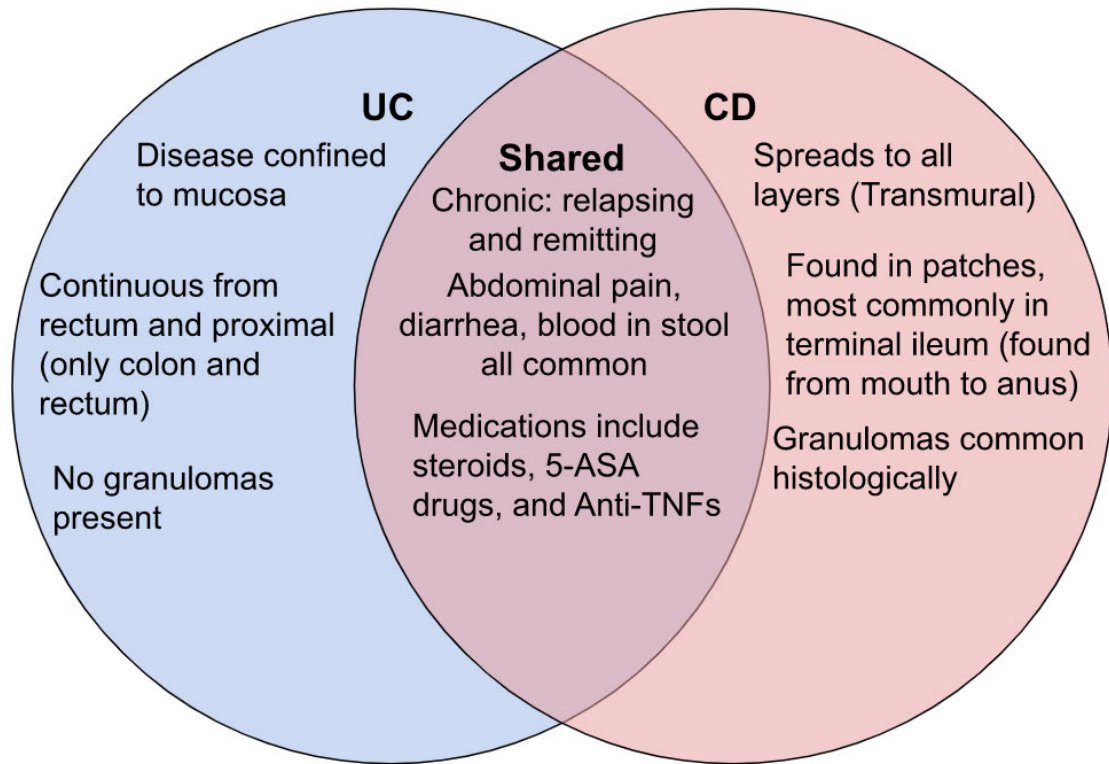


Figure 1: Comparing and contrasting common features of ulcerative colitis and Crohn's disease.

IBD is a heterogeneous disease and the differences observed with respect to clinical course and response to therapy are impacted by disease subtype, location of disease (Nikolaus, 2007), and genetic factors (Henderson, 2015). Variation can affect symptoms and biochemical markers of disease activity (Alper, 2017). Uniform consensus metrics that permit reliable disease classifications are essential for both clinical care and translational research. The Montreal Classification, as seen in Table 1, classifies disease activity based on age at diagnosis, location of disease, and anatomic changes the disease may cause, such as stricturing or fistulizing (Silverberg, 2005). As such, a patient with a patch of disease in her esophagus, another patch in her ileum, and fistulae would be

classified as L1+L4 B3 disease. A different patient with stricturing disease of the colon and perianal features would be classified as an L2 B2p disease. While these patients both fall under the diagnostic umbrella of Crohn's disease, they would likely benefit from differing medical and surgical approaches (Nikolaus, 2007).

Criteria	Class	Description
Age At Diagnosis	A1	≤16 Years Old
	A2	17-40 Years Old
	A3	≥40 Years Old
Location of Disease	L1	Ileal
	L2	Colonic
	L3	Ileocolonic
	L4	Isolated Upper Disease (Can be used alongside L1-L3)
Behavior of disease	B1	Non-Stricturing, Non-Penetrating
	B2	Stricturing
	B3	Penetrating
	p	Perianal Disease Modifier

Table 1: Montreal Classification of Crohn's Disease (Silverberg, 2005)

Some investigators contend that Crohn's disease should be further subdivided into two completely different diseases depending on whether the inflammation is confined to

the colon or is primarily in the small bowel (Liu, 2021; Gasche, 2005). New approaches are necessary to understand better the mechanisms driving inflammation and to develop new, novel technologies that will help more readily diagnose patients with IBD and assess their response to prescribed therapy. Some measurement modalities, such as laboratory tests, endoscopic evaluation, or patient questionnaires may prove to be more useful, when evaluating disease activity, in some IBD subtypes than others. Some treatments are also more useful in specific subtypes. Unfortunately, where one disease subtype starts and the next one ends can be difficult to define.

Measuring Disease Activity

Measuring disease activity in patients with IBD can be like aiming at an invisible target (Lewis, 2011). This is, in large part, due to the heterogeneity of the disease. A patient's own opinion of their disease depends on lifestyle. Children attending school, for example, may have limited time to use the bathroom. This becomes more pressing if they wish to participate in sports after school. Considering this subjective lens, it's apparent that the impact of IBD can be very different on different children.

PROMs

Patient-reported outcome measures (PROMs) are standardized metrics used by clinicians and researchers to elicit a patient's experience concerning a particular disease and its associated symptoms. Some of them are purely patient questionnaires, while others may also incorporate objective data, such as findings from a physical exam or results of laboratory testing (Holmes, 2017). Some PROMs are generic and therefore, useful for collecting data related to a variety of conditions. Other PROMs are more

disease-specific. While these measures have been used in clinical practice and research for decades (Fries, 1983), impressions of their utility are far from universal. Some adherents praise their ability to gauge a patient's functional status and the ability to pick the right PROM for the right patient. Detractors note their reliance on subjective responses and worry that individual patients may be misclassified due to reports that over or under-report their symptoms. Another concern related to PROMs is their lack of specificity (Holmes, 2017). For example, patients with IBD and a bacterial infection likely experience looser and more frequent stools. A PROM assessment may inadvertently score their IBD as being more severe than it is due to the clinical impact of the bacterial infection. This is not unique to PROMs, as the interpretation of general serologic markers of inflammation, including ESR and CRP, are limited by the same lack of specificity (Vermeire, 2006).

There are many PROMs used to assess disease severity in patients with IBD. They include broad, non-disease specific quality of life metrics, such as The WHO's International Classification of Functioning, Disability and Health (ICF). In contrast, the pediatric ulcerative colitis activity index (PUCAI) has been validated to assess disease activity in a specific age group and for a particular condition (Nelson, 2005). Similarly, the pediatric Crohn's disease activity index (PDAI) has been validated to assess disease activity in children with CD (Turner, 2009). The net impact of a particular disease or condition is typically assessed by asking patients direct questions that measure the impact it exerts on their daily lives (e.g. severity of abdominal pain). Higher scores reflect diseases or conditions that impact a patient's daily life more severely.

Category	Assessment	Resultant Score
Abdominal Pain	No pain	0
	Pain can be ignored	5
	Pain cannot be ignored	10
Rectal Bleeding	None	0
	Small amount only, in less than 50% of stools	10
	Small amount with most stools	20
	Large amount (>50% of the stool content)	30
Stool consistency of most stools	Formed	0
	Partially formed	5
	Completely unformed	10
Number of stools per 24 hours	0-2	0
	3-5	5
	6-8	10
	>8	15
Nocturnal stools (any episode causing wakening)	No	0
	Yes	10
Activity level	No limitation of activity	0
	Occasional limitation of activity	5
	Severe restricted activity	10

Table 2. PUCAI Scoring Criteria (Turner, 2009)

PUCAI and PCDAI are both reasonably easy to ascertain from a conversation with an inpatient or to reconstruct through careful chart review. PUCAI specifically has only six questions to be answered directly by the patient (Table 2). In contrast, PCDAI incorporates lab values and a physical assessment that must be administered by a skilled clinician (Hyams, 2005). Nonetheless, both metrics are relatively easy to obtain

longitudinally for use in clinical care or research. PUCAI and PCDAI scores are numeric, with higher scores reflecting greater disease severity. PUCAI scores range from 0-85, and PCDAI scores range from 0-100 (Turner, 2009).

While PUCAI and PCDAI are used in clinical practice and research, they are not flawless. They are subject to reporter bias. For example, both measures require a patient to determine if their stool contained a small amount or large amount of blood. For an anxious patient, or someone who is not used to passing bloody stools, the visual appearance of even a small amount of blood can seem magnified. The design of the PUCAI attempts to provide some structure to responses by defining a large amount of blood as “>50% of stool content,” but that is still difficult to quantify visually. Similarly, both PUCAI and PCDAI query activity modification. Sedentary and particularly active patients may be inclined to report a lower or higher score on the PCDAI simply due to their baseline lifestyle expectations.

PCDAI scores also rely on the number of stools per day, which may be less relevant for some patients. While it is true that patients with more active IBD often pass looser and more frequent stools, that is not always the case. Patients with Crohn’s disease may have strictures that cause bowel obstructions. These patients, while quite ill, may pass no stools at all. Similarly, PUCAI and PCDAI scores are difficult to obtain reliably in a patient with an ileostomy or colostomy, as their stool output does not fall into a traditional, discreet, quantifiable stooling pattern. PROM scores do not inform the root cause of a particular issue.

Laboratory Biomarkers

There are no consensus biomarkers for measuring disease activity in patients with IBD (Chen, 2020). The most commonly used serologic biomarkers of inflammation, including erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), can be elevated in the context of any inflammatory or infectious challenge. Some detractors refer to them as “unnecessary toys” (Vermeire, 2006). Nonetheless, most clinicians continue to rely on ESR and CRP measurements in clinical practice (Henderson, 2015). For ESR, the normal cutoff is 20mm/hr, and for CRP, the cutoff is 3mg/L. Higher values signify greater inflammation for both ESR and CRP (Alper, 2017). Serum anti-Saccharomyces cerevisiae (ASCA) and antineutrophil cytoplasmic autoantibodies (ANCA) are newer biomarkers for use in diagnosing IBD. These antibody concentrations can be measured in the blood and stool of patients with IBD. However, the lack of sensitivity and specificity has limited the use of these two biomarkers in clinical practice (Yorulmaz, 2022).

Oxidation-Reduction Potential

Reactive oxygen species are a diverse family of free radical (e.g. O_2^-) and nonradical (e.g. H_2O_2) chemicals that can oxidize other chemical species. The role of reactive oxidation species in disease has been clearly demonstrated in some diseases, such as various cancers (Liou, 2010). Their usage in the body, however, is not restricted to pathology. Reactive oxygen species are critical in paracellular signaling throughout the body. H_2O_2 , can be created internally within one cell and passively diffuse through membranes to affect nearby cells (Circu, 2011). This signaling requires a delicate

chemical balance between the release of reactive oxygen species from the signaling cell and antioxidant species and enzymes present in the receptive cells (Forman, 2014). There is some evidence that manipulating this otherwise tightly regulated balance between oxidant and antioxidant species contributes to the development of diabetes mellitus in mice (Forman, 2014).

Low ambient concentrations of ROS are useful in signaling and play a role in the defense against pathogens. Macrophage and neutrophil components of the innate immune system generate and release significant levels of ROS to defend against pathogens (Bhattacharyya, 2014). ROS can be made and secreted by a number of different cell types, including epithelial cells, endothelial cells, and leukocytes. In the context of chronic inflammation, including IBD or *H. Pylori* infection, resident macrophages and neutrophils release increased concentrations of ROS into the tissue and local milieu (Bhattacharyya, 2014). In this way, it has been hypothesized that reactive oxygen may be either the direct cause or the direct effect of IBD.

The oxidation-reduction potential of the gut has been an area of investigation since the 1940s, when investigators worked to elucidate gut function and the impact of diet on the GI tract (Bergeim, 1945). Data from initial studies were inconclusive or provided incorrect or misleading results concerning gastric oxidation-reduction potential. However, more recent basic science research strides have greatly broadened our understanding of the impact of oxidation-reduction potential on the pathogenesis of autoimmune and gastrointestinal diseases (Bhattacharyya, 2014). Macrophages are a major contributor to reactive oxygen potential (Murata, 2003) and can infiltrate the entire

bowel wall in a patient with active Crohn's disease (Xavier, 2007). In contrast, neutrophils appear to play a larger role in the pathogenesis of ulcerative colitis, particularly during the early phases of the disease (Xavier, 2007). Both macrophages and neutrophils produce ROS. There have been measurements of mucosal tissue that have found increased concentrations of ROS in both UC and CD (Bhattacharyya, 2014). The fact that different diseases have different leukocyte profiles opens the door for ROS to be used as a diagnostic tool if UC or CD have different oxidation-reduction potential readings.

In practice, oxidation-reduction potential is measured by an electronic oxidation-reduction potential (ORP) probe. This probe contains two electrodes, one that is soaked in a potassium chloride (KCl) solution and the other is in the tested solution. A voltage difference across the electrodes can be measured. Using the Nernst equation (Figure 2), a higher voltage corresponds with a higher ratio of oxidative species to reductive species within the sample (Bier, 2009).

$$E = E_o - \frac{R \cdot T}{n \cdot F} \ln \frac{C_{ox}}{C_{red}}$$

WHERE:

- E = measured potential (mV) between the platinum and the reference electrode
- E_o = measured potential (mV) between the platinum and the reference electrode at a concentration of $C_{ox} = C_{red}$
- R = Universal gas constant ($R = 8.314 \text{ J mol}^{-1} \text{ K}^{-1}$)
- T = Temperature in K (Kelvin), where $T \text{ (K)} = 273.15 + t^\circ\text{C}$ and t is the temperature of the measured solution
- F = Faraday constant (96485 C mol^{-1})
- n = electrical charge of the ion
- C_{ox} = oxidant concentration in moles/L
- C_{red} = reductant concentration in moles/L.

Figure 2: The Nernst equation, as used to calculate oxidation-reduction potential of a sample (Bier, 2009).

AIMS

This project aims to explore the many possible uses of stool, urine, blood, and intestinal aspirate oxidation-reduction potential in pediatric patients with inflammatory bowel disease. This includes finding possible differences between

- IBD and Non-IBD patients
- Ulcerative Colitis vs. Crohn's Disease vs. Indeterminant Colitis patients
- IBD patients with different severity of disease (remission vs. mild vs. moderate vs. severe disease)

This project also aims to see if stool, blood, and urine all provide useful (and comparable) data on oxidation-reduction potential. The results from this project may be useful in both initial patient diagnosis, predicting a possible flare, and population health monitoring.

MATERIALS AND METHODS

This project has been conducted by Dr. Rufo's research group in the Inflammatory Bowel Disease Center at Boston Children's Hospital in Boston, Massachusetts. The team was composed of graduate and undergraduate research students and was directed by Dr. Paul Rufo. The project began enrollment in 2018 (Weinbren, 2019), and has continued to the present (Ajithkumar, 2020; Ramos, 2021; Lu, 2022). Data collection for this thesis ceased in December 2022.

While oxidation-reduction potential has been used in biomedical (Bhattacharyya, 2014) and gastrointestinal (Ikeda, 2023) applications, its application in pediatric IBD patients remains an unexplored area. As such, we cast a wide net to capture a wide variety of patients in a wide variety of clinical circumstances. Patients were only excluded during screening if they were under two years old, were unable to consent (for example, due to a language barrier), or declined to participate. An upper age limit was not set, though being at a children's hospital effectively ensured all patients were under 25. All of the patients were recruited in person on the main campus of Boston Children's Hospital.

Patient Identification and Recruitment

All potential study subjects were screened using PowerChart, the hospital's electronic medical record system. Recruited patients generally fell into three categories: hospitalized patients, those coming in for an ambulatory visit, or patients completing scheduled endoscopic evaluations. Whenever possible, we attempted to collect contemporaneous blood, urine, and stool specimens from each enrolled patient.

Patients who were 18 and older provided their own consent. Patients who were over 12 but under 18 provided assent, acknowledging their desire to participate, and their parents/guardians provided the formal consent. In all instances, wet ink signatures were obtained on an IRB-approved consent form before any samples were collected. Consent forms were scanned into Boston Children's Hospital's central repository for research documents: "Children's Hospital Electronic Research Portal" (CHERP).

Inpatients were asked for stool and urine samples. These samples were collected at any time during their hospitalization. Samples were promptly refrigerated by nursing staff and were processed by our team within 24 hours. Ambulatory patients were asked for a urine sample, and if they were already having a blood draw as part of their routine care, an extra research tube was also sent for this study. Patients undergoing an endoscopic evaluation were asked for urine, blood, and cecal aspirate if possible. Cecal aspirates (also referred to more generally as intestinal aspirates) were collected from the material pooled in the cecum, the portion of the large intestine that connects with the small intestine. The physician performing the colonoscopy used a suction feature on the colonoscope to remove that fluid whenever it was present. In some circumstances, patients had a prior ileocectomy and had no cecum and in others no fluid was present. In other cases, no fluid was present in the cecum for collection.

Probe Measurement

An Arrowdox probe (Ax) and a Mettler Toledo probe (MT) were used in tandem. While the Arrowdox was the primary means of measurement, the MT probe provided a source of external validity and would ensure that other labs in the future could replicate

our general results without the need for using a specific (Arrowdox) probe. Both probes should produce similar results. Their most common application, currently, is in agricultural and environmental engineering applications to measure the oxidation-reduction potential of soil and water (Husson, 2013). The probe's output was measured in millivolts (mV), as mentioned in the "Oxidation-Reduction Potential" subsection of this thesis's introduction.

Before sample testing began on any given day, each probe was tested in ZoBell's ORP/Redox Standard Solution to ensure the probes were functioning correctly by measuring the redox potential of a standard solution. The Arrowdox probe consistently measured out of range (approximately 270 mV instead of 222-232 mV) for the last few months of data collection. Attempts to troubleshoot this variance with the manufacturer were unsuccessful. This period lasted less than five months over the life of a four year project and we do not anticipate these values will alter the overall trends in the data collected. Both probes were used for all samples, and a Pearson correlation was performed to examine the correlation between probes, as seen in the "Probe Measurement Correlation" subsection of the Results section.

Sample Processing and Testing

Previous work in this project explored the utility, efficacy, and replicability of different sample preparations, optimizing the protocols for collecting, storing, and measuring samples (Ajithkumar, 2020). Direct measurement obtained from formed stool led to inconsistent redox measurements, even when measured from within the same sample. However, more consistent results were obtained when stool samples were first

homogenized in deionized water, spun in a centrifuge, and the supernatant was subjected to redox measurement (Ramos, 2021). Similarly, redox measurements from whole blood samples were unreliable due to clotting. Sampling just blood plasma prevented that issue (Ramos, 2021). Ultimately, stool supernatant, urine, blood plasma, and cecal aspirate were determined to be the best samples to test (Lu, 2022). Prior work also found that freezing and thawing affected results (Weinbren, 2019). As such, all samples were refrigerated and tested within 24 hours of their production. Blood was obtained when it could be tested within an hour, as a longer interval may incur clotting and an inability to process and test the sample reliably.

When sampling stool, 0.1 mg of stool was placed in a conical tube with 1.0 mL of deionized water. The tube was vortexed to homogenize the liquid, then spun in a centrifuge at 7500 rotations per minute (RPM) at 4°C for 15 minutes. The supernatant was decanted to be measured. In some circumstances when a patient had watery diarrhea it was measured directly. This was determined qualitatively; if the sample was too watery to scoop into a centrifuge tube, then it would be measured directly.

Urine and cecal aspirates were measured directly by placing the probe in the sample cup where it was collected.

4 mL of blood was collected in lavender top tubes and kept on ice during all transport and processing. Lavender top tubes (also commonly referred to in the literature and clinic as purple top tubes) contain a standard amount of the calcium-chelating anticoagulant ethylenediaminetetraacetic acid (EDTA) that slows the clotting process of blood (Stanford Blood Center, 2021). The tubes were then inverted several times to mix

the blood, after which two 1.5 mL aliquots were placed in conical tubes. Tubes were spun in a pre-chilled centrifuge at 7500 rotations per minute (RPM) at 4°C for 15 minutes. Plasma was pipetted out and measured directly.

All samples were measured in triplicate, with the probe being washed with BioTrue contact solution and rinsed in deionized water between each measurement (Lu, 2022).

All biological samples were handled in contained laboratory spaces in accordance with their biosafety level. Steps were taken to minimize aerosol production with these biological samples. All samples were deidentified and disposed into proper hazard receptacles and the laboratory space was cleaned with 10% bleach and 70% ethanol. The laboratory passed its hospital-mandated Annual Laboratory Safety and Compliance Audit in October 2022.

Subdivision of Patients

First, patients were subdivided into two categories for analysis: patients with IBD and control patients. These control patients were further subdivided into those with a gastrointestinal indication for their time in the hospital (e.g. constipation) and those without a GI indication (e.g. scoliosis). The IBD patients were stratified by both disease type and severity.

Statistical Analysis

Samples were measured in triplicate, and the arithmetic mean (average) was used for analysis. Pearson correlation coefficients were determined to test the possible correlation of two continuous variables. The closer the absolute value of the correlation

coefficient to 1, the stronger the correlation. In some cases, graphs of the linear regression will be shown to visualize relationships. Linear regression was performed to see if a standard increase in PUCAI or PCDAI score causes a predictable increase in oxidation-reduction potential. If strong and significant correlations are found, then oxidation-reduction potential may be useful to directly predict PUCAI or PCDAI in the future. Any correlation was considered significant if its p-value is less than 0.05. This cutoff is seen as standard in scientific publications (Grabowski, 2016). Since this work is some of the first done exploring the use of redox in pediatric IBD patients, this project is largely hypothesis generating. A lower cutoff (e.g. $p < 0.01$) may have been less likely to suffer from type 1 error, but also may be overly restrictive at this stage in research.

When examining differences between multiple categorical variables (e.g. comparing mild disease to severe disease) an analysis of variance (ANOVA) was performed. First, a Shapiro-Wilk normality test determined if all categories were composed of normally distributed values. In this case, a one-way ANOVA was performed. If data were not normally distributed, then an independent-samples Kruskal-Wallis Test was performed. This non-parametric test is by rank and can accommodate for both outliers and skewed data. If the ANOVA indicates a significant difference among groups ($p < 0.05$), then an appropriate ad hoc test was performed. All statistics were performed with IBM® SPSS® Version 27.0.1.0

RESULTS

Enrollment and Demographics

Patients were recruited widely from multiple locations within the hospital by the Rufo Redox team, as detailed in the methods section of this thesis. At the time of enrollment, each patient was qualitatively considered to have active or inactive disease. This was determined by the enrolling coordinator, based in large part on whether a patient was admitted as an inpatient requiring acute management or seen in the ambulatory clinic for routine follow-up. Later, the qualitatively determined, subjective, and dichotomous designation would be supplanted by the use of PUCAI or PCDAI score to understand disease severity more granularly.

Due to a variety of factors, some patients were enrolled in the study but did not provide an analyzable sample. While the reason for failure to provide a sample was not specifically recorded, many were due to patients being discharged before being able to provide one.

An enrollment flow diagram (Figure 3) was used to both visualize enrollment and explore if there were any differential rates of enrollment without providing a sample. The percentages within the “No Sample Provided” side reflect the percentage of that group which did not provide a sample (e.g. for active CD, 55 sample-providing patients and 9 not providing a sample sums to 64 total patients enrolled with active CD. Those 9 who did not provide a sample are 14.1% of that group.) The percentage in the inactive group that did not provide a sample was low, as many were asked to provide a urine sample immediately upon enrollment in the ambulatory setting.

Some patients provided samples on more than one instance over the course of the study. There were only 22 occurrences of a sample being provided by a patient who had already given a sample, less than 10% of the total number of samples. That is why there are only 240 enrolled patients (see Figure 3) but 262 analyzed samples (see Table 4).

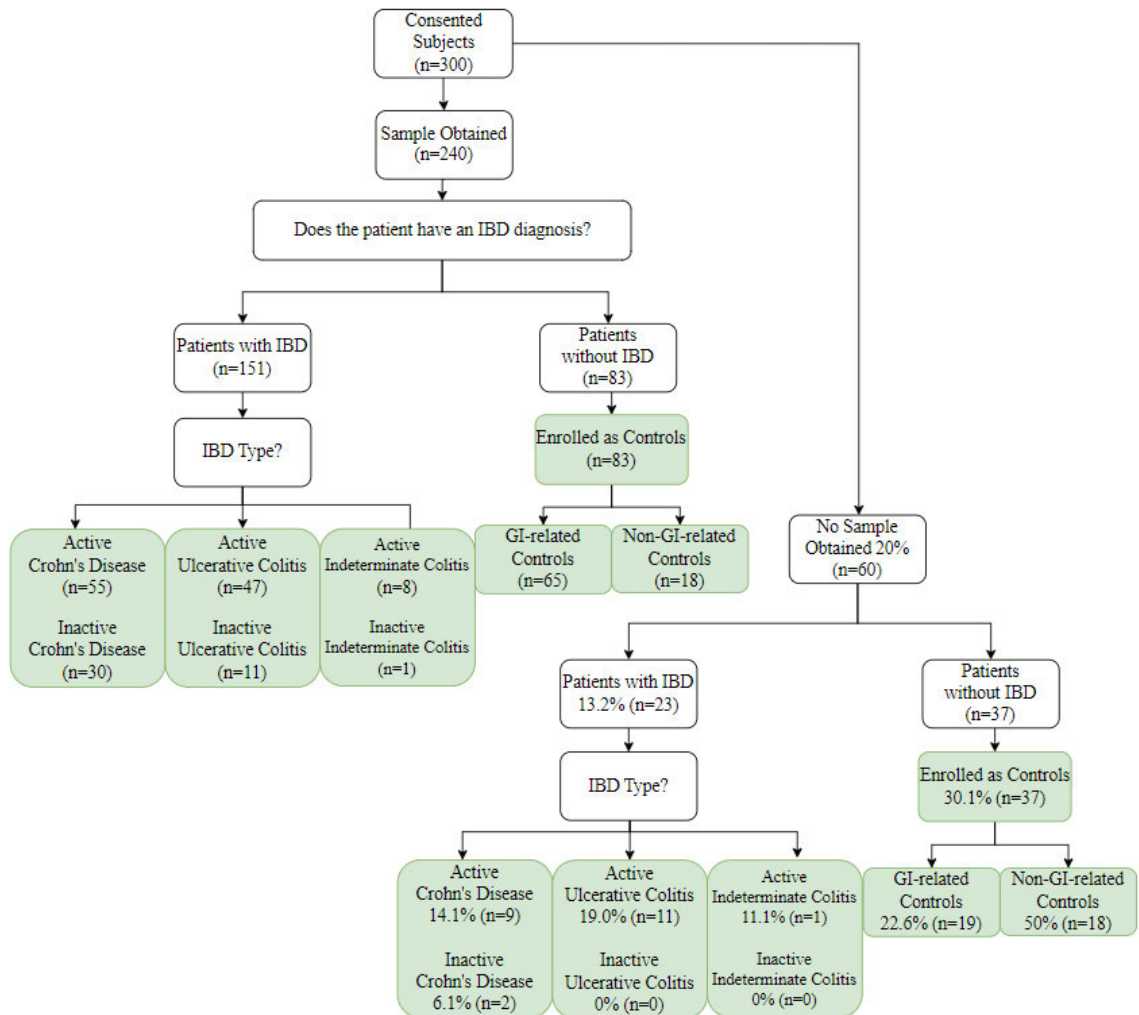


Figure 3: Enrollment flow diagram.

The disease severity of enrolled patients was assessed by relevant PROM metrics. Cut-off values abstracted from the literature were used to categorize patients as having mild, moderate, or severe disease (Dotson, 2016; Turner, 2009).

Disease Severity	PUCAI Score Range	PCDAI Score Range
Remission	>10	>10
Mild	10-34	10-27.5
Moderate	35-64	30-37.5
Severe	65-85	40-100

Table 3: Disease severity cutoffs for PUCAI and PCDAI (Dotson, 2016; Turner, 2009)

PUCAI and PCDAI metrics use different scales and ask different questions, so a PUCAI score of 40 and a PCDAI score of 40 are not equivalent nor are they directly comparable. For that reason, an established severity category allows the ability to normalize disease severity and compare across IBD subtypes, such as comparing moderate UC patients with moderate CD patients. Each patient was assigned a severity category based directly on their PUCAI or PCDAI score, using the cutoffs shown in Table 3. The frequency of each group is seen in Table 4.

Disease Severity	UC Patients	CD Patients	All Patients (n)
Control	NA	NA	89 (34%)
Remission	9 (10.7%)	34 (38.2%)	43 (16.4%)
Mild	34 (40.5%)	31 (34.8%)	65 (24.8%)
Moderate	30 (35.7%)	6 (6.7%)	36 (13.7%)
Severe	11 (13.1%)	18 (20.2%)	29 (11.1%)
Total	84 (100%)	89 (100%)	262 (100%)

Table 4: The frequency of each disease severity category (as assigned by PUCAI and PCDAI). This shows severity for each disease separately and the frequency of each severity category in the overall study population.

Probe Measurement Correlation

All patient samples were measured with both the Arrowdox and Mettler Toledo probes. This was done both for internal validity, and to ensure other labs interested in this field did not need to purchase one specific probe. If both probes provide strongly correlated data at all times, that allows for one probe to be used primarily and the other to be a backup. If the data did differ, that could signify that one probe can better measure ORP in biological samples.

Sample	n	Correlation Coefficient	p-value
Stool	71	0.611	<0.001
Urine	119	0.536	<0.001
Plasma	43	0.078	0.617
Intestinal Aspirate	12	0.776	0.003

Table 5: Pearson correlation data between the Arrowdox and Mettler Toledo probes for a given biological sample.

As seen in table 5, examining all patients, there are significant correlations between probes when stool, urine, or intestinal aspirates are measured. It is unclear if plasma has any specific properties that make it less reliable to measure for one probe or the other. Due to this discrepancy, and the fact that some correlations are only moderately

strong, data from both probes should be given equal weight at this time, with one not necessarily being seen as the better or primary probe. While there are some statistical outliers (as seen in Figure 5, represented by open circles and asterisks), this is still work in a new field, and it may be impossible to differentiate a true outlier from a valid datapoint. As such, all analysis is done with all available data points.

Localization of Oxidation-Reduction Potential

It remains uncertain whether the most relevant redox measurements should be obtained systemically or from specimens localized to the bowel. Inflammatory bowel disease can cause systemic and extraintestinal manifestations, including eye, joint, or skin involvement. Alternately, redox damage mediated by leukocytes in the bowel wall leukocytes may be best assessed locally.

To test the hypothesis that relevant changes in redox status are systemic, linear regression was first performed to explore the relationship between the oxidation-reduction potential of different samples from the same patient (Table 6 and Table 7). This analysis was done with all possible samples, including both IBD patients and controls. Some correlations were not able to be performed due to issues of mutual exclusivity. For example, intestinal aspirate is only obtainable when a patient is having a colonoscopy. Prior to that procedure, the patient has their bowels prepared (commonly known as a “clean out”) to remove all stool. As such, a patient could not provide a stool sample and an intestinal aspirate sample at the same visit.

Samples Correlated		n	Correlation Coefficient	p-value
Stool	Urine	101	0.634	<0.001
Urine	Plasma	38	0.715	<0.001
Urine	Intestinal Aspirate	16	-0.207	0.442
Plasma	Intestinal Aspirate	30	-0.424	0.020

Table 6: Pearson correlation coefficients for the redox values of different biological samples from all enrolled patients as measured with the Arrowdox probe

Samples Correlated		n	Correlation Coefficient	p-value
Stool	Urine	65	0.168	0.181
Urine	Plasma	34	0.264	0.132
Urine	Intestinal Aspirate	12	0.206	0.520
Plasma	Intestinal Aspirate	14	0.313	0.276

Table 7: Pearson correlation coefficients for the redox values of different biological samples from all enrolled patients as measured with the Mettler Toledo probe

Measurements of stool redox status are moderately correlated with urine redox status (Pearson correlation coefficient 0.634, $p < 0.001$) using measurements obtained with the Arrowdox probe. Urine and plasma redox statuses are strongly correlated (Pearson correlation coefficient 0.715, $p < 0.001$). However, these significant correlations were not supported by measurements obtained using the MT probe. While the Arrowdox data support the hypothesis that redox status is not localized to the bowel, the Mettler Toledo data does not. This further supports the hypothesis that there is some discordance between the probes when measuring biological samples. Due to differences in oxidation-reduction potential between probes and sample types, all biological samples will be analyzed with both probes in tandem.

Oxidation-Reduction Potential and Disease Severity Assessed by PROMs

To test the hypothesis that oxidation-reduction potential and disease severity are correlated, Pearson correlation coefficients were determined to compare stool oxidation-reduction potential with PUCAI and with PCDAI scores for each patient. Figure 4 is a visual representation of the linear regression of stool oxidation-reduction potential and PUCAI score. Tables 8 and 9 show the compiled results for the linear regressions of PUCAI and PCDAI scores with the redox values of any sample. For both PROM, for all samples, and for both probes, no significant correlations were found for stool, urine, plasma, or intestinal aspirates.

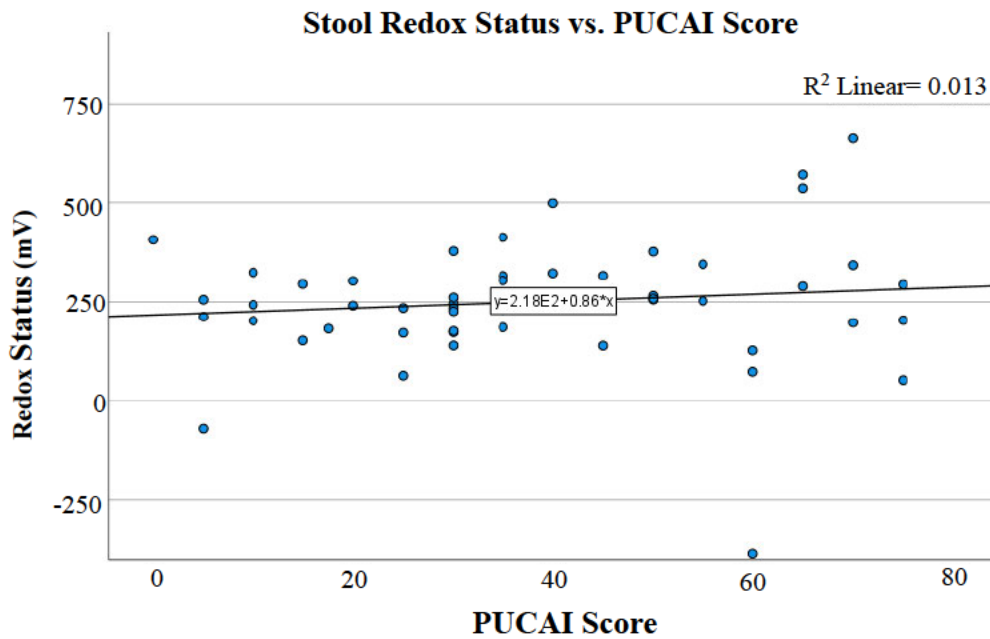


Figure 4: A visual representation of the relationship between PUCAI and stool oxidation-reduction potential score (n=56).

Samples Correlated		n	Correlation Coefficient	p-value
PUCAI vs.	Stool Oxidation- reduction potential	56	0.115	0.397
	Urine Oxidation- reduction potential	56	0.148	0.277
	Plasma Oxidation- reduction potential	9	0.206	0.594
	Intestinal Aspirate Oxidation- reduction potential	7	-0.404	0.368
PCDAI vs.	Stool Oxidation- reduction potential	42	0.136	0.391
	Urine Oxidation- reduction potential	72	0.003	0.983
	Plasma Oxidation- reduction potential	28	0.049	0.804
	Intestinal Aspirate Oxidation- reduction potential	12	0.187	0.561

Table 8: A summary of Pearson correlation coefficients for patient-reported disease severity (as indicated by PUCAI or PCDAI) vs. oxidation-reduction potential in mV of a given sample type measured with the Arrowdox Probe.

Samples Correlated		n	Correlation Coefficient	p-value
PUCAI vs.	Stool Oxidation-reduction potential	36	-0.116	0.501
	Urine Oxidation-reduction potential	31	-0.116	0.533
	Plasma Oxidation-reduction potential	6	0.102	0.848
	Intestinal Aspirate Oxidation-reduction potential	4	-0.497	0.503
	Stool Oxidation-reduction potential	28	0.083	0.673
PCDAI vs.	Urine Oxidation-reduction potential	56	-0.085	0.534
	Plasma Oxidation-reduction potential	19	0.424	0.071
	Intestinal Aspirate Oxidation-reduction potential	5	0.063	0.920

Table 9: A summary of Pearson correlation coefficients for patient-reported disease severity (as indicated by PUCAI or PCDAI) vs. oxidation-reduction potential in mV of a given sample type measured with the Mettler Toledo Probe.

Due to the somewhat imprecise nature of PUCAI and PCDAI, patients were also stratified by disease severity, either being control patients or someone with remittent, mild, moderate, or severe IBD based on the cutoffs established in the literature (Table 3).

A Shapiro-Wilk test was first performed to assess if oxidation-reduction potential, for each sample, was normally distributed within each disease severity category (Table 10 and Table 11). For a given sample, if at least one severity category has a significant p-value ($p < 0.05$), that category is not normally distributed. As such, the independent-samples Kruskal-Wallis test was used to test for differences among the patient groups. For samples where all severity categories tested by the Shapiro-Wilk test yield insignificant results ($p > 0.05$), then a one-way ANOVA was performed.

Sample	Severity Category	n	Skewness	Kurtosis	S-W Statistic ^a	S-W df ^b	p-value
Stool	Control	25	-1.335	3.743	0.886	25	0.009
	Remission	10	-0.681	0.204	0.905	10	0.249
	Mild	33	0.250	0.236	0.989	33	0.977
	Moderate	29	-2.239	7.265	0.788	29	<0.001
	Severe	23	1.166	1.534	0.889	23	0.014
Urine	Control	63	-0.084	-0.363	0.988	63	0.781
	Remission	30	-0.398	0.446	0.966	30	0.445
	Mild	43	0.007	-0.716	0.982	43	0.724
	Moderate	28	-0.596	-0.322	0.946	28	0.158
	Severe	22	0.100	1.112	0.981	22	0.932
Plasma	Control	29	-0.783	-0.623	0.872	29	0.002
	Remission	25	-0.397	-0.826	0.926	25	0.070
	Mild	10	-1.169	0.461	0.825	10	0.029
	Moderate ^c	1	NA ^c	NA ^c	NA ^c	NA ^c	NA ^c
Intestinal Aspirate	Control	12	-1.554	1.919	0.757	12	0.003
	Remission	11	-2.451	6.557	0.698	11	<0.001
	Mild	7	-0.811	-1.167	0.840	7	0.099

a: S-W Statistic: The Shapiro-Wilk Test Statistic

b: S-W df: The Shapiro-Wilk Degrees of Freedom

c: with only one data point within this category, analysis for kurtosis could not be performed

Table 10: A summary of normality tests used to see if a sample oxidation-reduction potential is normally distributed within a severity category. If any severity categories for a given sample have a significant p-value ($p < 0.05$), then the category is considered to not be normally distributed, and an independent-samples Kruskal-Wallis test is needed. ORP values were measured with the Arrowdox probe.

Sample	Severity Category	n	Skewness	Kurtosis	S-W Statistic ^a	S-W df ^b	p-value
Stool	Control	11	-0.006	0.448	0.949	11	0.636
	Remission	10	-1.664	1.555	0.728	10	0.002
	Mild	16	-2.043	6.658	0.806	16	0.003
	Moderate	20	-2.921	8.8689	0.578	20	<0.001
	Severe	18	-1.632	2.816	0.826	18	0.004
Urine	Control	39	0.382	0.737	0.969	39	0.353
	Remission	27	-0.048	-0.543	0.984	27	0.941
	Mild	24	-0.154	1.036	0.937	24	0.138
	Moderate	17	-1.519	2.204	0.848	17	0.010
	Severe	18	0.431	-0.623	0.960	18	0.594
Plasma	Control	19	1.582	3.316	0.864	19	0.012
	Remission	17	0.040	-0.576	0.968	17	0.791
	Mild	6	1.345	1.538	0.874	6	0.243
	Moderate ^c	1	NA ^c	NA ^c	NA ^c	NA ^c	NA ^c
Intestinal Aspirate	Control	4	0.001	-5.931	0.770	4	0.059
	Remission	5	-0.646	-2.868	0.800	5	0.080
	Mild	3	0.614	NA ^d	0.985	3	0.769

a: S-W Statistic: The Shapiro-Wilk Test Statistic

b: S-W df: The Shapiro-Wilk Degrees of Freedom

c: with only one data point within this category, normality tests could not be performed

d: with only 3 data points within this category, analysis for kurtosis could not be calculated

Table 11: A summary of normality tests used to see if a sample oxidation-reduction potential is normally distributed within a severity category. If any severity categories for a given sample have a significant p-value ($p < 0.05$), then the category is considered to not be normally distributed, and an independent-samples Kruskal-Wallis test is needed. ORP values were measured with the Mettler Toledo probe.

Each category was only normally distributed for urine Arrowdox, urine Mettler Toledo, and intestinal aspirate Mettler Toledo. As such, these samples could be tested by one-way ANOVA, as seen in Tables 12 and 13. In all cases, no significant differences were identified.

Sample	n	df ^a	F	p-value
Urine	181	4	1.197	0.314

a: Between groups Degrees of Freedom

Table 12: Summary of one-way ANOVA performed for samples from all IBD patients whose severity categories are normally distributed. No significant differences in sample redox values were found based on severity categories. The redox values were measured with the Arrowdox probe.

Sample	n	df ^a	F	p-value
Urine	120	4	0.201	0.937
Intestinal Aspirate	9	2	0.447	0.653

a: Between groups Degrees of Freedom

Table 13: Summary of one-way ANOVA performed for samples from all IBD patients whose severity categories are normally distributed. No significant differences in sample redox values were found based on severity categories. The redox values were measured with the Mettler Toledo probe.

An Independent-Samples Kruskal-Wallis Test was performed with the rest of the samples, which had at least one not normally distributed category. The data are summarized in Tables 14 and 15 below. In all cases, a significant result was not obtained. Figure 5 is a representative box plot visually showing the distributions of stool oxidation-reduction potential for the different severity categories, as measured with the Arrowdox probe.

Sample	n	Test Statistic	df ^a	p-value
Stool	120	6.488	4	0.166
Plasma	65	1.211	3	0.750
Intestinal Aspirate	30	0.148	2	0.929

a: df=Degrees of Freedom

Table 14: Summary of Independent-Samples Kruskal-Wallis Tests performed for each sample. No significant differences in sample oxidation-reduction potential were found based on severity categories. The redox values were measured with the Arrowdox probe.

Sample	n	Test Statistic	df ^a	p-value
Stool	75	4.025	4	0.403
Plasma	43	2.502	3	0.475

a: df=Degrees of Freedom

Table 15: Summary of Independent-Samples Kruskal-Wallis Tests performed for each sample. No significant differences in sample oxidation-reduction potential were found based on severity categories. The redox values were measured with the Mettler Toledo probe.

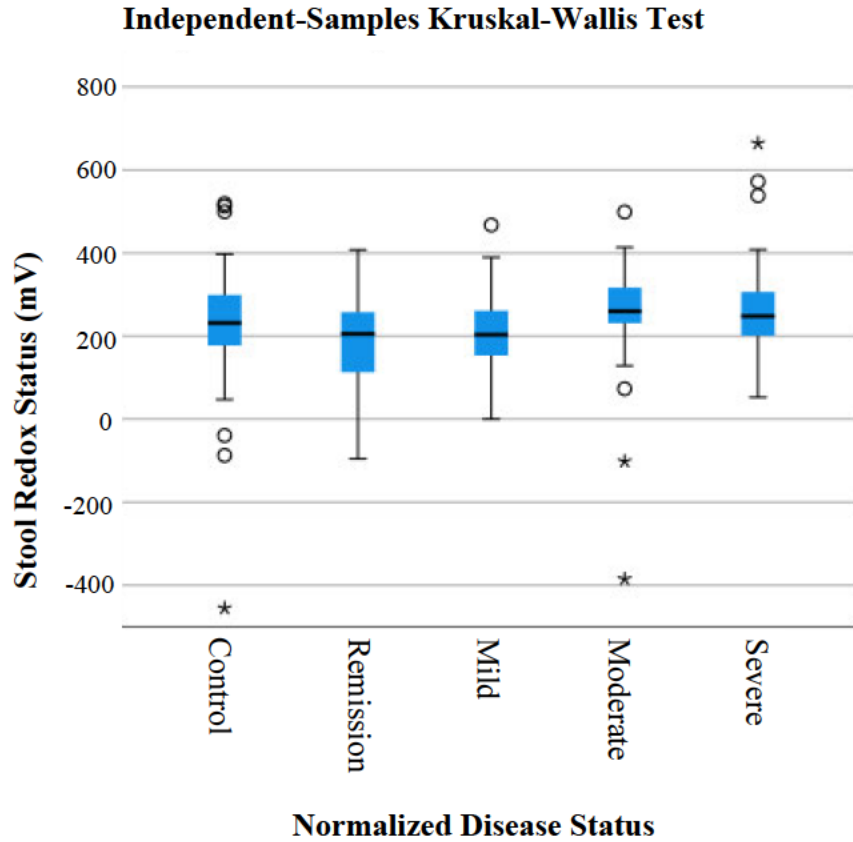


Figure 5: A box plot of stool redox values, in mV, measured with the Arrowdox probe for control and IBD disease severity categories. The horizontal black line is the median and the blue-shaded region is the interquartile range (25th percentile to 75th percentile). Open circles and asterisks are outlier data points.

A similar analysis was performed for UC and CD separately. Just as before, a Shapiro-Wilk test of normality was performed to see if a one-way ANOVA could be performed or if an Independent-Samples Kruskal-Wallis Test would be necessary. Tables 16, 17, 18, and 19 show the normality testing for measurements obtained using the Arrowdox and Mettler Toledo probes for patients with UC and CD. Samples which have all normally distributed disease severity categories ($p > 0.05$) will be compared with a one-

way ANOVA. The remainder, which are not normally distributed, will be tested with the independent-samples Kruskal-Wallis for group comparisons.

Sample	Severity Category	n	Skewness	Kurtosis	S-W Statistic ^a	S-W df ^b	p-value
Stool	Control	25	-1.335	3.743	0.886	25	0.009
	Remission	4	-0.932	1.784	0.943	4	0.675
	Mild	17	-0.007	1.076	0.974	17	0.890
	Moderate	23	-2.520	9.714	0.747	23	<0.001
	Severe	8	0.025	-0.907	0.956	8	0.772
Urine	Control	63	-0.084	-0.363	0.988	63	0.781
	Remission	6	-1.015	1.678	0.934	6	0.611
	Mild	19	-0.051	-0.611	0.964	19	0.659
	Moderate	20	-0.555	-0.141	0.950	20	0.360
	Severe	5	0.220	-0.537	0.976	5	0.913
Plasma	Control	29	-0.783	-0.623	0.872	29	0.002
	Remission	3	-1.511	NA ^c	0.880	3	0.325
	Mild	4	-1.849	3.505	0.774	4	0.063
Intestinal Aspirate	Control	12	-1.554	1.919	0.757	12	0.003
	Remission	2	NA ^d	NA ^c	NA ^e	NA ^e	NA ^e
	Mild	3	1.726	NA ^c	0.774	3	0.055

a: S-W Statistic: The Shapiro-Wilk Test Statistic

b: S-W df: The Shapiro-Wilk Degrees of Freedom

c: with 3 or fewer data points within this category, kurtosis could not be calculated

d: with only 2 data points within this category, skewness could not be calculated

e: with only 2 data points within this category, a Shapiro-Wilk test could not be performed

Table 16: A summary of normality tests used to see if a sample oxidation-reduction potential is normally distributed within a severity category for UC patients only. If any severity categories for a given sample have a significant p-value ($p < 0.05$), then the category is considered to not be normally distributed, and an independent-samples Kruskal-Wallis test is needed. ORP values were measured with the Arrowdox probe.

Sample	Severity Category	n	Skewness	Kurtosis	S-W Statistic ^a	S-W df ^b	p-value
Stool	Control	11	-0.006	0.448	0.949	11	0.636
	Remission	4	-0.1951	3.836	0.713	4	0.016
	Mild	9	0.401	0.522	0.953	9	0.724
	Moderate	17	-3.677	14.440	0.502	17	<0.001
	Severe	6	-2.300	5.391	0.644	6	0.001
Urine	Control	39	0.382	0.737	0.969	39	0.353
	Remission	4	-0.585	0.127	0.983	4	0.919
	Mild	9	0.198	-1.236	0.915	9	0.350
	Moderate	13	-1.061	1.573	0.927	13	0.310
	Severe	4	-0.648	-1.118	0.957	4	0.762
Plasma	Control	19	1.581	3.316	0.864	19	0.012
	Remission	2	NA ^d	NA ^c	NA ^e	NA ^e	NA ^e
	Mild	3	1.610	NA ^c	0.850	3	0.240
Intestinal Aspirate	Control	4	0.001	-5.931	0.770	4	0.059
	Remission	1	NA ^d	NA ^c	NA ^e	NA ^e	NA ^e
	Mild	2	NA ^d	NA ^c	NA ^e	NA ^e	NA ^e

a: S-W Statistic: The Shapiro-Wilk Test Statistic

b: S-W df: The Shapiro-Wilk Degrees of Freedom

c: with 3 or fewer data points within this category, kurtosis could not be calculated

d: with only 2 data points within this category, skewness could not be calculated

e: with only 2 data points within this category, a Shapiro-Wilk test could not be performed

Table 17: A summary of normality tests used to see if a sample oxidation-reduction potential is normally distributed within a severity category for UC patients only. If any severity categories for a given sample have a significant p-value ($p < 0.05$), then the category is considered to not be normally distributed, and an independent-samples Kruskal-Wallis test is needed. ORP values were measured with the Mettler Toledo probe.

Sample	Severity Category	n	Skewness	Kurtosis	S-W Statistic ^a	S-W df ^b	p-value
Stool	Control	25	-1.335	3.743	0.886	25	0.009
	Remission	5	-1.416	2.349	0.890	5	0.356
	Mild	13	0.685	-0.290	0.947	13	0.547
	Moderate	4	-1.067	-0.188	0.866	4	0.283
	Severe	14	0.125	1.720	0.956	14	0.651
Urine	Control	63	-0.084	-0.363	0.988	63	0.781
	Remission	22	-0.283	0.182	0.976	22	0.849
	Mild	21	0.124	-0.883	0.963	21	0.568
	Moderate	5	-0.183	-1.725	0.944	5	0.692
	Severe	16	-0.966	1.831	0.946	16	0.434
Plasma	Control	29	-0.783	-0.623	0.872	29	0.002
	Remission	21	-0.284	-0.831	0.939	21	0.213
	Mild	5	-0.953	-0.946	0.861	5	0.230
	Moderate ^c	1	NA ^c	NA ^c	NA ^c	NA ^c	NA ^c
Intestinal Aspirate	Control	12	-1.554	1.919	0.757	12	0.003
	Remission	8	-2.596	7.026	0.619	8	<0.001
	Mild	3	1.723	NA ^d	0.779	3	0.066

a: S-W Statistic: The Shapiro-Wilk Test Statistic

b: S-W df: The Shapiro-Wilk Degrees of Freedom

c: with only one data point within this category, normality tests cannot be performed

d: with 3 or fewer data points within this category, kurtosis could not be calculated

Table 18: A summary of normality tests used to see if a sample oxidation-reduction potential is normally distributed within a severity category for CD patients only. If any severity categories for a given sample have a significant p-value ($p < 0.05$) then the category is considered to not be normally distributed, and an independent-samples Kruskal-Wallis test is needed. ORP values were measured with the Arrowdox probe.

Sample	Severity Category	n	Skewness	Kurtosis	S-W Statistic ^a	S-W df ^b	p-value
Stool	Control	11	-0.006	0.448	0.949	11	0.636
	Remission	5	-1.917	4.058	0.763	5	0.039
	Mild	6	-1.258	2.179	0.913	6	0.458
	Moderate	2	NA ^c	NA ^c	NA ^c	NA ^c	NA ^c
	Severe	12	-0.639	-0.794	0.881	12	0.090
Urine	Control	39	0.382	0.737	0.969	39	0.353
	Remission	21	0.086	-0.698	0.978	21	0.895
	Mild	14	-0.888	2.334	0.918	14	0.205
	Moderate	3	-1.640	NA ^d	0.838	3	0.208
	Severe	14	0.517	-0.694	0.948	14	0.530
Plasma	Control	19	1.582	3.316	0.864	19	0.012
	Remission	14	-0.112	-0.785	0.958	14	0.687
	Mild	3	-0.435	NA ^d	0.993	3	0.838
	Moderate ^c	1	NA ^c	NA ^c	NA ^c	NA ^c	NA ^c
Intestinal Aspirate	Control	4	0.001	-5.931	0.770	4	0.059
	Remission	3	1.518	NA ^d	0.879	3	0.320
	Mild	1	NA ^c	NA ^c	NA ^c	NA ^c	NA ^c

a: S-W Statistic: The Shapiro-Wilk Test Statistic

b: S-W df: The Shapiro-Wilk Degrees of Freedom

c: with two or fewer data points within this category, normality tests could not be performed

d: with 3 or fewer data points within this category, kurtosis could not be calculated

Table 19: A summary of normality tests used to see if a sample oxidation-reduction potential is normally distributed within a severity category for CD patients only. If any severity categories for a given sample have a significant p-value ($p < 0.05$), then the category is considered to not be normally distributed, and an independent-samples Kruskal-Wallis test is needed. ORP values were measured with the Mettler Toledo probe.

For both UC and CD, each category was only normally distributed for urine

Arrowdox, urine Mettler Toledo, and intestinal aspirate Mettler Toledo. As such, these

samples could be tested by one-way ANOVA, as seen in Tables 20, 21, 23, and 23. In all

cases, no significant differences were identified.

Sample	n	df ^a	F	p-value
Urine	108	4	0.924	0.453

a: Between groups Degrees of Freedom

Table 20: Summary of one-way ANOVA performed for samples from UC patients whose severity categories are normally distributed. No significant differences in sample redox values were found based on severity categories. The redox values were measured with the Arrowdox probe.

Sample	n	df ^a	F	p-value
Urine	64	4	0.760	0.555
Intestinal Aspirate	4	2	0.800	0.510

a: Between groups Degrees of Freedom

Table 21: Summary of one-way ANOVA performed for samples from UC patients whose severity categories are normally distributed. No significant differences in sample redox values were found based on severity categories. The redox values were measured with the Mettler Toledo probe.

Sample	n	df ^a	F	p-value
Urine	122	4	0.481	0.750

a: Between groups Degrees of Freedom

Table 22: Summary of one-way ANOVA performed for samples from CD patients whose severity categories are normally distributed. No significant differences in sample redox values were found based on severity categories. The redox values were measured with the Arrowdox probe.

Sample	n	df ^a	F	p-value
Urine	86	4	0.461	0.764
Intestinal Aspirate	5	2	0.041	0.960

a: Between groups Degrees of Freedom

Table 23: Summary of one-way ANOVA performed for samples from CD patients whose severity categories are normally distributed. No significant differences in sample redox values were found based on severity categories. The redox values were measured with the Mettler Toledo probe.

The data from the independent-sample Kruskal-Wallis tests are shown below in Tables 24, 25, 26, and 27. No significant differences were observed in stool, urine, plasma, or intestinal aspirate ORP measurements obtained using the Arrowdox or Mettler Toledo probes in patients with UC or CD.

Sample	n	Test Statistic	df ^a	p-value
Stool	77	6.227	4	0.183
Plasma	36	3.439	2	0.179
Intestinal Aspirate	17	1.046	2	0.593

a: df=Degrees of Freedom

Table 24: Summary of Independent-Samples Kruskal-Wallis Tests performed for non-normally distributed samples for UC patients only. No significant differences in sample oxidation-reduction potential were found based on severity categories. The redox values were measured with the Arrowdox probe.

Sample	n	Test Statistic	df ^a	p-value
Stool	47	4.005	4	0.405
Plasma	24	0.036	2	0.982

a: df=Degrees of Freedom

Table 25: Summary of Independent-Samples Kruskal-Wallis Tests performed for non-normally distributed samples for UC patients only. No significant differences in sample oxidation-reduction potential were found based on severity categories. The redox values were measured with the Mettler Toledo probe.

Sample	n	Test Statistic	df ^a	p-value
Stool	61	3.645	4	0.456
Plasma	56	0.541	3	0.910
Intestinal Aspirate	23	0.213	2	0.899

a: df=Degrees of Freedom

Table 26: Summary of Independent-Samples Kruskal-Wallis Tests performed for non-normally distributed samples for CD patients only. No significant differences in sample oxidation-reduction potential were found based on severity categories. The redox values were measured with the Arrowdox probe.

Sample	n	Test Statistic	df ^a	p-value
Stool	36	4.319	4	0.365
Plasma	37	2.721	3	0.437

a: df=Degrees of Freedom

Table 27: Summary of Independent-Samples Kruskal-Wallis Tests performed for non-normally distributed samples for CD patients only. No significant differences in sample oxidation-reduction potential were found based on severity categories. The redox values were measured with the Mettler Toledo probe.

Redox and Other Laboratory Values

There are a variety of laboratory values that are used to measure inflammation. Of special clinical relevance are erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), as previously mentioned. Pearson correlation coefficients were calculated to assess the relationship between stool, urine, plasma, or intestinal aspirate ORP values and ESR or CRP. This analysis revealed only a weak negative correlation between urine redox status, measured by the Mettler Toledo probe, and ESR (Pearson correlation coefficient -0.287, p=0.015) as measured by the Metler Toledo probe.

Samples Correlated		n	Correlation Coefficient	p-value
ESR vs.	Stool Oxidation- reduction potential	63	0.045	0.724
	Urine Oxidation- reduction potential	98	-0.096	0.349
	Plasma Oxidation- reduction potential	49	0.116	0.428
	Intestinal Aspirate Oxidation- reduction potential	24	0.260	0.220
CRP vs.	Stool Oxidation- reduction potential	75	0.105	0.369
	Urine Oxidation- reduction potential	114	-0.101	0.283
	Plasma Oxidation- reduction potential	52	0.057	0.689
	Intestinal Aspirate Oxidation- reduction potential	26	-0.134	0.515

Table 28: A summary of Pearson correlation coefficients for laboratory biomarkers vs. oxidation-reduction potential of a given sample type measured with the Arrowdox probe.

Samples Correlated		n	Correlation Coefficient	p-value
ESR vs.	Stool Oxidation- reduction- potential	39	-0.045	0.785
	Urine Oxidation- reduction- potential	71	-0.287	0.015
	Plasma Oxidation- reduction- potential	34	0.049	0.784
	Intestinal Aspirate Oxidation- reduction- potential	12	0.223	0.486
CRP vs.	Stool Oxidation- reduction- potential	47	0.106	0.478
	Urine Oxidation- reduction- potential	83	-0.112	0.314
	Plasma Oxidation- reduction- potential	35	0.085	0.628
	Intestinal Aspirate Oxidation- reduction- potential	12	0.146	0.651

Table 29: A summary of Pearson correlation coefficients for laboratory biomarkers vs. oxidation-reduction potential of a given sample type measured with the Mettler Toledo probe.

DISCUSSION

Research focusing on applying new and novel biomarkers to assess disease severity in patients with IBD is of tremendous importance. In considering redox measurement, several questions must be addressed, including how the technology will be positioned. Should redox measurements be performed in the clinic, in the hospital laboratory, or even in a patient's home? Is there a biosample a patient can produce that is readily amenable for redox testing and provide data useful to their clinical care? Does the information provided by redox measurement differ from what is already available to the clinician from existing laboratory studies? If redox measurements correlated perfectly with PUCAI, PCDAI, ESR, or CRP, their utility would be limited. In that case, redox status would be, at best, a substitutive measure. On the other hand, a lack of correlation between redox measurements and existing biomarkers would require some explanation. Nonetheless, testing of this type (particularly for the urine and stool samples) could still be of value for patients that are non-communicative, needle-phobic, or unable not provide a blood sample.

The commercial availability of at-home redox platforms, such as REDOXSYS, would assist patients in discussions concerning their disease severity or response to therapy with their GI physicians. The REDOXSYS system is analogous to the Arrowdox and Mettler Toledo probes used in this thesis but is optimized for at-home use by patients. It is hoped that platforms of this type could be used to assess a variety of biosamples relevant to different acute and chronic diseases (Stagos, 2014). That being

said, the clinical uses of oxidation-reduction potential would be severely limited if there already existed a readily obtainable metric that gave the same answer.

The heterogeneous nature of IBD has long hindered the reliance on measured biomarker samples. For example, patients with elevated CRP at the time of diagnosis may have CRP values that change with disease activity. However, approximately one-quarter of patients with normal CRP at diagnosis will continue to have normal CRP irrespective of their disease status (Henderson, 2015). This is troublesome when investigating a new biomarker, as its efficacy is hard to measure without first defining the gold standard against which the biomarker can be compared.

The data presented here failed to demonstrate a significant signal when comparing redox measurements obtained from stool, urine, blood, or intestinal aspirates with relevant PROMs (PUCAI or PCDAI) or existing biomarkers of disease activity (ESR or CRP). Similarly, redox measurements did not predict disease severity in patients with UC or CD.

We had expected the oxidation-reduction potential of stool to be the most likely of the biological samples to correlate with disease severity, as stool is produced from the patient's inflamed bowel. Notably, Tables 7 and 8 show that there are not significant linear correlations when comparing PUCAI to stool redox values (Pearson correlation coefficient=0.115, $p=0.397$) or when comparing PCDAI to stool redox values (Pearson correlation coefficient=0.136, $p=0.391$). All other correlations did not yield significant results.

One outcome we expected was similar results from the Arrowdox and Mettler Toledo probes. They measured the same samples but did have inconsistencies. For example, there was no correlation between the oxidation-reduction potential measured for plasma with the two probes (Pearson correlation coefficient=0.078, $p=0.617$). When exploring correlations between different biological samples, the Mettler Toledo data did not exhibit the significant correlations observed with the Arrowdox probes (Tables 7 and 8). This raises the concern that these probes are not consistent with one another, and that any results found with one may not be replicable with the other. Other laboratories investigating the use of oxidative-reductive potential should consider using multiple probes to demonstrate the generalizability of their findings.

Changes in redox status have been previously measured in the mucosa of patients with inflammatory bowel disease (Bhattacharyya, 2014). Tissue oxidation-reduction potential can be measured in a variety of ways, from the use of liquid-chromatography coupled to mass-spectrometry to more simple probes, such as the ones used in this thesis (Petrova, 2021). This led to our lab's interest in measuring ambient ROS concentrations in blood, urine, and stool. Direct measurement of tissue redox status requires incurring the cost and morbidity associated with the performance of endoscopy and colonoscopy (Thakkar, 2016). In addition, the observations made during colonoscopy could make a mucosal redox measurement redundant. Endoscopy remains the gold standard for assessing disease activity in patients with IBD (Thakkar, 2016). We hypothesized that increased concentrations of mucosal ROS might lead to measurable changes in noninvasive testing performed on blood, stool, or urine specimens.

There are many reasons that patients may have elevated mucosal ROS concentrations, yet we fail to find significant differences between control patients and any severity category of IBD for stool, urine, plasma, or intestinal aspirate. One reason is that the reactive oxygen species may have reacted with other cells or materials before a sample can be obtained. An endogenous reducing agent, glutathione, is abundant in intestinal mucosa and is known to reduce H_2O_2 into H_2O (Bhattacharyya, 2014). If mucosal ROS is secreted but interacts with glutathione in the lumen of the bowel then it will not be excreted and measured in the lab. Another is dilution; a small amount of ROS may be diluted by other bulk in stool. These species could be different between active and inactive disease patients, or between IBD and control patients, but negligible by the time stool leaves a patient. It may also be that there is only a subset of patients where measurements of oxidation-reduction potential are useful, as is the case for CRP level. The concentration of ROS does appear to be affected by a patient's microbiome, which is another possible avenue for exploring the use of ORP (von Martels, 2020).

In biomedical sciences, there is a constant tug-of-war between rationalism and empiricism (Webb, 2018). Rationalists posit that special care must be taken to understand what the underlying physiology of a situation is and consider if a hypothesis is logically possible. An empiricist follows data above all. This thesis makes it clear that rationalism alone fails without empirical data to back it. It is reasonable to believe that an autoimmune condition in which leukocytes release an excess of reactive oxygen species should lead to stool with a more positive oxidative-reduction potential that correlates with disease severity. Empirically, though, this study does not support the use of oxidation-

reduction potential measurements from stool, urine, plasma, or intestinal aspirates to independently predict severity of disease in children with IBD.

CONCLUSION

Inflammatory bowel disease is a complex, debilitating disease whose pathogenesis is still not completely understood. Pediatric patients with IBD can have lifelong consequences of the disease, including malnutrition, abdominal pain, anemia, and growth delay. While there are multiple ways to measure disease activity for patients with IBD, each has its own shortcomings. There is a need for an easily obtainable, reliable, objective measure of disease activity to aid in diagnosis, monitoring response to therapy, and to aid in clinical decision-making. Oxidation-reduction potential, measured at home or in the clinic, has been considered to fill that need.

Leukocytes mediate the intestinal tissue damage observed in patients with Crohn's disease and ulcerative colitis by releasing reactive oxygen species. As such, we hypothesized that an increased concentration of reactive oxygen should be measurable when a patient has more severe disease.

This thesis found no correlation between oxidation-reduction potential of stool, urine, plasma, or intestinal aspirates when measured with an Arrowdox probe or a Mettler Toledo probe. When samples are stratified by disease severity and subtype of IBD, there were still not significant differences in oxidation-reduction potential among groups.

There are many factors that may explain the lack of relationship between disease severity and oxidation-reduction potential. There are mucosal compounds such as glutathione and enzymes that can reduce reactive oxygen species in the gut. Another possibility is that redox differences may be detectable for some sub-groups that are not seen at a population level, as is the case with C-reactive protein levels.

In the future, it may be useful to explore oxidation-reduction potential as a more longitudinal measure. It has the possibility to be useful to monitor a single patient's disease status over time, but this thesis shows conclusively that it is not a useful cross-sectional measure.

BIBLIOGRAPHY

Journal Abbreviations:

BMJ

BMJ: British Medical Journal

J Pediatr Gastroenterol Nutr

Journal of Pediatric Gastroenterology and Nutrition

Alper, A., Zhang, L., & Pashankar, D. S. (2017a). Correlation of Erythrocyte Sedimentation Rate and C-Reactive Protein With Pediatric Inflammatory Bowel Disease Activity. *Journal of Pediatric Gastroenterology and Nutrition*, 65(2), e25.

<https://doi.org/10.1097/MPG.0000000000001444>

Bergeim, O., Kleinberg, J., & Kirch, E. R. (1945). Oxidation-Reduction Potentials of the Contents of the Gastrointestinal Tract. *Journal of Bacteriology*, 49(5), 453–458.

<https://doi.org/10.1128/jb.49.5.453-458.1945>

Bhattacharyya, A., Chattopadhyay, R., Mitra, S., & Crowe, S. E. (2014). Oxidative Stress: An Essential Factor in the Pathogenesis of Gastrointestinal Mucosal Diseases. *Physiological Reviews*, 94(2), 329–354.

<https://doi.org/10.1152/physrev.00040.2012>

Bier, A. (2009). *Introduction to Oxidation Reduction Potential Measurement*. Hach.

<https://www.hach.com/p-zobell-s-orp-redox-standard-solution-500-ml/2316949>

Black, N. (2013). Patient reported outcome measures could help transform healthcare.

BMJ, 346, f167. <https://doi.org/10.1136/bmj.f167>

Center, S. B. (2021, January 15). Purple Top Tubes: Your Impact on Research. *Stanford*

Blood Center. <https://stanfordbloodcenter.org/purple-top-tubes-your-impact-on-research/>

Choi, Y. S., Kim, D. S., Lee, D. H., Lee, J. B., Lee, E. J., Lee, S. D., Song, K. H., & Jung,

H. J. (2018). Clinical Characteristics and Incidence of Perianal Diseases in Patients With Ulcerative Colitis. *Annals of Coloproctology*, *34*(3), 138–143.

<https://doi.org/10.3393/ac.2017.06.08>

Circu, M. L., & Aw, T. Y. (2011). Redox biology of the intestine. *Free Radical*

Research, *45*(11–12), 1245–1266. <https://doi.org/10.3109/10715762.2011.611509>

Dotson, J. L., Crandall, W. V., Zhang, P., Forrest, C. B., Bailey, L. C., Colletti, R. B., &

Kappelman, M. D. (2015). Feasibility and Validity of the Pediatric Ulcerative Colitis Activity Index in Routine Clinical Practice. *Journal of Pediatric*

Gastroenterology and Nutrition, *60*(2), 200–204.

<https://doi.org/10.1097/MPG.0000000000000568>

Effect of oral iron supplementation on oxidative stress and colonic inflammation in rats

with induced colitis. (n.d.). Retrieved September 6, 2022, from

<https://oce.ovid.com/article/00001716-200112000-00019>

El-Matary, W. (2014). Patient-reported outcome measures in inflammatory bowel

disease. *Canadian Journal of Gastroenterology & Hepatology*, *28*(10), 536–542.

Forman, H. J., Ursini, F., & Maiorino, M. (2014). An overview of mechanisms of redox

signaling. *Journal of Molecular and Cellular Cardiology*, *0*, 2–9.

<https://doi.org/10.1016/j.yjmcc.2014.01.018>

- Fries, J. F. (1983). Toward an Understanding of Patient Outcome Measurement. *Arthritis & Rheumatism*, 26(6), 697–704. <https://doi.org/10.1002/art.1780260601>
- Frontiers | Serum Biomarkers for Inflammatory Bowel Disease*. (n.d.). Retrieved February 20, 2023, from <https://www.frontiersin.org/articles/10.3389/fmed.2020.00123/full>
- Gasche, C., & Grundtner, P. (2005). Genotypes and phenotypes in Crohn’s disease: Do they help in clinical management? *Gut*, 54(1), 162–167. <https://doi.org/10.1136/gut.2003.035600>
- Grabowski, B. (2016). “P < 0.05” Might Not Mean What You Think: American Statistical Association Clarifies P Values. *JNCI Journal of the National Cancer Institute*, 108(8), djw194. <https://doi.org/10.1093/jnci/djw194>
- Henderson, P., Kennedy, N. A., Van Limbergen, J. E., Cameron, F. L., Satsangi, J., Russell, R. K., & Wilson, D. C. (2015). Serum C-reactive Protein and CRP Genotype in Pediatric Inflammatory Bowel Disease: Influence on Phenotype, Natural History, and Response to Therapy. *Inflammatory Bowel Diseases*, 21(3), 596–605. <https://doi.org/10.1097/MIB.0000000000000296>
- Holmes, M. M., Lewith, G., Newell, D., Field, J., & Bishop, F. L. (2017). The impact of patient-reported outcome measures in clinical practice for pain: A systematic review. *Quality of Life Research*, 26(2), 245–257. <https://doi.org/10.1007/s11136-016-1449-5>
- Husson, O. (2013). Redox potential (Eh) and pH as drivers of soil/plant/microorganism systems: A transdisciplinary overview pointing to integrative opportunities for

agronomy. *Plant and Soil*, 362(1), 389–417. <https://doi.org/10.1007/s11104-012-1429-7>

Hyams, J., Markowitz, J., Otley, A., Rosh, J., Mack, D., Bousvaros, A., Kugathasan, S., Pfefferkorn, M., Tolia, V., Evans, J., Treem, W., Wyllie, R., Rothbaum, R., Katz, A., Mezzoff, A., Oliva-Hemker, M., Lerer, T., & Griffiths, A. (n.d.). Evaluation of the Pediatric Crohn Disease Activity Index: A Prospective Multicenter Experience. *J Pediatr Gastroenterol Nutr*, 41(4), 6.

Kaplan, G. G., & Windsor, J. W. (2021). The four epidemiological stages in the global evolution of inflammatory bowel disease. *Nature Reviews Gastroenterology & Hepatology*, 18(1), Article 1. <https://doi.org/10.1038/s41575-020-00360-x>

Lewis, J. D. (2011). The Utility of Biomarkers in the Diagnosis and Therapy of Inflammatory Bowel Disease. *Gastroenterology*, 140(6), 1817-1826.e2. <https://doi.org/10.1053/j.gastro.2010.11.058>

Liou, G.-Y., & Storz, P. (2010). Reactive oxygen species in cancer. *Free Radical Research*, 44(5), 10.3109/10715761003667554. <https://doi.org/10.3109/10715761003667554>

Liu, T., Han, L., Tilley, M., Afzelius, L., Maciejewski, M., Jelinsky, S., Tian, C., McIntyre, M., Agee, M., Auton, A., Bell, R. K., Bryc, K., Elson, S. L., Fontanillas, P., Furlotte, N. A., Hinds, D. A., Huber, K. E., Kleinman, A., Litterman, N. K., ... the 23andMe Research Team. (2021). Distinct clinical phenotypes for Crohn's disease derived from patient surveys. *BMC Gastroenterology*, 21(1), 160. <https://doi.org/10.1186/s12876-021-01740-6>

- Lo Sasso, G., Khachatryan, L., Kondylis, A., Battey, J. N. D., Sierro, N., Danilova, N. A., Grigoryeva, T. V., Markelova, M. I., Khusnutdinova, D. R., Laikov, A. V., Salafutdinov, I. I., Romanova, Y. D., Siniagina, M. N., Vasiliev, I. Y., Boulygina, E. A., Solovyeva, V. V., Garanina, E. E., Kitaeva, K. V., Ivanov, K. Y., ... Abdulkhakov, S. R. (2020). Inflammatory Bowel Disease–Associated Changes in the Gut: Focus on Kazan Patients. *Inflammatory Bowel Diseases*, 27(3), 418–433. <https://doi.org/10.1093/ibd/izaa188>
- Mitchell, P. J., Rabau, M. Y., & Haboubi, N. Y. (2007). Indeterminate colitis. *Techniques in Coloproctology*, 11(2), 91–96. <https://doi.org/10.1007/s10151-007-0337-y>
- Murata, Y., Amao, M., & Hamuro, J. (2003). Sequential conversion of the oxidation-reduction potential of macrophages dictates the pathological progression of autoimmune diabetes. *European Journal of Immunology*, 33(4), 1001–1011. <https://doi.org/10.1002/eji.200323575>
- Nakase, H., Uchino, M., Shinzaki, S., Matsuura, M., Matsuoka, K., Kobayashi, T., Saruta, M., Hirai, F., Hata, K., Hiraoka, S., Esaki, M., Sugimoto, K., Fuji, T., Watanabe, K., Nakamura, S., Inoue, N., Itoh, T., Naganuma, M., Hisamatsu, T., ... Koike, K. (2021). Evidence-based clinical practice guidelines for inflammatory bowel disease 2020. *Journal of Gastroenterology*, 56(6), 489–526. <https://doi.org/10.1007/s00535-021-01784-1>
- Nelson, E. C., Eftimovska, E., Lind, C., Hager, A., Wasson, J. H., & Lindblad, S. (2015). Patient reported outcome measures in practice. *BMJ*, 350, g7818. <https://doi.org/10.1136/bmj.g7818>

- Nikolaus, S., & Schreiber, S. (2007). Diagnostics of Inflammatory Bowel Disease. *Gastroenterology*, 133(5), 1670–1689. <https://doi.org/10.1053/j.gastro.2007.09.001>
- Petrova B, Warren A, Vital NY, et al. Redox Metabolism Measurement in Mammalian Cells and Tissues by LC-MS. *Metabolites*. 2021;11(5):313.
doi:10.3390/metabo11050313
- Puntmann, V. O. (2009). How-to guide on biomarkers: Biomarker definitions, validation and applications with examples from cardiovascular disease. *Postgraduate Medical Journal*, 85(1008), 538–545. <https://doi.org/10.1136/pgmj.2008.073759>
- Rigottier-Gois, L. (2013). Dysbiosis in inflammatory bowel diseases: The oxygen hypothesis. *The ISME Journal*, 7(7), 1256–1261.
<https://doi.org/10.1038/ismej.2013.80>
- Silverberg, M. S., Satsangi, J., Ahmad, T., Arnott, I. D., Bernstein, C. N., Brant, S. R., Caprilli, R., Colombel, J.-F., Gasche, C., Geboes, K., Jewell, D. P., Karban, A., Loftus, E. V., Peña, A. S., Riddell, R. H., Sachar, D. B., Schreiber, S., Steinhart, A. H., Targan, S. R., ... Warren, B. F. (2005). Toward an Integrated Clinical, Molecular and Serological Classification of Inflammatory Bowel Disease: Report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Canadian Journal of Gastroenterology*, 19(suppl a), 5A-36A.
<https://doi.org/10.1155/2005/269076>
- Sipponen, T., Kärkkäinen, P., Savilahti, E., Kolho, K.-L., Nuutinen, H., Turunen, U., & Färkkilä, M. (2008). Correlation of faecal calprotectin and lactoferrin with an endoscopic score for Crohn's disease and histological findings. *Alimentary*

Pharmacology & Therapeutics, 28(10), 1221–1229. <https://doi.org/10.1111/j.1365-2036.2008.03835.x>

Spiceland, C. M., & Lodhia, N. (2018). Endoscopy in inflammatory bowel disease: Role in diagnosis, management, and treatment. *World Journal of Gastroenterology*, 24(35), 4014–4020. <https://doi.org/10.3748/wjg.v24.i35.4014>

Stagos, D., Goutzourelas, N., Bar-Or, D., Ntontou, A.-M., Bella, E., Becker, A. T., Statiri, A., Kafantaris, I., & Kouretas, D. (2015). Application of a new oxidation-reduction potential assessment method in strenuous exercise-induced oxidative stress. *Redox Report: Communications in Free Radical Research*, 20(4), 154–162. <https://doi.org/10.1179/1351000214Y.0000000118>

THAKKAR, K., EL-SERAG, H. B., MATTEK, N., & GILGER, M. (2008). Complications of Pediatric Colonoscopy: A Five-Year Multicenter Experience. *Clinical Gastroenterology and Hepatology : The Official Clinical Practice Journal of the American Gastroenterological Association*, 6(5), 515–520. <https://doi.org/10.1016/j.cgh.2008.01.007>

Turner, D., Hyams, J., Markowitz, J., Lerer, T., Mack, D. R., Evans, J., Pfefferkorn, M., Rosh, J., Kay, M., Crandall, W., Keljo, D., Otley, A. R., Kugathasan, S., Carvalho, R., Oliva-Hemker, M., Langton, C., Mamula, P., Bousvaros, A., LeLeiko, N., & Griffiths, A. M. (2009). Appraisal of the pediatric ulcerative colitis activity index (PUCAI): *Inflammatory Bowel Diseases*, 15(8), 1218–1223. <https://doi.org/10.1002/ibd.20867>

- Turner, D., Otley, A. R., Mack, D., Hyams, J., Bruijne, J. de, Uusoue, K., Walters, T. D., Zachos, M., Mamula, P., Beaton, D. E., Steinhart, A. H., & Griffiths, A. M. (2007). Development, Validation, and Evaluation of a Pediatric Ulcerative Colitis Activity Index: A Prospective Multicenter Study. *Gastroenterology*, *133*(2), 423–432.
<https://doi.org/10.1053/j.gastro.2007.05.029>
- Vermeire, S., Van Assche, G., & Rutgeerts, P. (2006). Laboratory markers in IBD: Useful, magic, or unnecessary toys? *Gut*, *55*(3), 426–431.
<https://doi.org/10.1136/gut.2005.069476>
- von Martels, J. Z. H., Bourgonje, A. R., Klaassen, M. A. Y., Alkhalifah, H. A. A., Sadaghian Sadabad, M., Vich Vila, A., Gacesa, R., Gabriëls, R. Y., Steinert, R. E., Jansen, B. H., Bulthuis, M. L. C., van Dullemen, H. M., Visschedijk, M. C., Festen, E. A. M., Weersma, R. K., de Vos, P., van Goor, H., Faber, K. N., Harmsen, H. J. M., & Dijkstra, G. (2020). Riboflavin Supplementation in Patients with Crohn's Disease [the RISE-UP study]. *Journal of Crohn's & Colitis*, *14*(5), 595–607.
<https://doi.org/10.1093/ecco-jcc/jjz208>
- Webb, W. M. (2018). Rationalism, Empiricism, and Evidence-Based Medicine: A Call for a New Galenic Synthesis. *Medicines*, *5*(2), Article 2.
<https://doi.org/10.3390/medicines5020040>
- Xavier, R. J., & Podolsky, D. K. (2007). Unravelling the pathogenesis of inflammatory bowel disease. *Nature*, *448*(7152), Article 7152.
<https://doi.org/10.1038/nature06005>

Yorulmaz, E., Adalı, G., Yorulmaz, H., Taşan, G., Gürses, S., Ayaş, M. R., & Tuncer, İ. (2022). The Correlation between New Serological Markers and Disease Phenotype and Activation in Inflammatory Bowel Disease. *Middle East Journal of Digestive Diseases*, *14*(3), 294–303. <https://doi.org/10.34172/mejdd.2022.286>

CURRICULUM VITAE

