

# Steroid Resistance and Inflammatory Bowel Disease

A thesis submitted to the

University of Dublin

In fulfilment for the degree of Doctor in Medicine (M.D.)

March 2021

By Dr Mary Hussey

MB BCh BAO MRCPI

On research carried out with the School of Medicine,

Trinity College Dublin, Trinity Academic

Gastroenterology Group, and the Department of

Gastroenterology Tallaght University Hospital

Supervised by Professor Deirdre McNamara

# Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other University and it is entirely my own work. I agree to deposit this thesis in the University's open access intuitional repository or allow the library to do so on my behalf, subject to Irish Copyright Legislation and Trinity College Library conditions of use and acknowledgement. I consent to the examiner retaining a copy of this thesis beyond the examining period, should they wish to do so.

Signed: Mary Hussey

Trinity College Dublin Student Number: 15308074

Date: 18/03/2021

# Summary

Glucocorticoids are the mainstay of treatment in managing an acute flare in Inflammatory Bowel Disease as well as an acute relapse of disease. The primary objective of this dissertation has been to expand upon the current understanding of steroid metabolism in IBD patients overall, and in particular, to try and provide a better understanding of steroid resistance amongst IBD patients and to build upon the current knowledge of mechanisms underlying resistance. We have demonstrated that despite advancements in IBD therapeutics over the years, steroid resistance remains significant at 22% and we have accurately identified and reaffirmed clinical predictors of GCS failure including an elevated CRP, significant anaemia, hypoalbuminaemia, severe endoscopic disease and extensive disease. Indeed, major clinical trials like the SONIC trial supports the lack of concordance between clinical remission and concurrent endoscopic inflammation which strengthens the argument to use objective markers of inflammation such as those outlined above<sup>1</sup>. In addition, we have also shown the potential use of an immunomodulator may reduce rates of steroid resistance. Early recognition of these critical clinical indicators of corticosteroid failure may avoid prolonged corticosteroid exposure and allow for earlier introduction of rescue therapies and improve overall outcomes for the patients.

The underlying molecular mechanisms of corticosteroid resistance in the literature to date have been explored. Possible explanations of resistance including alterations relative to tissue cortisol metabolism including alterations in the glucocorticoid receptor (GR), reduced affinity of the ligand for the GR, reduced affinity of the GR to bind DNA and altered expression of transcription factors and/ or cytokines have all been examined in detail. In addition, this dissertation has outlined that in addition to the hypothalamic pituitary adrenal axis, tissue control of cortisol is under the control of the  $11\beta$ -HSD enzyme system.  $11\beta$ -HSD 1 activates cortisol at a tissue level and  $11\beta$ -HSD 2 inactivates cortisol. This enzyme system in relation to IBD has been explored and attempts have been made to build upon the understanding of the role and regulation of the  $11\beta$ -HSD enzyme system and cortisol metabolism amongst IBD subjects. Our initial data concluded that  $11\beta$ -HSD2 gene expression was downregulated in patients with IBD with an increased  $11\beta$ -HSD 1:2 ratio correlating with inflammatory activity in IBD patients. We hypothesised that potential dysregulation in this pathway may contribute to our understanding of GCS resistance amongst IBD patients. We later went on to examine the role  $11\beta$ -HSD enzyme system specifically amongst steroid responders and non-responders. We identified that resistant subjects demonstrated reduced levels of  $11\beta$ -HSD1, H6PDH and GR with an apparent upregulation of inflammatory cytokines compared with

responders. Our results suggest a potential role for alternative GCS use amongst IBD patients such as dexamethasone whose metabolism is less influenced by the 11 $\beta$ -HSD enzyme system. Further work on mechanisms of GR upregulation and increased 11 $\beta$ -HSD1 expression may provide a potential therapeutic pathway to overcome glucocorticoid resistance.

## **Table of Contents**

|                                           |           |
|-------------------------------------------|-----------|
| <b>Dedication</b>                         | <b>13</b> |
| <b>Acknowledgements</b>                   | <b>14</b> |
| <b>List of Abbreviations</b>              | <b>15</b> |
| <b>List of Tables</b>                     | <b>20</b> |
| <b>List of figures</b>                    | <b>22</b> |
| <b>Published papers and presentations</b> | <b>23</b> |
| -Publications                             | 23        |
| - Poster Presentations                    | 23        |
| - Oral Presentations                      | 23        |
| - Awards                                  | 24        |

# **Chapter 1- Introduction**

|                                                       |           |
|-------------------------------------------------------|-----------|
| <b>1.1 Inflammatory Bowel Disease</b>                 | <b>26</b> |
| 1.1.1 General Overview                                | 26        |
| 1.1.2 Prevalence and Incidence                        | 27        |
| 1.1.3 Pathogenesis                                    | 28        |
| 1.1.4 Disease Phenotyping and Activity                | 29        |
| 1.1.5 Biomarkers of Inflammation                      | 35        |
| <b>1.2 Management of IBD</b>                          | <b>37</b> |
| 1.2.1 General Overview                                | 37        |
| 1.2.2 Role of 5-aminosalicyclates                     | 39        |
| 1.2.3 Role of Immunomodulators                        | 40        |
| 1.2.4 Anti-Tumour Necrosis factor- $\alpha$ treatment | 42        |
| 1.2.5 Other Biologics                                 | 46        |
| 1.2.6 Role of Ciclosporin in Acute Severe UC          | 50        |
| <b>1.3 Corticosteroids and IBD</b>                    | <b>51</b> |

|                                              |           |
|----------------------------------------------|-----------|
| <b>1.4 Steroid Dependence and Resistance</b> | <b>56</b> |
| <b>1.5 Predictors of Steroid Response</b>    | <b>58</b> |
| <b>1.6 Goals of Dissertation</b>             | <b>61</b> |

## **Chapter 2**

### **Related Risk Factors associated with Steroid Resistance in an Irish IBD population**

|                                  |           |
|----------------------------------|-----------|
| <b>2.1 Introduction</b>          | <b>63</b> |
| <b>2.2 Aims</b>                  | <b>66</b> |
| <b>2.3 Materials and Methods</b> | <b>66</b> |
| <b>2.4 Results</b>               | <b>68</b> |
| 2.4.1 Baseline Characteristics   | 69        |
| 2.4.2 Response Rates             | 72        |
| 2.4.3 Predictors of Non-Response | 73        |



|                       |           |
|-----------------------|-----------|
| <b>2.5 Discussion</b> | <b>77</b> |
|-----------------------|-----------|

|                        |           |
|------------------------|-----------|
| <b>2.6 Conclusions</b> | <b>80</b> |
|------------------------|-----------|

## **Chapter 3: Molecular Mechanisms of Steroid Resistance**

|                         |           |
|-------------------------|-----------|
| <b>3.1 Introduction</b> | <b>82</b> |
|-------------------------|-----------|

|                              |           |
|------------------------------|-----------|
| <b>3.2 Normal Physiology</b> | <b>85</b> |
|------------------------------|-----------|

|                                               |           |
|-----------------------------------------------|-----------|
| <b>3.3 Molecular Mechanisms of Resistance</b> | <b>88</b> |
|-----------------------------------------------|-----------|

|                                                 |    |
|-------------------------------------------------|----|
| 3.3.1 Glucocorticoid Abnormalities & Resistance | 89 |
|-------------------------------------------------|----|

|                                         |    |
|-----------------------------------------|----|
| 3.3.2 Transcription factors & cytokines | 88 |
|-----------------------------------------|----|

|                                                  |           |
|--------------------------------------------------|-----------|
| <b>3.4 Genetics of Glucocorticoid Resistance</b> | <b>91</b> |
|--------------------------------------------------|-----------|

|                        |           |
|------------------------|-----------|
| <b>3.5 Conclusions</b> | <b>94</b> |
|------------------------|-----------|

## **Chapter 4: An introduction to the 11 $\beta$ -hydroxysteroid dehydrogenase enzyme system**

|                                                                     |            |
|---------------------------------------------------------------------|------------|
| <b>4.1 Introduction</b>                                             | <b>95</b>  |
| <b>4.2 11<math>\beta</math>-HSD Enzyme System</b>                   | <b>97</b>  |
| <b>4.3 11<math>\beta</math>-HSD &amp; Other Autoimmune Diseases</b> | <b>100</b> |
| <b>4.4 11<math>\beta</math>-HSD &amp; IBD, Current Knowledge</b>    | <b>101</b> |
| <b>4.5 Conclusions</b>                                              | <b>103</b> |

## **Chapter 5: Role & Regulation of 11 $\beta$ -HSD Enzyme System in patients with IBD**

|                                  |            |
|----------------------------------|------------|
| <b>5.1 Introduction</b>          | <b>105</b> |
| <b>5.2 Aims and Endpoints</b>    | <b>106</b> |
| <b>5.3 Materials and Methods</b> | <b>107</b> |
| 5.3.1 Patient Selection          | 107        |

|                                                     |            |
|-----------------------------------------------------|------------|
| 5.3.2 Processing of biopsies                        | 108        |
| 5.3.3 Reverse Transcriptase PCR                     | 109        |
| 5.3.4 Statistical Analysis                          | 111        |
| <b>5.4 Results</b>                                  | <b>112</b> |
| 5.4.1 Study Population                              | 112        |
| 5.4.2 11 $\beta$ -HSD 1 and 2 Expression            | 113        |
| 5.4.3 Expression of GR $\alpha$ , H6PDH & cytokines | 114        |
| <b>5.5 Discussion</b>                               | <b>117</b> |
| <b>5.6 Conclusions</b>                              | <b>121</b> |

## **Chapter 6: Comparing 11 $\beta$ -HSD Enzyme System amongst Glucocorticoid responders and Non-responders.**

|                         |            |
|-------------------------|------------|
| <b>6.1 Introduction</b> | <b>123</b> |
|-------------------------|------------|

|                                                          |            |
|----------------------------------------------------------|------------|
| <b>6.2 Aims and Objectives</b>                           | <b>125</b> |
| <b>6.3 Materials and Methods</b>                         | <b>126</b> |
| <b>6.4 Results</b>                                       | <b>130</b> |
| 6.4.1 Study Population                                   | 130        |
| 6.4.2 Responders and Non-responders                      | 132        |
| 6.4.3 $11\beta$ -HSD1 & 2, $GR\alpha$ , H6PD & cytokines | 134        |
| <b>6.5 Discussion</b>                                    | <b>136</b> |
| <b>6.6 Conclusions</b>                                   | <b>139</b> |
| <br><b>Chapter 7:</b>                                    |            |
| <b>Conclusions and Future Directions</b>                 | <b>140</b> |
| <br><b>References</b>                                    | <b>143</b> |

# Dedication

*To my wonderful, selfless, hardworking parents*

*Bridget and Alan, and to Joseph, my very*

*patient and tolerant husband.*

# Acknowledgements

This dissertation submitted for the degree of Doctor in Medicine, to the University of Dublin, describes the research work carried out in the School of Medicine Trinity College, the Trinity Academic Gastroenterology group, and the Department of Gastroenterology Tallaght University Hospital, under the supervision of Professor Deirdre McNamara. Several individuals and organisations contributed to the successful completion of this work. Firstly, I would like to extend a big thank you to my supervisor Professor Deirdre McNamara. From a very junior stage in my career, Professor McNamara encouraged me to develop a career in gastroenterology and she has been instrumental in directing my career from the onset. I am extremely grateful to her for giving me the opportunity to undertake this MD under her supervision and expert guidance. She has provided invaluable support, dedication, and advice throughout this project as well as my career to date and continues to do so when the occasion calls for it. I will always be indebted to her for all the mentoring and teaching she has provided to me over the years.

I would also like to thank the other gastroenterologists at Tallaght hospital, Professor Barbara Ryan, Dr Niall Breslin and Dr Anthony O'Connor for allowing me to recruit patients from their service and for their support and

encouragement throughout the project. I am grateful to the IBD nurse specialist Dr Yvonne Bailey and all the Gastroenterology admin staff including Ms Mary Gregg for all their help with co-ordinating the various clinical aspects of this project.

Thank you to my all my gastroenterology colleagues working with me throughout my time in Tallaght Hospital, especially Dr Grainne Holleran who has been a huge mentor to me throughout the years and during the completion of this dissertation. They have all be instrumental with patient recruitment and data collection. Thank you to our wonderful scientists, Dr Sinead Smith, and Dr Aoife Cannon for all their help with the scientific and laboratory aspects of my project.

I am also extremely grateful to Professor Mark Sherlock, Consultant endocrinologist, for all his expertise and input throughout this project. In addition, thank you to the Adelaide and Meath foundation for their financial support throughout the project.

Finally, a huge thank you to my wonderful family. They have shown me tremendous support throughout my research and career to date and I am eternally grateful for their continued patience and encouragement.

# List of Abbreviations

IBD: Inflammatory Bowel Disease

CD: Crohn's Disease

UC: Ulcerative Colitis

TI: Terminal Ileum

GIT: Gastrointestinal Tract

OCP: Oral Contraceptive Pill

QOL: Quality of Life

CDEIS: Crohn's Disease Endoscopic Index of Severity

SES-CD: Simplified Endoscopic activity Score for Crohn's  
Disease

5-ASA: 5-Aminosalicylate Acid

AZA: Azathioprine

TNF- $\alpha$ : Tumour Necrosis Factor-alpha

ADA: Adalimumab



|       |                                 |
|-------|---------------------------------|
| JAK:  | Janus Kinase                    |
| ASUC: | Acute Severe Ulcerative Colitis |
| CS:   | Corticosteroids                 |
| GCS:  | Glucocorticosteroids            |
| RCT:  | Randomised Controlled Trial     |
| MMX:  | Multi-Matrix                    |
| IV:   | Intravenous                     |
| HBI:  | Harvey Bradshaw Index           |
| CRP:  | C-Reactive Protein              |
| FBC:  | Full Blood Count                |
| LFT:  | Liver function Test             |
| U/E:  | Urea and Electrolytes           |
| ESR:  | Erythrocyte Sedimentation Rate  |
| OR:   | Odds Ratio                      |
| AXR:  | Abdominal Xray                  |

|               |                                     |
|---------------|-------------------------------------|
| 6-MP          | 6-Mercaptopurine                    |
| HIPE:         | Health Inpatient Enquiry            |
| LOS:          | Length of stay                      |
| MR:           | Mineralocorticoid Receptor          |
| BSG:          | British Society of Gastroenterology |
| SC:           | Subcutaneously                      |
| TNF- $\alpha$ | Tumour Necrosis Factor- $\alpha$    |
| LOR:          | Loss of Response                    |
| SLE:          | Systemic Lupus Erythematosus        |
| HPAA:         | Hypothalamic Adrenal Axis           |
| CRH:          | Corticotrophin Releasing Hormone    |
| ACTH:         | Adrenocorticotrophic Hormone        |
| GR:           | Glucocorticoid Receptor             |
| HSP:          | Heat Shock Proteins                 |
| GRE:          | Glucocorticoid Response elements    |

|                 |                                             |
|-----------------|---------------------------------------------|
| NFKB:           | Nuclear Factor Kappa B                      |
| PBMC:           | Peripheral Blood Mononuclear Cell           |
| TGF- $\beta$ 3  | Transforming Growth Factor Beta 3           |
| IL-10           | Interleukin-10                              |
| IL-8            | Interleukin 8                               |
| MRNA            | Messenger RNA                               |
| MDR1            | Multi-Drug Resistant Gene-1                 |
| 11 $\beta$ -HSD | 11Beta-Hydroxysteroid Dehydrogenase         |
| IL-1 $\beta$    | Interleukin-1Beta                           |
| IL-6            | Interleukin-6                               |
| NADPH           | Nicotinamide Adenine Dinucleotide Phosphate |
| H6PDH           | Hexose-6-Phosphate-Dehydrogenase            |
| RT-PCR          | Real Time-Polymerase Chain Reaction         |
| AU              | Arbitrary Units                             |

# List of Tables

|                                                               |     |
|---------------------------------------------------------------|-----|
| Table 1:1 Montreal and Paris Classification in UC             | 30  |
| Table 1.2 Montreal and Paris Classification in CD             | 31  |
| Table 1.3 Mayo Score for UC                                   | 33  |
| Table 1.4 Harvey Bradshaw Index in CD                         | 34  |
| Table 1.5 Summary of Glucocorticoids                          | 52  |
| Table 1.6 Indices predictive of Steroid failure in ASUC       | 60  |
| Table 2.1 Basic Characteristics                               | 71  |
| Table 2.2 Predictors of Non-Response                          | 75  |
| Table 2.3 Responders vs. Steroid Dependence                   | 76  |
| Table 3.1 Rates of Steroid Resistance Current Evidence        | 84  |
| Table 4.1 Effects of Glucocorticoids                          | 96  |
| Table 5.1 Demographic & Clinical Data IBD vs. Controls        | 113 |
| Table 5.2 MRNA expression of 11 $\beta$ HSD1&2 and regulators | 115 |
| Table 5.3 Expression of 11 $\beta$ HSD1&2 based on Disease    | 116 |

|                                                                 |     |
|-----------------------------------------------------------------|-----|
| Table 5.4 Relative expression of 11 $\beta$ HSD 2 and cytokines | 117 |
| Table 6.1 Study Population                                      | 132 |
| Table 6.2 Base Characteristics Responders & Non-Responders      | 133 |
| Table 6.3 11 $\beta$ HSD 1&2, H6PDH, GR                         | 135 |
| Table 6.4 Cytokine expression Responders &Non-Responders        | 135 |

# List of Figures

|                                                          |     |
|----------------------------------------------------------|-----|
| Figure 1:1 Endoscopic views of intestinal mucosa in IBD  | 27  |
| Figure 1:2 Glucocorticoids                               | 53  |
| Figure 3:1 HPA-A axis and regulation of intracellular GR | 87  |
| Figure 3.2 Molecular Mechanisms of Steroid Resistance    | 88  |
| Figure 4.1 Tissue regulation of Glucocorticoid action    | 100 |
| Figure 5.1 Reverse Transcriptase PCR                     | 110 |

# Published Papers and Presentations

Part of this thesis has been published and or presented at both national and international meetings as follows:

1. **Hussey, Mary** & Holleran, Grainne & Smith, Sinead & Sherlock, Mark & McNamara, Deirdre. (2017). The Role and Regulation of the 11 Beta-Hydroxysteroid Dehydrogenase Enzyme System in Patients with Inflammatory Bowel Disease. Digestive Diseases and Sciences. 62. 10.1007/s10620-017-4753-1.

## Presentations at National Meetings:

1. **Hussey M**, Cannon A, Holleran G, Kiat C, Hall B, O'Sullivan J, Sherlock M, McNamara D; Altered Tissue Glucocorticoid metabolism is associated with inflammatory Bowel disease Irish Society of Gastroenterology Summer 2015.  
Oral Presentation
2. **Hussey M**, Holleran G, Keating D, Whelan S, Devitt P, Healy J, Ryan B , McNamara D; Related risk factors associated with steroid resistance in an Irish IBD population, Irish Society of Gastroenterology 2016

# Presentations at International Meetings:

1. **Hussey M**, Holleran G, Smith S, Sherlock M, McNamara D: Inflammatory cytokines and their regulation of 11-beta-hydroxysteroid dehydrogenase type 2 in IBD patients, United European Gastroenterology Week (UEGW), October 2017
2. **Hussey M**, Cannon A, Holleran G, Kiat C, Hall B, O'Sullivan J, Sherlock M, McNamara D  
Altered Tissue Glucocorticoid metabolism is associated with inflammatory Bowel disease, United European Gastroenterology Week (UEGW), October 2015
3. **Hussey M**, Holleran G, Keating D, Whelan S, Devitt P, Healy J, Ryan B, McNamara D; Related risk factors associated with steroid resistance in an Irish IBD population, United European Gastroenterology Week (UEGW) October 2016

## Awards

1. **Hussey M**, Cannon A, Holleran G, Kiat C, Hall B, O'Sullivan J, Sherlock M, McNamara D; Altered Tissue Glucocorticoid metabolism is associated with inflammatory Bowel disease Irish Society of Gastroenterology Summer 2015.



Oral Presentation: **Second prize for oral presentation at Irish Society of Gastroenterology 2015**

2. **Professor O'Morain Research Medal** awarded by the Meath Foundation, Steroid Metabolism, and Inflammatory Bowel Disease

# Chapter 1: Introduction

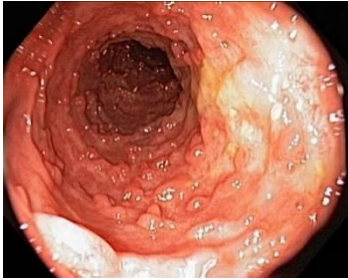
## 1.1 Inflammatory Bowel Disease

### 1.1.1 General Overview

Inflammatory bowel disease (IBD) is a chronic relapsing immune mediated condition which is commonly classified as either Crohn's disease (CD) or ulcerative colitis (UC). About 10% of colitis cases are not initially distinguishable and are termed indeterminate. Although CD can more commonly affect the terminal ileum (TI), it can potentially involve any region of the gastrointestinal tract (GIT), from the mouth to the rectum of the affected individual. Crohn's disease can often be a segmental disease which can be associated with penetrating complications such as stricture formation, fistulae as well as ulcers, and granulomas in the bowel mucosa (**Figure 1.1**). UC generally only involves the large intestine and typically manifests as a continuous colitis. Clinical manifestations of IBD can include diarrhoea or bloody diarrhoea, malnutrition, abdominal pain, and weight loss<sup>2</sup>. Other extraintestinal complications can include arthropathies, ophthalmic and dermatological manifestations<sup>3</sup>. The diagnosis of IBD can

involve a combination of clinical, biochemical, endoscopic, radiological, and histological domains.

**Figure 1.1 Endoscopic views of inflamed intestinal mucosa in IBD**



## **1.1.2 Prevalence and Incidence**

At the turn of the 21st century, the prevalence of IBD remains high at 0.3%, with an accelerating incidence<sup>4</sup> in newly industrialised countries whose societies have become more westernised<sup>4</sup>. A recent systematic analysis published in the lancet reported 6.8 million cases of IBD globally in 2017<sup>5</sup>. The age-standardised prevalence rate increased from 79.5 per 100 000 population in 1990 to 84.3 per 100 000 population in 2017<sup>5</sup>. Additionally, an epidemiological study of an Irish cohort of paediatric patients in 2017 also demonstrated incidences of early onset IBD (EO-IBD, <10 years) are also on the rise<sup>6</sup>. The incidence of EO-IBD increased by 0.6 per 100 000 per year, with a significant increase in UC by 0.06 per 100 000 per year. This study also demonstrated that male patients have more extensive and

severe disease phenotypes, and younger patients have higher relapse rates than older children<sup>6</sup>. IBD potentially can affect people of all ages but usually begins before age 30, with peak incidence from 14 to 24. It can also have a second smaller peak between ages 50 and 70<sup>7</sup>.

### **1.1.3 Pathogenesis**

The pathogenesis is generally considered multifactorial with different environmental, genetic, immune, and gut microbial mediated factors all hypothesized to play an interactive role <sup>7,8</sup>. Although not completely understood, it is suggested to be a result of an uncontrolled immune response to a trigger in genetically prone individuals<sup>9</sup>. For example genetic susceptibility has been observed to play an important role in the pathogenesis of CD, with a five-fold increased risk reported amongst first-degree relatives of affected individuals <sup>10,11</sup>. Excessive production of local inflammatory cytokines such as IL-12, IL-17, TNF- $\alpha$ , and IFN- $\gamma$ , has also been implicated in the chronic intestinal inflammation observed in CD patients <sup>12,13</sup>. Dysregulation in the body's microflora has also been hypothesised as a potential trigger in driving the proinflammatory cascade which occurs in IBD<sup>8</sup>. A number of environmental factors such as smoking, appendicitis, Oral contraceptive pill (OCP) use, diet, breastfeeding, infections/vaccinations, antibiotics, helminths, and childhood hygiene, have all been implicated as potential triggers in IBD pathogenesis <sup>14</sup>.

There are recognisable pathological distinctions between UC and CD. Typically, CD features transmural inflammation involving the whole thickness of the bowel wall, skip lesions often with rectal sparing and an inflammatory response which is associated with lymphoid aggregates and granulomas <sup>15</sup>. Overtime recurrent transmural inflammation can result in fibrosis resulting in obstructing complications such as strictures, which are quite distinguishable from UC. Penetrating complications are perhaps associated with a more severe phenotype of CD, and often result in the development of abnormal fistulous tracts between bowel segments and other potential organs <sup>16</sup> with often considerable implications on patient care and quality of life (QOL).

Conversely UC typically demonstrates a continuous pattern of submucosal inflammation with inflammation typically beginning in the rectum and extending proximally. Like CD, UC patients will experience periods of relapse and remission. Reports of up to 90% relapse rates after the first attack are reported in the literature and early relapse or active disease in the first 2 years is associated with a worse disease course subsequently<sup>17-19</sup>.

#### **1.1.4 Phenotypic classification and disease activity scoring**

Disease activity in IBD is determined using several different assessment tools which incorporate clinical, endoscopic, histological, and sometimes

biochemical parameters. The Montreal classification<sup>20</sup> in adults and Paris classification<sup>21</sup> in children (**Tables 1.1 & 1.2**) are widely used to outline key phenotypic features of patients with UC and CD<sup>16</sup>. These classifications are widely employed in day-to-day clinical practice as well as research fields. The Montreal classification incorporates age of onset of disease (A), disease location (L) and disease behaviour (B) for both UC and CD.

**Table 1.1 Montreal and Paris classification in UC**

|                 | Montreal <sup>20</sup> |                                                  | Paris <sup>26</sup> |                                                  |
|-----------------|------------------------|--------------------------------------------------|---------------------|--------------------------------------------------|
| <b>Extent*</b>  | <b>E1</b>              | <b>Ulcerative proctitis</b>                      | <b>E1</b>           | <b>Ulcerative proctitis</b>                      |
|                 | <b>E2</b>              | <b>Left-sided UC (distal to splenic flexure)</b> | <b>E2</b>           | <b>Left-sided UC (distal to splenic flexure)</b> |
|                 | <b>E3</b>              | <b>Extensive (proximal to splenic flexure)</b>   | <b>E3</b>           | <b>Extensive (hepatic flexure distally)</b>      |
|                 |                        |                                                  | <b>E4</b>           | <b>Pancolitis (proximal to hepatic flexure)</b>  |
| <b>Severity</b> | <b>S0</b>              | <b>Clinical remission</b>                        | <b>S0</b>           | <b>Never severe†</b>                             |
|                 | <b>S1</b>              | <b>Mild UC</b>                                   | <b>S1</b>           | <b>Ever severe†</b>                              |
|                 | <b>S2</b>              | <b>Moderate UC</b>                               |                     |                                                  |
|                 | <b>S3</b>              | <b>Severe UC</b>                                 |                     |                                                  |

*\*Extent defined as maximal macroscopic inflammation.*

*†Severe defined by Paediatric Ulcerative Colitis Activity Index (PUCAI) ≥65*

**Table 1.2 Montreal and Paris classification in Crohn's disease**

|                                 | Montreal <sup>20</sup> |                                              | Paris <sup>21</sup>  |                                                                                        |
|---------------------------------|------------------------|----------------------------------------------|----------------------|----------------------------------------------------------------------------------------|
| <b>Age at diagnosis (years)</b> | <b>A1</b>              | <b>&lt;17</b>                                | <b>A1a</b>           | <b>&lt;10</b>                                                                          |
|                                 |                        |                                              | <b>A1b</b>           | <b>10–17</b>                                                                           |
|                                 | <b>A2</b>              | <b>17–40</b>                                 | <b>A2</b>            | <b>17–40</b>                                                                           |
|                                 | <b>A3</b>              | <b>&gt;40</b>                                | <b>A3</b>            | <b>&gt;40</b>                                                                          |
| <b>Location</b>                 | <b>L1</b>              | <b>Terminal ileal±limited caecal disease</b> | <b>L1</b>            | <b>Distal 1/3 ileum±limited caecal disease</b>                                         |
|                                 | <b>L2</b>              | <b>Colonic</b>                               | <b>L2</b>            | <b>Colonic</b>                                                                         |
|                                 | <b>L3</b>              | <b>Ileocolonic</b>                           | <b>L3</b>            | <b>Ileocolonic</b>                                                                     |
|                                 | <b>L4</b>              | <b>Isolated upper disease</b>                | <b>L4a</b>           | <b>Upper disease proximal to ligament of Treitz</b>                                    |
|                                 |                        |                                              | <b>L4b</b>           | <b>Upper disease distal to ligament of Treitz and proximal to distal 1/3 ileum</b>     |
| <b>Behaviour</b>                | <b>B1</b>              | <b>Non-stricturing, non-penetrating</b>      | <b>B1</b>            | <b>Non-stricturing, non-penetrating</b>                                                |
|                                 | <b>B2</b>              | <b>Stricturing</b>                           | <b>B2</b>            | <b>Stricturing</b>                                                                     |
|                                 | <b>B3</b>              | <b>Penetrating</b>                           | <b>B3</b>            | <b>Penetrating</b>                                                                     |
|                                 |                        |                                              | <b>B2B3</b>          | <b>Both penetrating and stricturing disease, either at the same or different times</b> |
|                                 | <b>P</b>               | <b>Perianal disease modifier</b>             | <b>P</b>             | <b>Perianal disease modifier</b>                                                       |
| <b>Growth</b>                   |                        |                                              | <b>G<sub>0</sub></b> | <b>No evidence of growth delay</b>                                                     |
|                                 |                        |                                              | <b>G<sub>1</sub></b> | <b>Growth delay</b>                                                                    |

Additionally, the Mayo Score for UC (**Table 1.3**) is a disease assessment tool which incorporates clinical and endoscopic parameters when determining disease severity<sup>22</sup>. The score of 0–12 measures stool frequency, rectal bleeding, a physician's global assessment and a measure of mucosal inflammation at endoscopy<sup>17</sup>. The partial Mayo score is a non-invasive measure of clinical activity and has been shown to demonstrate comparable accuracy in determining patient perceived disease activity and response<sup>23</sup>. It essentially includes all the parameters used in the full Mayo score but does not require endoscopic assessment. Clinical response is determined by reduction of baseline Mayo score by  $\geq 3$  points and a decrease of 30% from the baseline score with a decrease of at least one point on the rectal bleeding subscale or an absolute rectal bleeding score of 0 or 1<sup>22</sup>. Clinical remission is defined as a Mayo score  $\leq 2$  and no individual subscore  $> 1$ <sup>22</sup>.



**Table 1.3 Mayo score for Ulcerative Colitis<sup>23</sup>**

| Mayo index                    | 0                          | 1                                                                    | 2                                                                                  | 3                                                 |
|-------------------------------|----------------------------|----------------------------------------------------------------------|------------------------------------------------------------------------------------|---------------------------------------------------|
| Stool frequency               | Normal                     | 1–2/day more than normal                                             | 3–4/day more than normal                                                           | 5/day more than normal                            |
| Rectal bleeding               | None                       | Streaks of blood with stool <50% of the time                         | Obvious blood with stool most of time                                              | Blood passed without stool                        |
| Mucosa (endoscopic subscore)  | Normal or inactive disease | Mild disease (erythema, decreased vascular pattern, mild friability) | Moderate disease (marked erythema, lack of vascular pattern, friability, erosions) | Severe disease (spontaneous bleeding, ulceration) |
| Physician's global assessment | Normal                     | Mild disease                                                         | Moderate disease                                                                   | Severe disease                                    |

**\*Disease activity: Mild 3–5; Moderate 6–10; Severe 11–12.**

Similarly, the Harvey-Bradshaw index (HBI) is a comparable, simple, and reliable clinical assessment tool used in CD. This score considers five parameters: patient well-being, abdominal pain, stool frequency, presence of an abdominal mass or complications (**Table 1.4**). Clinical response is typically defined as  $HBI \leq 3$  or a 30% reduction from index scores. A HBI

score  $\leq 4$  is often used to define clinical remission<sup>24</sup>. There are several endoscopic scoring systems employed for CD endoscopic assessment. The Crohn's Disease Endoscopic Index of Severity (CDEIS)<sup>25</sup> and the Simplified Endoscopic activity Score for Crohn's disease (SES-CD)<sup>26</sup> are the two most frequently used, particularly when it comes to clinical trials. Both are used to assess for complete mucosal healing as an endpoint in clinical trials<sup>17</sup>.

**Table 1.4 Harvey Bradshaw Index for Crohn's Disease<sup>24</sup>**

|                                                                                 |
|---------------------------------------------------------------------------------|
| <b>General well being</b>                                                       |
| 0=very well                                                                     |
| 1=slightly below average                                                        |
| 2=poor                                                                          |
| 3=very poor                                                                     |
| 4=terrible                                                                      |
| <b>Abdominal pain</b>                                                           |
| 0=none                                                                          |
| 1=mild                                                                          |
| 2=moderate                                                                      |
| 3=severe                                                                        |
| <b>Number of liquid stools per day</b>                                          |
| 0=0–1                                                                           |
| 1=2–3                                                                           |
| 2=4–5                                                                           |
| 3=6–7                                                                           |
| 4=8–9                                                                           |
| 5=10+                                                                           |
| <b>Abdominal mass</b>                                                           |
| 0=none                                                                          |
| 1=dubious                                                                       |
| 2=definite                                                                      |
| 3=tender                                                                        |
| <b>Complications</b>                                                            |
| Arthralgia, uveitis, erythema nodosum, aphthous ulcers,                         |
| pyoderma gangrenosum, anal fissures, new fistulas, abscesses (1 point for each) |

**\*Disease activity: <4 remission, Mild 5-7, Moderate 8-16, Severe>16**

### **1.1.5 Biomarkers of inflammation and disease activity in IBD**

Objective biochemical markers of disease activity provide a non-invasive means of monitoring disease activity amongst IBD patients. Previously biomarkers such as C-reactive protein (CRP), serum albumin and faecal calprotectin have all been shown to correlate with both clinical and endoscopic disease activity. For example, Solem et al. demonstrated that in CD patients, moderate-severe clinical activity (Odds Ratio (OR), 4.5) , active disease at colonoscopy (OR, 3.5), and histologically severe inflammation (OR, 10.6) were all significantly associated with CRP elevation<sup>27</sup>. Furthermore, in UC patients, CRP elevation was also associated with severe clinical and endoscopic activity but not with histological inflammation. They also noted there was a correlation between worsening disease activity, anaemia and hypoalbuminaemia and CRP<sup>27</sup>. In another study, a high baseline CRP (>50 mg/L) and a low serum albumin (<35 g/L) which were representative of severe disease activity, independently correlated with reduced infliximab concentrations from week 0–6, amongst UC patients with moderate-severe disease<sup>28</sup>.

Faecal calprotectin is a useful first line investigative tool to use in patients presenting with gastrointestinal symptoms and a normal value has been shown to have a very high negative predictive value for diagnosing IBD<sup>29</sup>.

In more recent years faecal calprotectin has become a reliable marker of disease activity in patients with established IBD and in particular for detection of early recurrence. For example, in the POCER trial, faecal calprotectin with a level  $>100\text{ }\mu\text{g/g}$  stool, correlated well with endoscopic recurrence in CD patients with an overall sensitivity of 89% and negative predictive value of 91%<sup>30</sup>. The TOPPIC trial also demonstrated correlation between faecal calprotectin and clinical recurrence of CD<sup>31</sup>. Furthermore, a study by Cassinotti et al. in 2017 showed that despite also investigating the use of full blood count (FBC), CRP and protein electrophoresis at 3 month intervals, faecal calprotectin remained the only reliable indicator of clinical relapse upon cessation of azathioprine in both UC and CD patients<sup>32</sup>.

## **1.2 Management of IBD and Treatment options**

### **1.2.1 General Overview**

Given the heterogeneity of IBD, the degree of symptomatic and inflammatory burden varies from patient to patient. Thus, optimal medical management of IBD has increasingly required a multifaceted and individualized approach to patient care <sup>33</sup>. IBD therapy has progressed significantly in the last number of years and developments in both pharmacological and non-pharmacological options, as well as surgical techniques continue to advance daily. Treatment goals overall are directed towards; improving patient QOL, disease maintenance and remission, prevention and minimalization of disease associated complications, avoiding disease related malnutrition as well as providing adequate psychosocial support for these patients throughout their lifetime<sup>33</sup>.

More recently, a treat-to-target approach, involving close monitoring of specific and objective measures of inflammation, has been advocated for IBD to improve long-term patient outcomes and reduce the need for surgeries<sup>34,35</sup>. Treatment decisions are influenced by a number of disease and patient related characteristics including disease phenotype, extent and severity, age of onset, gender, serology and sometimes even genetic

markers<sup>36</sup>. These factors guide clinician decisions regarding the need for more aggressive therapeutic strategies in those high-risk individual patient groups. For example, an increased adoption of a “top-down approach” has seen an early introduction of biologic agents, often in combination with immunomodulators, for more complex and aggressive disease phenotypes<sup>37</sup>. Patients with extensive UC often require this approach, as colectomy rates are higher in patients with more extensive disease. A systematic review showed that the 10 year colectomy rate is 19% for those with extensive colitis, 8% with left-sided colitis and 5% with proctitis; and male gender, young age and elevated inflammatory markers at diagnosis also increase the likelihood of colectomy<sup>38</sup>.

The ultimate treatment target for IBD patients has been extensively debated amongst expert groups and to date no consensus has been agreed upon regarding the definition of disease remission. Multiple definitions have been suggested however which include clinical, endoscopic, or even histological parameters. Variations exist amongst rates of remission depending upon which parameter is used. Utilising mucosal healing has been challenged in the past as it often places increased pressure on services in clinical practice with the need for more regular endoscopic assessment and potential for treatment escalation in asymptomatic individuals<sup>39</sup>. More recently, histological remission has been utilised as a primary end point in a lot of the

newer clinical trials but opinions on the precise definition on histological remission varies<sup>40,41</sup>. However, increasing evidence suggests that the persistence of endoscopic and histological disease activity correlate with elevated risks of disease relapse, increasing need for corticosteroids as well as surgery<sup>42-44</sup>. IBD is associated with an increased incidence of colorectal cancer with a younger median age of onset compared with sporadic colorectal cancer<sup>45</sup>. Mucosal healing has been shown to reduce the risk of colorectal cancer in these patients<sup>46</sup>.

### **1.2.2 5-aminosalicylates (5-ASA)**

5-aminosalicylates are among one the first line therapies used in IBD. Although their mechanism of action is not entirely understood, they have been shown to have anti-inflammatory effects in IBD and rheumatological disorders. Meta-analyses support the use of oral 5-ASA as a standard induction therapy of mild to moderately active UC<sup>47-49</sup>. Remission rates over 90% have been reported in cases of mild-moderate distal or left sided disease<sup>50</sup>. They predominantly exert their anti-inflammatory effects in the large bowel, and they do not typically have a role in the management of CD. A Cochrane systematic literature review showed that oral 5-ASA has no efficacy in maintaining clinical remission in Crohn's disease<sup>51</sup>. The

ASCEND trials demonstrated that UC patients with more severe disease benefited from a higher dose of 4.8g/day compared with those with milder disease, achieving similar response rates with the reduced rate of 2.4g<sup>52-54</sup>. Following initiation of oral 5-ASA, 10–30% of patients are in symptomatic remission at week 2, 30–45% by week 4 and 35–50% by week 8<sup>55-58</sup>. Superior response rates are noted when oral and topical 5-ASA therapies are combined even in patients with extensive disease<sup>59</sup>. A recent Cochrane analysis advocated maintenance 5-ASA therapy in UC patients at doses > 2g to reduce relapse rates and sustain remission<sup>49</sup>. Despite multiple formulations of 5-ASA on the market no single agent has been advocated as more efficacious and preferences are often driven by patient selection and tolerance. In addition to its anti-inflammatory properties, 5-ASAs have been shown to reduce the risk of colorectal cancer in IBD patients<sup>60</sup>. A systematic review by Bonovas et al. in 2017 supported the chemopreventive effects in IBD noting a strong reduction in colorectal neoplasia in UC (RR = 0.50, 95% CI: 0.38-0.64), but nonsignificant in Crohn's disease (RR = 0.76, 95% CI: 0.43-1.33). Furthermore, they noted mesalazine use was protective (RR = 0.70, 95% CI: 0.51-0.94) with evidence of a dose-effect<sup>61</sup>.

### **1.2.3 Immunomodulators in IBD**



Thiopurines such as Azathioprine (AZA) and its pro-drug 6-mercaptopurine (6-MP) are some of the most consistently used treatments to maintain disease remission in IBD<sup>62,63</sup>. A 2016 Cochrane review by T

immer et al. included 232 UC patients from four maintenance studies of azathioprine versus placebo and showed a benefit of azathioprine over placebo (44% vs 65% failure rate, respectively, RR 0.68, 95% CI 0.54 to 0.86)<sup>64</sup>. Although meta-analyses continue to support thiopurine use in maintenance care in UC, longer term data however does not recommend their use as a treatment option for induction of remission in UC patients<sup>65</sup>. This is explained largely by the fact that it can take up to 3 months before the full therapeutic effects are felt.

The British Society of Gastroenterology (BSG) have recently updated their guidelines on the management of IBD and a strong recommendation has been issued for thiopurine use for maintenance of remission in CD patients<sup>17</sup>. They can be used as monotherapy or in combination with a biologic agent as a “top down” treatment approach in patients with more severe disease. The SONIC trial has shown that the combination of infliximab and azathioprine is superior to infliximab alone for the induction and maintenance of remission in early CD<sup>66</sup>. Studies have also demonstrated that they may have a role in reducing recurrence rates postoperatively for small bowel CD patients<sup>67</sup>. There are considerations however, in relation to

potential side effects, duration of therapy and appropriate patient selection when considering thiopurines.

Methotrexate is an alternative option to thiopurines for maintaining remission in CD patients. A dose of at least 15mg per week is recommended<sup>17</sup>. Feagan et al.<sup>68</sup> showed increased clinical remission rates compared with placebo at 16 weeks, with reduced prednisolone requirements in patients receiving intramuscular methotrexate 25 mg weekly with chronic active CD despite at least 3 months of prednisolone therapy. Additionally, a retrospective cohort study by Kopylov et al. which included 118 patients, showed benefit of methotrexate in both induction and maintenance treatment for CD, with steroid-free remission being achieved in 37.2% of patients, and maintained relapse-free in 63.6% for a median of 12 months<sup>69</sup>. Superior efficacy with subcutaneous (SC) methotrexate has been consistently reported and initiation of therapy via subcutaneous route is recommended initially. Patients can switch to oral treatment during later maintenance stages.

### **1.2.4 Anti-Tumour Necrosis Factor-alpha therapy**

Biologic therapy with anti-Tumour Necrosis Factor-alpha (TNF- $\alpha$ ) blockers has revolutionized the therapeutic management of IBD. Tumour Necrosis factor-alpha (TNF- $\alpha$ ) is a key inflammatory cytokine involved in

the inflammatory cascade that is triggered in IBD<sup>70</sup>. TNF- $\alpha$  levels have been shown to be increased in IBD patients and higher concentrations have been noted in the intestinal mucosa and lumen<sup>71,72</sup>. Biologic therapies such as infliximab, adalimumab and golimumab are some of the more commonly used antibody agents which directly target TNF- $\alpha$ , and they play an essential role in altering the natural history of IBD for many patients.

Infliximab is an infusion based chimeric mouse/human monoclonal antibody which is given at a typical dose of 5mg/kg at week 0, week 2 and week 6 at induction. Depending on response rates maintenance intervals can range from 4-8 weekly intervals. In certain situations, accelerated infliximab may be required and this method has been shown to be both safe and efficacious in the past in select patient cohorts<sup>73</sup>. UC patients have shown superior response rates at induction (67% vs. 33%) as well as sustained maintenance remission rates (30% vs. 13%), when compared with placebo in both the ACT1 and ACT2 clinical trials<sup>74</sup>. Additionally, Of the patients who were receiving corticosteroids at baseline, approximately 22 percent of patients treated with infliximab had discontinued corticosteroids by week 30 among 269 patients in both studies or by week 54 among 143 patients in ACT 1 while maintaining clinical remission<sup>74</sup>. Similarly, cohort studies have demonstrated steroid free remission rates of 47% for UC patients treated with infliximab and up to 77% of patients were colectomy free at 41.5

months<sup>75,76</sup>. Again, superior efficacy rates were noted in those patients who received combination therapy with an immunomodulator. The ACCENT I trial also demonstrated the efficacy of infliximab as an induction and maintenance therapy in patients with active luminal CD with response rates of 58 % and 39% at induction and maintenance intervals, respectively<sup>77</sup>. Additionally, this trial also demonstrated that patients on corticosteroids who received maintenance infliximab were able to reduce steroid use, and a third of these patients were able to stop steroids with maintenance of clinical benefit<sup>77</sup>.

Adalimumab (ADA) is SC fully humanised monoclonal antibody and it too has proven efficacy in both establishing and maintaining remission in IBD patients. Remission rates of 19% vs. 9% (UC vs. placebo) at week 8, and sustained remission rates of 22% vs. 12% at week 52, were demonstrated in the ULTRA1 and ULTRA2 trials, which looked at the use of ADA in moderate-severe active UC patients<sup>78,79</sup>. Overall, steroid free remission rates were recorded at 13.3 % at week 52 in the adalimumab group compared with 5.7% in the placebo group,  $p=0.03$ <sup>80</sup>. Longer term remission rates remained at 25% at year 4 in the ULTRA 3 trial<sup>81</sup>. The CLASSIC I study in moderate to severe Crohn's disease naïve to anti-TNF therapy showed remission was achieved in 36% of patients receiving standard induction ADA dosing

compared with 24% in placebo<sup>82</sup>. Maintenance of clinical remission was achieved by 36% of subjects who receive fortnightly 40mg SC ADA at week 56 in the In the CHARM study<sup>83</sup>. At week 56, 6%, 29%, and 23% of patients treated with placebo, adalimumab 40 mg every other week, and adalimumab 40 mg weekly, respectively, achieved corticosteroid-free remission<sup>83</sup>. Golimumab is the latest subcutaneous anti-TNF- $\alpha$  antibody agent to be licensed for use in UC. There is no efficacy with CD. The PURSUIT-SC study demonstrated response rates at week 6 were 51% and 54.9% amongst patients receiving 200mg/100mg and 400mg/200mg golimumab, respectively vs. 30.3% of those in the placebo arm of the study<sup>84</sup>. Sustained remission has been demonstrated in a follow-up study in patients with moderate to severe UC<sup>85</sup>.

In recent years therapeutic drug monitoring has been encouraged as a means of optimising therapy and early identification of treatment failure and poor compliance amongst patients. This involves the measurement of drug and drug antibody levels to guide the need for dose adjustments, detection of immunogenicity and potentially switch drug therapy if patients are relapsing despite an optimal detected drug level. In addition, if significant antibodies are detected, addition of immunomodulators or dose escalations may be employed to reduce drug antibody levels. Drug levels can vary depending on the assay used and varied opinions exist regarding the optimal therapeutic

targets, especially in patients with more severe phenotypic features of disease. Therapeutic drug monitoring has been shown to be very effective in clinical practice<sup>86</sup>.

### **1.2.5 Other Biologic agents**

Although anti-TNF- $\alpha$  agents play an integral role in IBD management, due to increasing immunogenicity and resultant loss of response newer biologic agents which target alternative molecules involved in the inflammatory cascade have emerged in recent years.

#### ***Ustekinumab***

Ustekinumab is a monoclonal antibody to the p40 subunit of interleukin-12 and interleukin-23 and has been evaluated in the UNITI and IM-UNITI studies in patients with CD<sup>17</sup>. UNITI-1 enrolled patients who had prior anti-TNF failure, either as result of intolerance or primary/secondary loss of response (LOR). The week 8 clinical response rates were 37.8% in those receiving Ustekinumab 6 mg/kg, 33.5% with 130 mg and 20.2% with placebo<sup>87</sup>. UNITI2 enrolled patients who were anti-TNF naïve and response

rates at week 8, were 57.9% for 6 mg/kg, 47.4% for 130 mg and 32.1% for placebo ( $p < 0.001$  vs both doses). The UNITI-IM study later went on to show that 41.1% of subjects were still in remission at week 44 on Ustekinumab 90 mg subcutaneously 8-weekly compared with 26.2% on placebo. In addition, steroid free remission rates were statistically higher in the Ustekinumab treatment groups compared with controls at week 52, 46% vs. 29%,  $p = 0.04$ .<sup>87</sup> More recently, data presented in abstract form from the UNIFI trial, examined the use of Ustekinumab as induction and maintenance therapy in moderate to severely active UC in adults who had failed or were intolerant to alternative therapies including corticosteroids, anti-TNF agents, anti-integrins and immunomodulators. Week 8 remission rates were as follows: 15.6% on 130 mg IV Ustekinumab, 15.5% on the approximately 6 mg/kg IV dose and 5.3% on placebo ( $p < 0.001$ )<sup>88</sup>.

### ***Vedolizumab***

Vedolizumab is one of the newer monoclonal antibodies licensed for use in UC and CD as a first or second-line therapy. It blocks the binding of the  $\alpha 4 \beta 7$  integrin to the mucosal addressin cell adhesion molecule-1 (MAdCAM-1) in gut-selective tissue resulting in anti-inflammatory activity<sup>89</sup>. One of the major advantages of ant-integrin

therapies such as vedolizumab is that they are gut selective. They therefore do not carry the same potential for systemic infections that alternative biologics such may carry.

Vedolizumab has been demonstrated as effective in inducing and maintaining remission in the GEMINI studies for both UC and CD<sup>90-92</sup>. CD patient outcomes were better at week 10 of treatment with TNF-naïve patients responding better (48.4% on vedolizumab vs 30.1% on placebo) compared with those with prior TNF-failure (39.7% on vedolizumab vs 22.5% on placebo)<sup>93</sup>. Remission rates at week 10 were also better for TNF-naïve patients (26.6% on vedolizumab vs 15.4% on placebo) compared with those with prior TNF-failure (21.8% on vedolizumab vs 11.0% on placebo). Week 52 clinical remission was maintained in 39% of patients receiving 8-weekly infusions and 36.4% of patients receiving 4-weekly infusions vs. 21.6% of patients in the placebo group<sup>93</sup>. Additionally, glucocorticoid-free remission at week 52 were higher among patients receiving vedolizumab every 8 weeks or every 4 weeks than among CD patients who were switched to placebo<sup>92</sup>. GEMINI1 also demonstrated vedolizumab as an effective treatment in UC patients with prior exposure to anti-TNFs although outcomes were also superior in those who were anti-TNF naïve<sup>91</sup>. Vedolizumab responders also appear to maintain response, with long-term follow-up data from the GEMINI-2 study 83% of responders at



week 6, were in remission after 2 years and 89% after 3 years<sup>92</sup>. Overall steroid free remission rates were 31.4% of the patients who received vedolizumab every 8 weeks and 45.2% of those who received vedolizumab every 4 weeks, as compared with 13.9% of patients who received placebo<sup>90</sup>.

### ***Tofacitinib***

Tofacitinib is the latest drug therapy used in the management of UC. It is an oral, partially selective Janus kinase (JAK) inhibitor which works intracellularly to inhibit JAK-dependent cytokine signalling. JAK1 and JAK3 mediate the intracellular effects of several inflammatory cytokines, which are the major therapeutic targets of the drug and their inhibition results in downregulation of the immune and inflammatory response<sup>94</sup>. The major clinical trials which have demonstrated tofacitinib's effectiveness in inducing and maintaining remission in patients with UC include the OCTAVE 1 and 2 trials and the OCTAVE Sustain trial<sup>95</sup>. Real-world experience demonstrated by Weisshof et al. also demonstrated efficacy. In this cohort of patients with moderate-to-severe, anti-TNF resistant IBD (53 UC, 4 CD, 1 pouchitis), tofacitinib induced clinical response in 69% of the patients, 27% were in clinical, steroid-free remission by 1 year<sup>96</sup>. One of the major limitations of this therapy however is the potential association with

venous thromboembolism and its use is therefore contraindicated in patients on combined hormonal contraceptives or hormone replacement therapy, previous venous thromboembolism, either deep venous thrombosis or pulmonary embolism, inherited coagulation disorders, active malignancy or patients undergoing major surgery.

### **1.2.6 Role of Ciclosporin**

Ciclosporin is a calcineurin inhibitor selectively inhibiting T-cell mediated IL-2 production; an inflammatory cytokine involved in the inflammatory response which occurs in IBD. Ciclosporin and infliximab are two potential medical options used as rescue therapies in the treatment of acute, severe UC (ASUC). A RCT conducted by Van Aasche et al. in 2006 reported response rates of 82% with 2 mg/kg intravenous ciclosporin in ASUC patients<sup>97</sup>. However, long term colectomy rate still varies between 60-88% among patients in whom Ciclosporin initially induced remission<sup>98</sup>. During the initial stages of intravenous dosing, target trough ciclosporin concentration of 150–250 ng/mL should be targeted<sup>99</sup>. Responders should later receive an oral dose in divided doses, with a target trough concentration of 100–200 ng/ml. The major limiting factor for ciclosporin use is the potential for toxic side effects. Significant side effects in the form of serious infections have been

reported as 5% and potential mortality of 1–3%<sup>98,100,101</sup>. Other major adverse events related to ciclosporin use include nephrotoxicity (6.3%), seizures (3.6%), anaphylaxis (0.9%) and death (1.8%)<sup>98</sup>. In spite of this, with appropriate patient selection it remains a viable rescue therapeutic option for ASUC patients.

### 1.3 Corticosteroids and IBD

Corticosteroids (CS) or glucocorticosteroids (GCS) are a potent non-selective anti-inflammatory treatment used in multiple autoimmune conditions to downregulate systemic inflammation. They are highly effective in inducing remission in IBD and have been the mainstay of the acute management of IBD flare-ups since the 1950s<sup>102</sup>. Disease extent and severity are important variables when it comes to decisions relating to CS administration. Variations in CS formulation, route of administration, treatment duration as well as the potential for significant side effects, are all important considerations when prescribing CS. A summary of first- and second-generation GCS can be found in **Table 1.5**. Prednisolone is one of the most prescribed oral systemic CS and has been shown to be superior to 5-ASA for induction of remission in UC patients<sup>103,104</sup>. However, in an effort to minimise steroid exposure, patients with mild-moderately active UC are only recommended to receive a course of oral prednisolone if they have

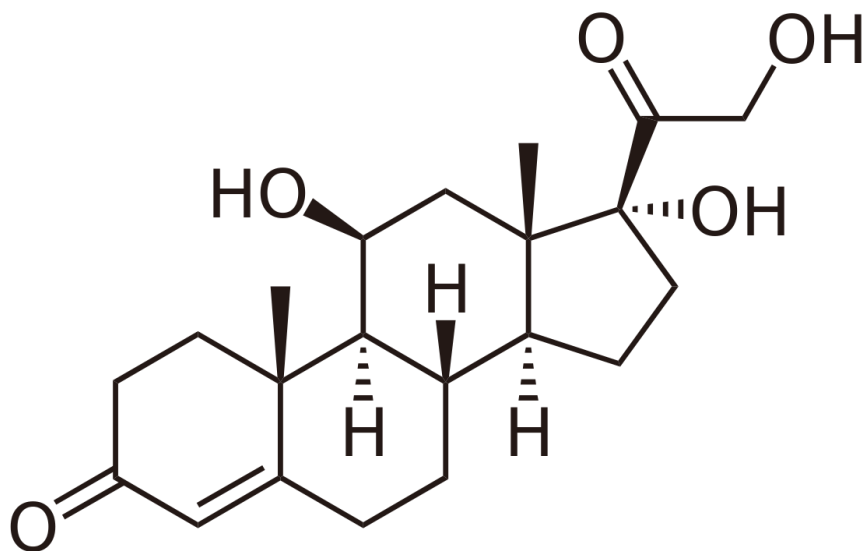
failed or have been intolerant to induction therapy with topical or systemic 5-ASA<sup>17</sup>.

**Table 1.5 Summary of Glucocorticoids of 1<sup>st</sup> and 2<sup>nd</sup> generation<sup>105</sup>**

| <b>Glucocorticoids</b>           | <b>Component</b>                                        | <b>Indications</b>                            |
|----------------------------------|---------------------------------------------------------|-----------------------------------------------|
| <b>1<sup>st</sup> generation</b> | <b>Prednisone</b>                                       | <b>Moderate to Severe IBD</b>                 |
|                                  | <b>Methylprednisolone</b>                               | <b>Moderate-Severe IBD</b>                    |
|                                  | <b>Hydrocortisone</b>                                   | <b>Moderate-Severe IBD<br/>Short Duration</b> |
| <b>2<sup>nd</sup> generation</b> | <b>Budesonide</b>                                       | <b>Moderate CD cases</b>                      |
|                                  | <b>Budesonide MMX</b>                                   | <b>Mild to moderate UC cases</b>              |
|                                  | <b>Beclomethasone dipropionate</b>                      | <b>Topical administration</b>                 |
|                                  | <b>Erythrocyte - Mediated Delivery of Dexamethasone</b> | <b>In research for long term treatments</b>   |

Previous meta-analysis of multiple randomised controlled trials (RCT) demonstrated CS to be superior at inducing remission in UC patients compared with placebo<sup>106</sup>. Additionally, previous studies suggested a dose of 40mg superior to 20mg, and higher doses of 60mg/day were associated with greater side effects such as skin changes, insomnia, mood disturbance, glucose intolerance, osteoporosis, increased risk of cardiovascular disease, higher rates of infection etc<sup>106,107</sup>. Countless side effects relating to continued or repeated exposure to CS are well established in the literature with the potential for significant impact on a patient's QOL. Treatment should therefore ideally be weaned over a period of 6-8 weeks.

**Figure 1.2: Glucocorticoid compound**



In recent years more locally acting oral CS have been developed to avoid systemic side effects. Locally acting budesonide MMX (multi-matrix system), ileal-release budesonide and beclomethasone dipropionate are potential options. A Cochrane review which looked at the use of budesonide MMX as an induction option for mild-moderate UC concluded that the quality of evidence was moderate, and benefit was considerable for those with left sided disease but benefit for those with more extensive disease was unclear<sup>108</sup>. Steroid suppositories are an effective option for patients with more limited disease such as ulcerative proctitis, but they are generally reserved for patients who are intolerant to or who fail 5-ASA therapy<sup>109</sup>. Previous trials have also demonstrated that daily ileal-release budesonide 9mg given for 8 weeks was as effective as systemic oral prednisolone in patients with mild-moderately active ileo-caecal CD, with response rates of 51% and 52.5% at 8 weeks for budesonide and prednisolone respectively, however significantly fewer side effects were noted in the budesonide group<sup>110</sup>. Benefits for more proximal colonic disease in CD has been noted but no benefits have been reported for distal colonic Crohn's activity<sup>110</sup>.

Patients with more moderate-severe UC should be considered for oral systemic CS such as prednisolone 40 mg daily weaning over 6–8 weeks<sup>17</sup>. Oral prednisolone is generally continued in those patients with acute severe

disease requiring hospital admission who may have responded initially to intravenous (IV) CS until maintenance treatment has been established. As well as UC, systemic CS such as prednisolone are also effective in inducing remission in mild-severely active colonic CD<sup>111-113</sup>. Benchimol et al. demonstrated CS to be significantly more effective than placebo at inducing remission in CD patients (RR 1.99; 95% CI 1.51 to 2.64;  $P < 0.00001$ )<sup>111</sup>.

Severe UC can be determined based on the Mayo Score outlined above or clinical parameters such as fever, tachycardia, stool frequency, elevation of inflammatory markers or anaemia as outlined by the Truelove and Witts criteria<sup>114</sup>. Figures suggest up to 25%-30% of UC patients will require hospitalisation for management of an acute severe flare throughout their lifetime and IV CS, mainly in the form of hydrocortisone, play an integral role in avoiding surgery in these patients<sup>115</sup>. Hydrocortisone 100mg IV four times a day or methylprednisolone 60mg once daily are the recommended IV CS to be used for ASUC. Response rates to steroids reviewed during a systematic review of 1991 ASUC patients were reported at 67% , with 29% of patients requiring a colectomy<sup>116</sup>. Alarming mortality rates were reported at 1%. The CONSTRUCT trial which compared the efficacy of infliximab and ciclosporin as rescue therapy for ASUC reported no significant differences in response rates between the two but did conclude steroid response rates overall were 49%<sup>99</sup>. Treatment response determined

by clinical and biochemical markers should generally be assessed at day 3 of IV corticosteroids to ascertain if salvage medical or surgical therapy is required<sup>116</sup>. Therapy exceeding 7-10 days has been shown to have no additional benefit and carries a greater risk of adverse events<sup>117,118</sup>.

Appropriate and timely decision-making is therefore integral in order to avoid delays or prolongation of medical therapy, as those patients with refractory medical disease and undergoing colectomy have higher rates of postoperative complications after delayed surgery<sup>118</sup>.

## **1.4 Steroid Dependence and Refractory Disease**

It is important to note that whilst CS have a vital role in inducing remission in IBD patients, they are not an appropriate maintenance treatment strategy<sup>106</sup>. As outlined above prolonged steroid use can be associated with significant side effects and it is often defined as continuous use for 3 months or more. Indeed even 2 or more courses of CS can be considered as steroid excess and a significant risk factor for the development of long-term side effects. Patients can develop steroid dependent or resistant disease. Steroid dependence refers to those individuals who initially respond to CS but then relapse with tapering of CS (usually 10mg and 3mg of prednisolone and budesonide respectively) or shortly after discontinuation (within 3 months)



and require reintroduction of CS to maintain control of symptoms<sup>119</sup>. Steroid resistant disease refers to active disease despite taking up to 1 mg/kg/day of prednisolone for 4 weeks<sup>17</sup>.

A study by Khan et al. explored the use of CS and long-term outcomes after the index CS exposure amongst 1038 patients. Prevalence of CS use was 45% and of these CS exposed patients, 464 were followed over 3.4 years. Amongst these, 65% required CS reintroduction, 38% were classified as CS dependent, and 11% were classified as CS refractory mostly within 2 years after the index CS course<sup>120</sup>. More recently, a UK audit reported 14.9% of IBD patients had steroid dependency or excess in the UK, more commonly in UC than CD<sup>121</sup>. Prior to the availability of antibody treatments like infliximab or the usage of calcineurin inhibitors like Ciclosporin for acute severe disease, colectomy was the only option once steroid resistance was determined<sup>122</sup>.

Whilst generally considered inexpensive, and used for a diverse array of conditions, long-term use is known to be associated with certain toxicities which have considerable direct and indirect economic implications for the healthcare sector. Rice et al. recently conducted a systematic literature review looking at the economic impact of long-term corticosteroid use. They reported dose-related increases in health care resource utilization and costs with CS use, with per-annum incremental costs relative to non-users ranging

from \$5700 in low-dose users (<7.5 mg/d) to \$29,000 in high-dose users (>15 mg/d)<sup>123</sup>.

Similarly, Manson et al. examined factors linked to the incidence of oral CS related adverse events found that dose, age, gender, duration of use, treatment history, smoking habits or cholesterol level were influential in determining risk. Additionally, they conducted a cumulative economic analysis of selected adverse events and found the annual cost of treating these events in the UK to be at least 165 pounds per patient taking oral CS<sup>124</sup>.

## 1.5 Predictors of Steroid response

The clinical and economic burden of corticosteroid related adverse events highlights the need for effective steroid sparing strategies to be employed. Earlier recognition of potential steroid failure could ideally avoid undue exposure and allow for earlier treatment escalation and patient selection as well as reduce potential long term clinical and financial implications associated with their use. Some clinical and biochemical potential predictors of CS failure have been summarised in the BSG guidelines published in 2019 but they are largely centred around clinical and serological markers of CS failure (**Table 1.6**). Limited understanding exists relating to rates of CS failure here in Ireland and globally very little is known about tissue specific

mechanisms of steroid resistance in IBD patients or if tissue specific markers of CS failure exist.

**Table 1.6 Indices predictive of Corticosteroid failure in ASUC<sup>17</sup>**

| Assessment at day 3 of corticosteroids                   |   |        | Chance of treatment failure | Reference                     |
|----------------------------------------------------------|---|--------|-----------------------------|-------------------------------|
| BO >8/day or BO 3–8/day and CRP >45 mg/L                 |   |        | 85%                         | Travis et al <sup>125</sup>   |
| Mean stool frequency day 1–3                             |   | Total: |                             | Ho et al <sup>126</sup>       |
| <4                                                       | 0 |        |                             |                               |
| 4–6                                                      | 1 | 0–1    | 11%                         |                               |
| 7–9                                                      | 2 | 2–3    | 45%                         |                               |
| >9                                                       | 4 | ≥4     | 85%                         |                               |
| Transverse colonic dilatation on abdominal X-ray ≥5.5 cm | 4 |        |                             |                               |
| Albumin on admission <30 g/L                             | 1 |        |                             |                               |
| Number of stools in 24 hours + (0.14×CRP (mg/L)) >8      |   |        | 72%                         | Lindgren et al <sup>127</sup> |
| CRP/albumin ratio >0.85 combined plus stool frequency >3 |   |        | 74%                         | Gibson et al <sup>128</sup>   |

## 1.6 Goals of dissertation

The overall objectives and aims of this dissertation are as follows:

- (1) Examine the prevalence of steroid resistance and associations in Ireland.
- (2) Explore the literature for potential molecular mechanisms of steroid resistance in IBD patients.
- (3) Provide an introduction into the role of the 11 $\beta$ -hydroxysteroid dehydrogenase system in IBD
- (4) To examine tissue specific glucocorticoid regulators in IBD
- (5) Characterise these regulators of glucocorticoid action in the colon of patients with IBD in an Irish Cohort
- (6) Provide pilot data and predictive value of resistance markers in Irish Cohort.

In summary, while there have been significant advances in the management of IBD with disease modifying agents and biologic agents, steroids remain a mainstay of therapy and there is still no accurate way of predicting in which patients' steroids will be effective. The main objective for this dissertation

is to therefore examine the literature for mechanisms of steroid resistance in IBD patients and investigate tissue specific glucocorticoid metabolism for a potential explanation of steroid resistance in this complex disease. The hypothesis of this work is that we may be able to provide a better understanding of these tissue specific regulators of steroid resistance in IBD, which would be advantageous. It could potentially pave the way for the development of novel mechanisms to re-sensitize individuals, through optimal patient selection, development of novel drug delivery systems as well as dosing optimisation. In addition, earlier identification of steroid resistance may herald a timelier and more tailored introduction of alternative therapy with a view to improving patient care and reducing long term complications for our patients as well as the economic burden on our healthcare system.

# **Chapter 2: Related risk factors associated with steroid resistance in an Irish IBD population.**

## **2.1 Introduction**

The therapeutic uses of GCS are extremely vast and some of their most important functions include their role as an anti-inflammatory and immunosuppressant agent. They have been used as a treatment for Inflammatory Bowel Disease since the 1950s<sup>114</sup> and as outlined in chapter 1, continue to play a critical role in the management of an acute disease relapse. Despite their efficacy, some IBD patients may be deemed steroid dependent (clinical relapse of symptoms upon withdrawal of steroids) or steroid resistant (inadequate or no response to steroid treatment).

Up to 25-30% of patients with UC or CD will require hospitalisation for an acute severe flare of disease at some point throughout their disease course, often at initial presentation. All patients admitted with a suspected IBD flare generally receive baseline bloods (Full Blood count (FBC), C-Reactive Protein (CRP), Urea and electrolytes (U&E), Liver Function Tests (LFT) and magnesium), stool culture and Clostridium difficile assay, radiological imaging (AXR or CT) and flexible sigmoidoscopy, with close monitoring

after admission<sup>17</sup>. These investigations will aid clinicians to assess likely prognosis and to ideally recognise CS failure and the potential need for treatment escalation or colectomy<sup>115,129</sup>. Early flexible sigmoidoscopy is also important to confirm diagnosis and obtain histology including evaluation for cytomegalovirus. The literature suggests up to half of hospitalised patients can potentially fail corticosteroids<sup>99</sup> so timely consideration should be given to a pre-biologic screen.

Previous independent studies have reported that 15-30 % of patients respond poorly or not at all to GCS treatment<sup>116,126,130</sup>. One such study by Munkholm et al. prospectively examined the outcome of the first steroid course in 109 patients with Crohn's Disease<sup>131</sup>. Thirty-day outcomes were reported as follows; 36% were steroid dependent and 20% were steroid resistant. A high rate of surgical intervention was reported in the steroid dependent (26%) and the steroid resistant (59%) groups within 1 month of GCS treatment.

Similarly, a retrospective study by Faubion et al. reported steroid dependency rates of 28% and 22% in CD and Ulcerative Colitis (UC) patients respectively whereas no response was observed in 16% of patients<sup>130</sup>. Additionally, a systematic review of 1991 acute UC patients requiring admission from 1974-2006 by Turner et al. reported an overall response rate to intravenous steroids of 67% and a short term colectomy rate of 29%<sup>116</sup>.



This variability in response rates is unpredictable and can often result in prolonged courses of GCS amongst IBD patients, especially amongst the resistant and dependent groups of patients. Consequently, these patients are at an increased risk of common complications often associated with their use such as osteoporosis, cardiovascular disease, skin changes and metabolic syndromes. Response to steroids has been variously described but it is often indicated by an improvement in the patient's symptoms (stool frequency, general wellbeing and abdominal pain) as well as improved laboratory markers including serum CRP, Erythrocyte Sedimentation Rate (ESR), haemoglobin (Hb) and albumin levels<sup>132</sup>. Some studies have attempted to identify potential predictive factors of steroid resistance in the hope that early identification may result in a more efficient introduction of alternative therapies with a view to improving patient care and reducing long term complications.

The precise molecular mechanism of steroid resistance in inflammatory bowel disease remains unclear, but several mechanisms have been proposed. Possible mechanisms behind this steroid resistance include abnormalities in steroid absorption/metabolism, altered numbers of glucocorticoid receptors, altered affinity of the ligand for glucocorticoid receptors, reduced affinity of the glucocorticoid receptor to bind DNA and altered expression of

transcription factors and/ or cytokines<sup>133</sup>. However, to date none of these above mechanisms have fully explained steroid resistance in these patients.

The emergence of biologic agents as well as immunomodulatory therapies has revolutionised the management of IBD and they are commonly used as long-term maintenance therapy and steroid sparing agents in IBD patients. Whether they have truly impacted upon the natural history of IBD as well as overall secondary response rates to GCS is not well known.

## **2.2 Aims**

1. To assess response rates to Corticosteroid therapy in a tertiary referral centre
2. To provide an update of potential predictive risk factors associated with GCS resistance in an Irish population.
3. To ascertain if concomitant therapies alter the levels of response rates to GCS.

## **2.3 Materials and Methods**

This was an observational retrospective cohort, single centre study performed at Tallaght Hospital, Dublin, Ireland. Following ethical approval,

a review of patients from the Hospital In-Patient Enquiry (HIPE) data base at a this tertiary IBD centre was conducted. Eligible patients included those aged 18-80 years requiring hospitalisation with an acute flare of IBD from 2010-2015. Medical, endoscopic, and clinical data was reviewed to determine patient treatment courses and clinical outcomes. All included patients received a minimum of 3 days of high dose intravenous hydrocortisone at minimum dose of 100mg QDS during their admission.

Further inclusion criteria included those patients who had negative stool sample for clostridium difficile during their admission. Exclusion therapy included patients with incomplete records or records which did not include at least 3 months of follow-up, those patients given immediate anti-TNF therapy, patients with toxic colitis or those requiring immediate surgery and non IBD associated colitis. Demographics & clinical details including disease phenotype, extent, duration, concomitant therapies, length of stay (LOS) were analysed. Patients had serum biochemical and haematological markers measured during their admission and these included CRP, albumin, and haemoglobin. All admission levels and day 3 levels were all recorded. A normal CRP was considered <5mg/l and anaemia was defined as <11.5g/dl in women and <13g/dl in men as per local laboratory reference ranges. Significant anaemia was defined as Hb of < 10.5g/dl. Hypoalbuminemia was defined as an albumin level <35g/ as per our local

laboratory reference guidelines. Disease severity was also recorded according to endoscopic parameters where available (Mayo and SES CD). As this was a retrospective study, disease severity was defined based on endoscopic data available using the Mayo or SES CD score. In addition, where recorded patient symptoms were used to determine clinical levels of disease activity using the Mayo score or Harvey-Bradshaw score where applicable.

Resistance rates were determined by a lack of improvement or deterioration in day 3 clinical status, day 3 CRP or if the patient was determined to require rescue medical therapy in form of a biologic agent or ciclosporin or surgery during admission. Steroid dependence was defined as either the impossibility of reducing the corticosteroid dose to less than 10 mg/day after 3 months of treatment initiation or disease relapse within 3 months after steroid discontinuation.

Results were compared amongst responders & non-responders using a student t-test and p value of  $\leq 0.05$  was considered significant. Statistical analysis was performed using MedCalc Statistical Software version 17.4 (MedCalc Software bvba, Ostend, Belgium, <http://www.medcalc.org>; 2017)

## **2.4 Results**

## 2.4.1 Baseline Characteristics

In all 475 patients requiring hospitalisation for acute inflammatory colitis were identified. A total of 19% (n=90) of patients were excluded due to insufficient information. Of the remaining 385, 57% (n=219) were female and the overall mean age was 39 years (range 15-84 years). Additionally, the mean disease duration was 4.5 years (range 0-27 years). Baseline characteristics can be summarised in **Table 2.1**.

Amongst hospitalised patients, 37% (n=142) had a new diagnosis of IBD. Of those patients with an established diagnosis of IBD (n=243), 31 % (n=75) had prior treatment with a 5 ASA, 43% (n=105), an immunomodulator and 32% (n=77) an anti-TNF alpha agent. There were statistically more patients with Crohn's Disease (CD), than Ulcerative Colitis (UC) requiring admission, 61% (n=236) vs. 38% (n=149),  $p \leq 0.0001$ , 95% CI 0.16 - 0.30. Amongst the UC patients, the majority (76%, n=113) had pancolitis (E3). Anatomical distribution of Crohn's disease in the study cohort was 27% (n=64) ileal (L1), 37% (n=87) colonic (L2) and 36% (n=85) ileocolonic(L3). During the course of admission, 84% (n=323) of patients underwent endoscopic disease assessment and of these 35% (n=113) had severe, 46% (n=150) moderate and 19% (n=61) mild disease activity. Overall mean LOS was 10 days (range 4-49). The mean admission CRP, Hb and albumin across

the patient cohort was 59 mg/l (range 1-307.9mg/l), 12.2g/dl (range 6.2-15.9 g/dl) and 38 g/dl (range 11-44 g/dl) respectively. The mean day 3 CRP was 25mg/l (range 1-260mg/l), mean day 3 Hb was 11.2 (range 7.3-16.1g/dl) and mean day 3 albumin was 34g/dl (16-45g/dl).

## Table 2.1 Baseline Characteristics

|                                     |                     |
|-------------------------------------|---------------------|
| <b>Patient Number</b>               | <b>N=385 (100%)</b> |
| <b>Crohns Disease</b>               | N=236 (61%)         |
| <b>Mean Disease Duration (yrs.)</b> | 4.5 (0-27)          |
| <b>% Female</b>                     | N=219 (57%)         |
| <b>Mean Age</b>                     | 39 (15-84 )         |
| <b>Mean LOS (days)</b>              | 10 (3-49)           |
| <b>New IBD Diagnosis</b>            | 142 (37%)           |
| <b>Mild Disease</b>                 | 61 (19%)            |
| <b>Moderate Disease</b>             | 150 (46%)           |
| <b>Severe Disease</b>               | 113 (35%)           |
| <b>Prior 5ASA</b>                   | 75 (31%)            |
| <b>Prior Immunomodulator</b>        | 105 (43%)           |
| <b>Prior Biologic agent use</b>     | 77 (32%)            |
| <b>Mean CRP (mg/L)</b>              | 59 (1-307.9)        |
| <b>Mean Hb (g/dl)</b>               | 12.2 (6.2-15.9)     |
| <b>Mean Albumin (g/dl)</b>          | 38 (11-44)          |

## 2.4.2 Response Rates

Overall, amongst this cohort, 68% (n=262) were responders to CS, 23% (n=88) non-responders and 9% (n=35) steroid dependent, respectively. Amongst the responders and non-responders, 65% (n=169) and 57% (n=50) had CD (p=0.1), and 35% (n=93) and 43% (n=38) had UC respectively (p=0.1). However overall, amongst the non-responders, more patients had CD, 57% (n=50) compared to UC, 43% (n=38),  $P \leq 0.03$ , 95% CI 0.01 - 0.31.

In terms of concomitant therapies amongst responders and non-responders 20% (n=53) vs. 25% (n=22) were on a 5 ASA, 27% (n=63) vs. 31% (n=27) were on a biologic agent prior to admission. Interestingly however, 36% (n=94) of responders vs. 12.5% (n=11) of non-responders were on an immunomodulator prior to admission, ( $p \leq 0.00001$ , 95% CI 0.13-0.34) given an overall OR of 3.9.

Endoscopic assessment data was available on 77% (n=206) of responders, 100% (n=88) of non-responders and 82% (n=29) of steroid dependent patients, respectively. Not surprisingly overall non-responders had more severe disease endoscopically compared with responders, 61% (n=54) vs. 53% (n=109)  $p \leq 0.0001$ , OR 4.07, 95% CI 2.33 to 7.09.



### 2.4.3 Predictors of non-response

Mean admission CRP for responders and non-responders was 48mg/l vs. 89mg/l and mean day 3 CRP for responders and non-responders was 10mg/l vs. 48mg/l respectively. A CRP level >45mg/l on day 3 appeared to be predictive of steroid resistance, 58 % (n=51) vs. 23% (n=60), OR 4.6,  $p \leq 0.0001$ , 95% CI 2.78-7.74. Overall non-responders had higher rates of anaemia, 55% (n=48) vs. 39% (n=102) ( $p \leq 0.01$ , 95% CI 0.04 - 0.28) and hypoalbuminemia, 41% (n=36) vs. 34% (n=89), ( $p=0.2$ ) on admission. However significant anaemia (38% (n=33) vs. 26% (n=68)), OR 1.7,  $P \leq 0.03$ , 95% CI 1.03-2.86) and hypoalbuminemia (36% (n=32) vs. 24% (n=62), OR 2.2,  $P \leq 0.02$ , 95% CI 1.09-3.09) on day 3 appeared to be predictive of response

On subgroup analysis disease duration, age and gender did not appear to influence response rates, **Table 2.2**. Overall, amongst patients with a new diagnosis of IBD (n=142), 73% (n=104) were responders. In relation to disease extent, the only significant finding was amongst UC patients with more extensive disease. Pancolitic patients had higher rates of resistance, 84% (n=32) vs. 66% (n=61), OR 2.7,  $p \leq 0.03$ , 95%CI 1.06-7.39. Additionally, as mentioned above, patients with more severe disease

endoscopically tended to have higher rates of steroid failure. Overall, amongst Crohn's patients, stricturing disease phenotype was associated with a higher risk of non-response, 40% (n=30) vs. 26% (n=44), OR 1.9,  $p \leq 0.04$ , 95% CI 1.00-3.79.

Overall, 91% (n=80) of the resistant patients received rescue biologic therapy, 16% (n=13) adalimumab (milder group), 55% (n=60) infliximab and 9% (n=9) IV ciclosporin. In all 44% (n=39) of the resistant patients required surgical intervention, of which 38% (n=30) had failed rescue biologic therapy.

In terms of the steroid dependent patients, 51% (n=18) had CD. There was a trend towards a higher percentage of females amongst steroid dependent patients compared with responders, but this did not quite reach statistical significance, **Table 2.3**. Furthermore, patients in the responder group appeared to have more severe disease but this may be related to patient sample size amongst the dependent group and the available endoscopic data amongst this group, 53% vs 14%.

Overall, there were no identifiable predictors of steroid dependence in this group. Median admission and day 3 CRP, albumin and Hb were similar across responders and steroid dependent groups. In all, 43 % (n=15) went on to commence immunomodulator therapy and 37% (n=13) an anti-TNF agent.

**Table 2.2 Predictors of Steroid Non-Response**

|                                                 | <b>Responders<br/>n=262</b> | <b>Resistant<br/>n=88</b> | <b>P value, 95 % CI</b> | <b>Odds<br/>Ratio</b> |
|-------------------------------------------------|-----------------------------|---------------------------|-------------------------|-----------------------|
| <b>Gender (% females)</b>                       | 152 (58%)                   | 41 (47%)                  | 0.8                     |                       |
| <b>Age (yrs.)</b>                               | 42 (19-74)                  | 37(15-84)                 | 0.2                     |                       |
| <b>Disease duration<br/>(years)</b>             | 4.8 (0-24)                  | 5.2 (0-27)                | 0.4                     |                       |
| <b>Disease subtype (CD)</b>                     | 169 (65%)                   | 50 (57%)                  | 0.1                     |                       |
| <b>Disease severity (n (%)<br/>severe)</b>      | 109 (53%)                   | 54 (61%)                  | ≤0.0001, 2.33-7.09      | 4.0                   |
| <b>Prior exposure to 5ASA</b>                   | 53 (20%)                    | 22 (25%)                  | 0.1                     |                       |
| <b>Prior exposure to<br/>Thiopurines</b>        | 94(36%)                     | 11(12.5%)                 | ≤0.00001,0.13-0.34      |                       |
| <b>Prior exposure to<br/>biologics</b>          | 63 (27%)                    | 27 (31%)                  | 0.7                     |                       |
| <b>Day 3 Haemoglobin<br/>(≤10.5g/dL)</b>        | 68 (26%)                    | 33 (38%)                  | ≤0.03, 1.03-2.86        | 1.7                   |
| <b>Day 3 C-Reactive<br/>Protein(&gt;45mg/L)</b> | 60 (23%)                    | 51 (58%)                  | ≤0.0001, 2.78-7.74      | 4.6                   |
| <b>Day 3 albumin<br/>(&lt;35g/dl)</b>           | 62 (24%)                    | 32 (36%)                  | ≤0.01, 1.09-3.09        | 2.2                   |
| <b>Extensive Disease UC</b>                     | 8 (7%)                      | 21(28%)                   | ≤0.0001, 1.06-7.39      | 2.7                   |
| <b>Stricturing Disease<br/>phenotype CD</b>     | 44 (26%)                    | 30 (40%)                  | ≤0.04, 1.00-3.79        | 1.9                   |

**Table 2.3 Responders vs. Steroid Dependent groups**

|                                            | <b>Responders,<br/>n=262</b> | <b>Dependent<br/>n=35</b> | <b>P value, 95 % CI</b>  |
|--------------------------------------------|------------------------------|---------------------------|--------------------------|
| <b>Gender (% females)</b>                  | <b>152 (58%)</b>             | <b>26 (74%)</b>           | <b>0.06</b>              |
| <b>Age (yrs.)</b>                          | <b>42 (19-74)</b>            | <b>37(15-84)</b>          | <b>0.2</b>               |
| <b>Disease duration (years)</b>            | <b>4.8 (0-24)</b>            | <b>3.2 (0-14)</b>         | <b>0.4</b>               |
| <b>Disease subtype (CD)</b>                | <b>169 (65%)</b>             | <b>17 (49%)</b>           | <b>0.06</b>              |
| <b>Disease severity (n (%)<br/>severe)</b> | <b>109 (53%)</b>             | <b>5(14%)</b>             | <b>≤0.0017, 0.1-0.44</b> |
| <b>Prior exposure to 5ASA</b>              | <b>53 (20%)</b>              | <b>5(14%)</b>             | <b>0.4</b>               |
| <b>Prior exposure to<br/>Thiopurines</b>   | <b>94(36%)</b>               | <b>9(26%)</b>             | <b>0.2</b>               |
| <b>Prior exposure to<br/>biologics</b>     | <b>63 (27%)</b>              | <b>5 (14%)</b>            | <b>0.7</b>               |
| <b>Mean Day 3<br/>Haemoglobin g/dl</b>     | <b>12.1</b>                  | <b>11.4</b>               | <b>0.4</b>               |
| <b>Mean Day 3 Crp mg/l</b>                 | <b>31</b>                    | <b>27</b>                 | <b>0.3</b>               |
| <b>Mean Day 3 albumin g/dl</b>             | <b>38</b>                    | <b>34</b>                 | <b>0.4</b>               |
| <b>Extensive Disease</b>                   | <b>135 (66%)</b>             | <b>15 (53%)</b>           | <b>0.6</b>               |

## 2.5 Discussion

The most widely accepted criterion for steroid refractoriness is the lack of clinical improvement after 7-10 days of IV prednisolone at a dose of 1mg/kg/day<sup>134</sup> or persistent active disease after treatment with oral prednisolone 0.75mg/kg/day for 4 weeks<sup>135</sup>. Clinicians generally cannot afford to delay 7-10 days to establish if a patient is deemed steroid resistant or not due to risks of clinical deterioration and development of disease related complications. Their primary objective is to identify patients unlikely to respond to IV GCS as early as possible to allow for more appropriate and timely escalation of alternative therapies.

Like our study, efforts have been made to identify potential clinical and biochemical parameters as useful early predictors of outcome in hospitalised IBD patients. A prospective study conducted by Travis et al.<sup>125</sup> identified the presence of  $\geq 8$  stools/day or 3-8 stools/day plus a CRP  $>45\text{mg/L}$  at day 3 amongst 51 patients with severe UC predicted a colectomy rate of 85%. Similarly, two retrospective studies by Ho et al.<sup>126</sup> and Ananthakrishnan et al.<sup>136</sup> identified that number of stools/day, hypoalbuminemia, colonic dilatation  $>5.5\text{cm}$ , anaemia, malnutrition and need for blood transfusion and total parenteral nutrition were all reliable parameters in predicting the need for a colectomy amongst UC patients. Another study by Kim et al. in which clinical outcomes at 1 month, 4 months, and 1 year after GCS treatment

amongst 96 CD patients reported a less severe disease phenotype and a lower CD activity index demonstrated clinical significance for mid-term or mid- and long-term steroid responses, respectively<sup>137</sup>. Similarly Chow et al. retrospectively identified thrombocytosis at diagnosis predicted corticosteroid-dependency in IBD<sup>138</sup>. Similar to our study, they also reported that a stricturing phenotype of CD (OR1.9,  $p \leq 0.04$ , 95% CI 1.00-3.79) and the presence of anaemia in UC predicted subsequent corticosteroid refractoriness.

Our larger study which includes both patients with UC and CD requiring admission would appear to be in agreement with other published data in that it illustrates that early clinical and biochemical parameters are reliable in predicting response rates to steroid therapy. In hospitalised patients with a flare of IBD, like those studies listed above, day 3 CRP >45mg/l (OR 4.6,  $p \leq 0.0001$ , 95% CI 2.78-7.74), significant anaemia (OR 1.7,  $p \leq 0.03$ , 95% CI 1.03-2.86) and hypoalbuminemia (OR 2.2,  $p \leq 0.02$ , 1.09-3.09) all predicted steroid non-response. In addition, disease severity also appeared to be associated with higher rates of resistance (84% vs. 66%, OR 4.2  $P \leq 0.0001$ ) with similar rates amongst CD and UC patients.

Severe endoscopic activity (deep ulcers, extensive loss of mucosal layers, well-like ulcers or large erosions) has been associated with steroid resistance and need for colectomy<sup>139-141</sup>. Corte et al. conducted a study including 89

ASUC patients treated with IV CS and/or infliximab or ciclosporin. They illustrated that almost all patients with a UCEIS score of 7 or more on admission required rescue therapy<sup>142</sup>

Our study does not control for previous steroid exposure but reflects the clinical need for careful interpretation and prediction in all patients irrespective of medication history. Of note our findings would suggest that prior Immunomodulator treatment amongst our group appeared to be protective in terms of steroid response rates amongst those with established IBD, with 36% vs. 12.5% ( $p \leq 0.00001$ , 95% CI 0.13-0.34) of responders and non-responders demonstrating prior exposure.

Interestingly similar response rates were observed in those patients taking a biologic agent as those who were biologic naïve prior to admission. This would suggest that there remains a clear role for steroid use within this cohort. In addition, despite advancements in IBD therapeutics over the years, 44% (n=39) of our resistant patients still required surgical intervention, of which 38% (n=30) had failed rescue biologic therapy.

In terms of steroid dependence, the overall rate was 9% (n=35). This was quite a small sample size in relation to steroid dependence and insufficient endoscopic data was available in some of these patients. There was a trend towards a higher percentage of females with steroid dependent disease compared with responders, 74% vs. 58% but this did not quite reach

statistical significance. No other obvious predictors of dependence were noted in this study.

The other overall weaknesses in our study would be related to its retrospective design and its sole inclusion of inpatients only. This would naturally represent a more severe cohort and may not necessarily be reflective of IBD steroid response overall in our cohort. Additionally, the use of clinical response and remission as an end-point can represent a potential limitation in itself , given the sometimes lack of concordance between clinical symptoms and the degree of endoscopic disease observed, such was the case in up to 30% of patients noted in other studies including the SONIC trial in 2009<sup>1</sup>.

## **2.6 Conclusions**

In summary GCS resistance rates in our cohort are similar to previously published figures and remain significant at 23%. A high CRP, anaemia, hypoalbuminemia on day 3, as well as severe disease& pancolitis are predictive of GCS resistance in line with previous published works. Clinicians should be aware of the high rate of resistance should use day 3 CRP, albumin and Hb as previously recommended. In addition, our study supports the role for endoscopic assessment amongst these patients and



supports the role of immunomodulator use in high-risk patients to reduce resistance rates.

Further work on mechanisms of steroid resistance is needed as a significant number of patients continue to require surgical rescue despite advancements in biologic therapy.

# Chapter 3: Molecular Mechanisms of Steroid Resistance in IBD

## 3.1 Introduction

Glucocorticoids have a well-established role in the management of a diverse array of inflammatory and autoimmune conditions<sup>143-145</sup>. Despite their reliability and efficacy, for most patients, response rates may be variable and often unpredictable. Failure to respond to GCs can often render patients requiring surgery acutely or electively, with as many as 20% of patients with Ulcerative Colitis and up to 50% of patients with Crohn's disease requiring surgery throughout their lifetime, largely as a result of poor response to GCs<sup>146</sup>.

Earlier chapters referred to rates of steroid dependence and resistance amongst IBD cohorts and the potential clinical challenges clinicians are faced with. Prolonged steroid use in these patients can understandably result in an increased risk of treatment-related complications such as glucose intolerance, hypertension, obesity, osteoporosis, cardiovascular disease, and myopathy<sup>147-149</sup>.

Following first exposure to corticosteroids, previous studies have shown a thirty-day outcome of complete remission in 48-58%, partial remission in

26-32% and no response in 16-20% for CD patients<sup>130,131,150</sup>. In UC, immediate outcomes were complete remission in 54%, partial remission in 30% and no response in 16% of the patients<sup>130</sup>. The variability in response rates could possibly be accredited to factors such as sub-therapeutic dosing or even potentially reduced bioavailability of oral glucocorticoid in patients with active IBD, particularly those with severe diarrhoea<sup>146</sup>. However, one study by Reinsh et al. reported comparable steroid dependency and resistance rates of 63% and 13% respectively amongst 48 patients with active CD treated with higher doses of oral glucocorticoids and a slower tapering regime than used in the previous studies<sup>151</sup>. Additionally, a retrospective study by Lindgren et al. showed that 34% of 97 hospitalized patients with severe UC required a colectomy within 30 days of presentation, despite higher doses of intravenous glucocorticoid therapy<sup>127</sup>. Similarly, our own study in chapter 2 demonstrated a resistance rate of 23% despite a minimum of 3 days of IV hydrocortisone. This would suggest dose intensification does not provide a solution for these CS resistant and dependent groups and perhaps suggests drug bioavailability may not be a factor in early non-response.

Steroid resistance has also been observed in other inflammatory conditions such as Rheumatoid Arthritis, Asthma, and Systemic Lupus Erythematosus (SLE) and like IBD, can significantly affect clinical outcomes for these

patients<sup>152-154</sup>. Exploring potential mechanisms of steroid resistance is of significant clinical importance given the vital therapeutic role corticosteroids play on a day-to-day basis.

The precise mechanisms behind steroid resistance in inflammatory conditions remains unclear, but several have been proposed. To fully appreciate these mechanisms, we must first review the basic physiology underlying steroid metabolism.

**Table 3.1 Rates of steroid resistance in IBD-evidence from trials**

| Study                                         | Design                      | Patient number | Disease        | Steroid type used        | Disease severity          | Steroid Resistance | Steroid Dependent |
|-----------------------------------------------|-----------------------------|----------------|----------------|--------------------------|---------------------------|--------------------|-------------------|
| <b>Truelove and Witts et al<sup>134</sup></b> | Retrospective cohort        | 87             | UC             | IV Steroids              | Severe                    | 25%                | N/A               |
| <b>Munkholm et al<sup>131</sup></b>           | Prospective cohort          | 109            | CD             | Oral steroids            | Moderate/Severe           | 20%                | 36%               |
| <b>Chakravarty et al<sup>155</sup></b>        | Retrospective cohort        | 89             | UC             | IV Steroids              | Severe                    | 28%                | N/A               |
| <b>Faubion et al<sup>130</sup></b>            | Retrospective observational | 137            | 63 UC<br>74 CD | Oral and IV<br>Oral only | Moderate/Severe           | 16%                | 22%               |
| <b>Lindgren et al<sup>127</sup></b>           | Retrospective cohort        | 97             | UC             | IV steroids              | Severe                    | 34%                | N/A               |
| <b>Travis et al<sup>125</sup></b>             | Prospective observational   | 49             | UC             | IV steroids              | Severe                    | 27%                | N/A               |
| <b>Khan et al<sup>120</sup></b>               | Retrospective cohort        | 464            | UC             | Oral                     | OPD cohort (severity N/A) | 11%                | 38%               |

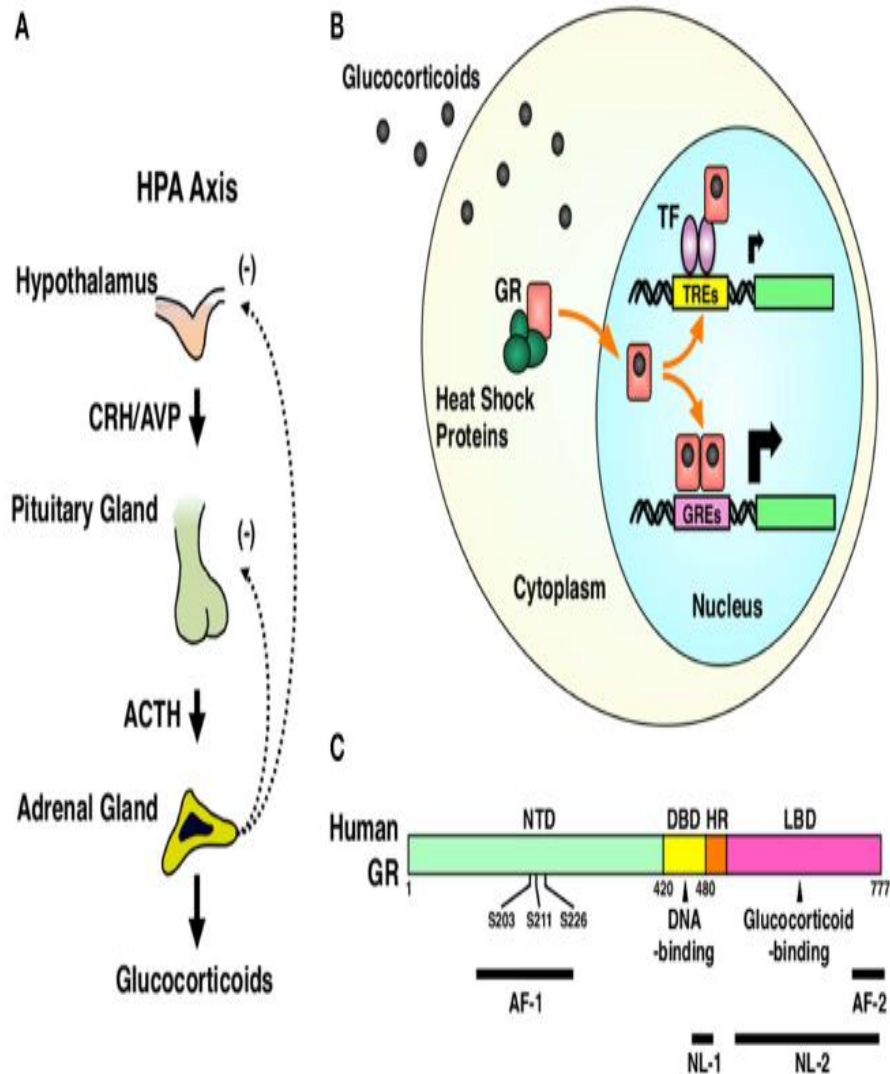
### 3.2 Normal Physiology

Cortisol (the main active glucocorticoid in humans) is secreted under the tight control of the hypothalamic-pituitary-adrenal axis (HPA-A). Corticotrophin releasing hormone (CRH) is synthesized and secreted from the hypothalamus, which leads to ACTH (corticotrophin) synthesis and secretion from the pituitary gland, which acts on the zona fasciculata of the adrenal cortex. Classical endocrine feedback loops are in place to control the secretion of GC through a tightly regulated mechanism in the hypothalamus and pituitary gland<sup>148</sup>. The effects of glucocorticoids are mediated by the GC receptor (GR) which is a ligand regulated receptor, also known as the classic GR or GR $\alpha$ , a phosphorylated 92-kDa protein, which is a member of the nuclear receptor superfamily<sup>156</sup>.

In the absence of its GC ligand, the GR remains bound to other proteins within the cytoplasm such as the 90 kDa and 70 kDa heat shock proteins (HSP90, HSP70)<sup>157</sup>. GC binding to the GR leads to dissociation of the HSPs and translocation of the GR-ligand complex into the cell nucleus. Following translocation to the nucleus, gene transcription is altered following binding of the dimerised GR/ ligand complex to the specific DNA sequences known as glucocorticoid response elements (GREs) in the promoter region of the target gene<sup>156</sup>.

This sequence-specific DNA binding results in the inhibition of the promoter regions of genes, such as nuclear factor kappa B (NFKB) and activator protein-1 (AP-1). These act as potent transcription factors for many pro-inflammatory cytokines and adhesion genes. One of the integral anti-inflammatory actions of glucocorticoids is achieved via the induction of inhibitor kappa B alpha ( $\text{IKB}\alpha$ ) which binds to and inhibits NF-KB by sequestering it in the cytoplasm<sup>158</sup>. In addition, glucocorticoids increase the expression of other cytokines that inhibit the production of other inflammatory mediators: such as transforming growth factor- $\beta$ 3 (TGF- $\beta$ ) and IL-10, increasing its anti-inflammatory function. They also inhibit T and B lymphocyte proliferation, all resulting in an anti-inflammatory response.

**Figure 3.1: Hypothalamic-Pituitary Adrenal Axis (HPA) (A) Organisation of the HPA Axis, (B) Intracellular regulation of the Glucocorticoid Receptor (GR)** <sup>159</sup>

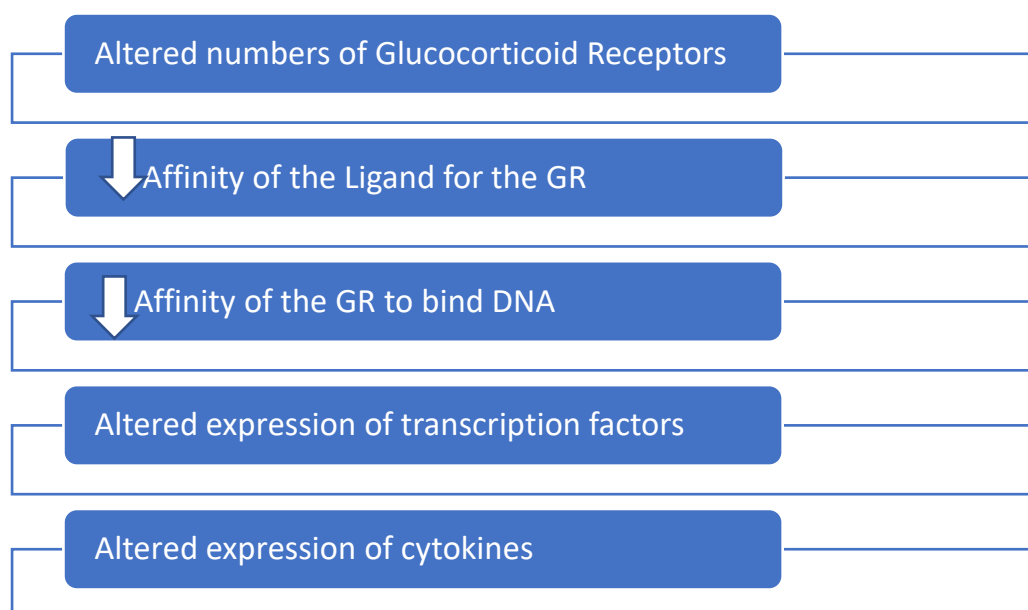


*ACTH, adrenocorticotrophic hormone; AF-1 and -2, activation function-1 and -2; AVP, arginine vasopressin; CRH, corticotropin-releasing hormone; DBD, DNA-binding domain; GR, glucocorticoid receptor; GREs, glucocorticoid response elements; HPA axis, hypothalamic-pituitary-adrenal axis; HR, hinge region; HSPs, heat shock proteins; LBD, ligand-binding domain; NL-1 and -2, nuclear localization signal-1 and -2; NTD, N-terminal domain; S203, 211, and 226, serine at amino acid position 203, 211, and 226; TF, transcription factor; TREs, transcription factor response elements.*

### 3.3 Molecular mechanisms of steroid resistance

The precise molecular mechanism of steroid resistance in inflammatory diseases remains unclear, but several mechanisms have been proposed. Possible mechanisms include altered numbers of glucocorticoid receptors, altered affinity of the ligand for glucocorticoid receptors, reduced affinity of the glucocorticoid receptor to bind DNA and altered expression of transcription factors and/ or cytokines<sup>133</sup>. However, to date none of these above mechanisms have fully explained steroid resistance in these patients. This review describes the molecular basis for GC resistance in inflammatory bowel disease.

**Figure 3.2 Molecular Mechanisms of Steroid Resistance**





### ***3.3.1 Glucocorticoid receptor abnormalities and resistance mechanisms***

To date, glucocorticoid resistance research on T-lymphocytes and other select inflammatory cells have proposed several GR abnormalities as potential mechanisms impacting response rates to glucocorticoids. Primary GR congenital abnormalities have been described in the literature <sup>160</sup>, but generally represent a very rare cause of glucocorticoid resistance in inflammatory conditions overall, and in fact have never been identified amongst resistant IBD patients.

Variations in concentrations of GR has been hypothesized as a potential mechanism for steroid resistance in the past. Glucocorticoid receptor numbers in peripheral blood mononuclear cells (PBMC) have been examined amongst subjects with steroid-resistant asthma, with the majority of studies demonstrating no significant differences in receptor concentrations<sup>161,162</sup>. Amongst IBD patients, Flood et al. showed the concentration of GR mRNA in peripheral leukocytes was higher in UC patients with quiescent disease compared with controls, however no significant differences were shown in GR mRNA levels between glucocorticoid responders and glucocorticoid non-responders<sup>163</sup>. In contrast,

Raddatz et al reported significantly lower tissue concentrations of GR mRNA from mucosal biopsies in patients with steroid resistant UC<sup>164</sup>.

Interestingly, GR exists in different isoforms, and these differences in isoforms have been forwarded as a potential explanation for steroid resistance<sup>163</sup>. GR- alpha (GR- $\alpha$ ) is the most dominant isoform and is physiologically active<sup>165</sup>. In contrast, GR- beta (GR- $\beta$ ) is a truncated splice variant, which is unable to bind to the ligand and is transcriptionally inactive, and exerts a dominantly negative effect by interfering with binding of GR- $\alpha$  to the DNA<sup>166-170</sup>. The presence of GR- $\beta$  has been suggested as a mechanism of steroid resistance. In a Japanese study by Honda et al, GR- $\beta$  mRNA was detectable in more patients with steroid-resistant UC (83%) compared to patients with steroid-sensitive UC (9%), 10% of healthy volunteers and 10% of chronic active CD patients<sup>171</sup>. However, in IBD, GR $\beta$  is expressed 100-1000 times less than GR $\alpha$ , which has challenged its role in the mechanism of steroid resistance in the past.

In addition to variations of the GR, altered glucocorticoid binding affinity to the GR as well as GR binding affinity to DNA have also been suggested as a means of resistance in the past. One study by Cho<sup>172</sup> et al. which examined patients with severe asthma, demonstrated that steroid resistant patients had decreased GC/GR binding affinity detected in peripheral mononuclear blood

cells, compared with the steroid sensitive patients as well as healthy controls. Another study which examined the ability of nuclear translocated GRs to bind their DNA-binding sites, showed impaired binding in steroid-resistant asthmatics compared with steroid-sensitive asthmatics and healthy controls, and that this was as a result of reduced numbers of receptors available for binding to DNA<sup>173</sup>.

Therefore, an inability of the GR–ligand complex to activate key genes may be responsible for a poor response to glucocorticoid action. Specifically, however, there have been no studies looking at differences in ligand binding affinity to the GR amongst IBD subjects.

### ***3.3.2 Altered expression of transcription factors and inflammatory cytokines***

The role of inflammatory cytokines in glucocorticoid resistance has also been examined in the past. Pro-inflammatory cytokines are involved in the pathogenesis of several chronic inflammatory diseases such as rheumatoid arthritis, osteoarthritis, asthma and IBD<sup>174,175</sup>. Their excessive upregulation is typically overcome by GCs, however steroid resistant subjects often demonstrate increased concentrations of local and/or systemic pro-inflammatory cytokines<sup>176</sup>. As glucocorticoid resistance is more commonly

noted in those patients with more severe disease, it is difficult to distinguish, whether glucocorticoid resistance is an intrinsic phenomenon or whether the anti-inflammatory capacity of glucocorticoids is simply overcome by a surge in pro-inflammatory cytokines during an inflammatory event<sup>146</sup>. This in turn, increases the activity of various intracellular transcription factors which, may ultimately reduce the affinity of GR for its intracellular ligand. Both mechanisms involve an upregulation of inflammatory molecules and a positive feedback system with a secondary upregulated inflammatory response.

Overexpression of Interleukin-6 (IL-6) and Interleukin-8 (IL-8) have been reportedly associated with non-responsiveness to GC treatment in some studies<sup>177</sup>. In paediatric patients with UC, patients who did not respond to GC had higher levels of IL-6, compared to responders<sup>178</sup>. Similarly, in CD a correlation between cytokine production and resistance to GC was found. A clinical study showed that in intestinal mucosa from GC-resistant patients, the rate of apoptosis of T and B cells, presence of caspase-3 and IL-10 production were diminished compared to healthy and GC-responsive individuals<sup>179</sup>.

As outlined above, GR- $\alpha$  interacts with potent transcription factors such as NF-KB, which consequently inhibits cytokine production during an inflammatory cascade<sup>146</sup>. Conversely, NF-KB and GR- $\alpha$  can mutually

repress each other's transcriptional activity. Previous studies have shown different NF-KB activity in biopsy specimens from steroid-resistant and steroid-sensitive patients with CD and UC who all had severe disease activity<sup>176</sup>. In sensitive patients, NF-KB activation was mainly found in lamina propria macrophages and in single scattered endothelial cells. However, over 60% of steroid-resistant patients had a different staining pattern with active NF-KB predominantly localized in epithelial cells<sup>176</sup>. Differences in the distribution of such key molecules may be an important consideration in exploring the mechanisms of steroid resistance.

### **3.4 Genetic mechanisms of Glucocorticoid Resistance**

Although this review has predominantly focused on the molecular mechanisms of steroid resistance, one important genetic factor thought to contribute to steroid resistance in a significant way, is alterations in the P-glycoprotein (P-gp) which is encoded for by the multi-drug resistant gene-1 (*MDR1*)<sup>180</sup>. P-gp is responsible for the absorption, distribution, metabolism and excretion of various drugs including GC<sup>181</sup>. A relationship between increased P-gp levels in circulating lymphocytes and intestinal epithelial cells with GC-resistant IBDs patients has been reported<sup>181</sup>. Polymorphisms in the *MDR1* gene, responsible for P-gp function have been thought to influence response rates to GCs in the past.

### 3.5 Conclusion

The proposed mechanisms involved in steroid resistance are extremely vast and initial enthusiasm for any specific mechanism has failed to identify a distinct genotype or phenotype that is applicable clinically. To date it remains unclear as to the exact reasons underlying resistance amongst IBD patients. This presents a unique challenge to clinicians when trying to identify patients who are likely to be resistant to steroid treatment.

Interestingly in recent years it has become evident that in addition to the control of glucocorticoid levels by the HPA Axis, there is also a local control of tissue specific action of glucocorticoids by the 11 $\beta$ -Hydroxysteroid Dehydrogenase enzyme system (11 $\beta$ -HSD). Tissue regulation of GC function represents a novel approach to understanding resistance mechanisms and may potentially offer a clinically applicable early marker of GC resistance. There is very little in the literature regarding the role of the 11 $\beta$ -HSD enzyme system in IBD. In the next chapter we discuss the role and regulation of the 11 $\beta$ -HSD enzyme system in steroid metabolism and we review the current literature in relation to its role in IBD.

# Chapter 4: An introduction to 11 $\beta$ -Hydroxysteroid Dehydrogenase enzyme system

## 4.1 Introduction

Adrenal corticosteroids are essential for life and can be divided into three main types: glucocorticoids (synthesised in the zona fasciculata), mineralocorticoids (synthesised in the zona glomerulosa) and sex steroids (synthesised in the zona reticularis)<sup>157</sup>. Cortisol (the main active glucocorticoid in humans) is secreted under the tight control of the hypothalamic-pituitary-adrenal axis.

Glucocorticoids affect every organ system with the main physiological and pathophysiological effects being on energy metabolism, the cardiovascular/ blood pressure system, bone, connective tissue (including muscle), the immune system, the central nervous system, the gastrointestinal and the endocrine system **Table 4.1**

**Table 4.1. Effects of Glucocorticoids**

| METABOLISM                                                                                                                                                                                                                          | BONE                                                                                                                                                                  | VASCULAR/<br>BLOOD PRESSURE                                                                                                                                                                                                                                              | IMMUNE                                                                                                                                                                                                                                    |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <ul style="list-style-type: none"><li>↑ Serum Glucose</li><li>↑ Liver Glycogen</li><li>↑ Glycogen synthase</li><li>↑ Gluconeogenesis</li><li>↑ Circulating chol</li><li>↑ Circulating TG</li><li>↓ Glycogen phosphorylase</li></ul> | <ul style="list-style-type: none"><li>↑ Renal excretion of Ca<sup>++</sup></li><li>↑ PTH</li><li>↓ Osteoblast activity</li><li>↓ Ca<sup>++</sup> absorption</li></ul> | <ul style="list-style-type: none"><li>↑ Sensitivity to pressor agents</li><li>↑ GFR</li><li>↑ Angiotensinogen</li><li>↑ Proximal Na<sup>+</sup> epithelium transport</li><li>↑ Free water clearance</li><li>↑ MR activity</li><li>↓ NO mediated vasodilatation</li></ul> | <ul style="list-style-type: none"><li>↑ Apoptosis</li><li>↓ Circulating lymphocytes</li><li>↓ Ig synthesis</li><li>↓ Cytokine production</li><li>↓ Monocyte differentiation</li><li>↓ Phagocytosis</li><li>↓ Cytotoxic activity</li></ul> |

Glucocorticoid release is often triggered by an environmental or immunological stressor resulting in immunoregulatory activities, mostly immunosuppressive, on distant tissues and cells, in particular in immune cells <sup>182</sup>. Within the immune system, glucocorticoids lead to increased apoptosis and decreased circulating lymphocytes, immunoglobulin synthesis, cytokine production, monocyte differentiation, phagocytosis and



cytotoxic activity which underpin the efficacy of glucocorticoids as an immunomodulatory therapy<sup>157</sup>. GCs exert effects on vast array of cell subtypes in the body to ensure reactions to normal diurnal changes in metabolism and stress responses are in equilibrium<sup>183,184</sup>. Interestingly inflammatory cytokines including interleukin-1 beta (IL-1 $\beta$ ), IL-6, and tumor necrosis factor alpha (TNF) have also been shown to trigger the secretion of ACTH and CRH suggesting a further bidirectional feedback between immune and neuroendocrine systems<sup>185</sup>

#### **4.2 11-beta-hydroxysteroid dehydrogenase enzyme system**

In addition to the control of GC levels by the HPA axis, there is also tissue specific regulation. Local GC tissue concentrations can be further modified through their pre-receptor metabolism, a process which is significantly influenced by the 11-beta-hydroxysteroid dehydrogenase (11 $\beta$ -HSD) enzyme system.

Two distinct isoforms of this intracellular enzyme exist, 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2. 11 $\beta$ -HSD1 is expressed in an array of tissue types including the liver, placenta, adipose tissue as well as epithelial and non-epithelial cells of colonic tissue<sup>186,187</sup> **Figure 4.1.** It acts predominantly as an oxoreductase, converting inactive cortisone into active cortisol and thus increases the local

concentration of active glucocorticoid within tissues<sup>188</sup>. This oxoreductase activity is determined by nicotinamide adenine dinucleotide phosphate (NADPH) co-factor availability produced by endoplasmic reticulum specific enzyme hexose-6-phosphate dehydrogenase (H6PDH)<sup>189</sup>.

In contrast, 11 $\beta$ -HSD2 is a high affinity nicotinamide adenine dinucleotide (NAD) dependent enzyme which is predominantly found in mineralocorticoid sensitive tissues, such as the colon, kidney, and placenta. 11 $\beta$ -HSD2 acts as a dehydrogenase, converting the active glucocorticoid cortisol to inactive cortisone and thus decreases the local concentration of active glucocorticoids<sup>190</sup>. This is vital in tissues that have a mineralocorticoid receptor (MR) as cortisol has equal affinity to the MR as aldosterone and the conversion to cortisone protects the MR from illicit occupancy by cortisol. Mutations in this enzyme may result in the syndrome of apparent mineralocorticoid excess, with sodium retention, severe hypertension, growth delay and renal dysfunction<sup>191,192</sup>.

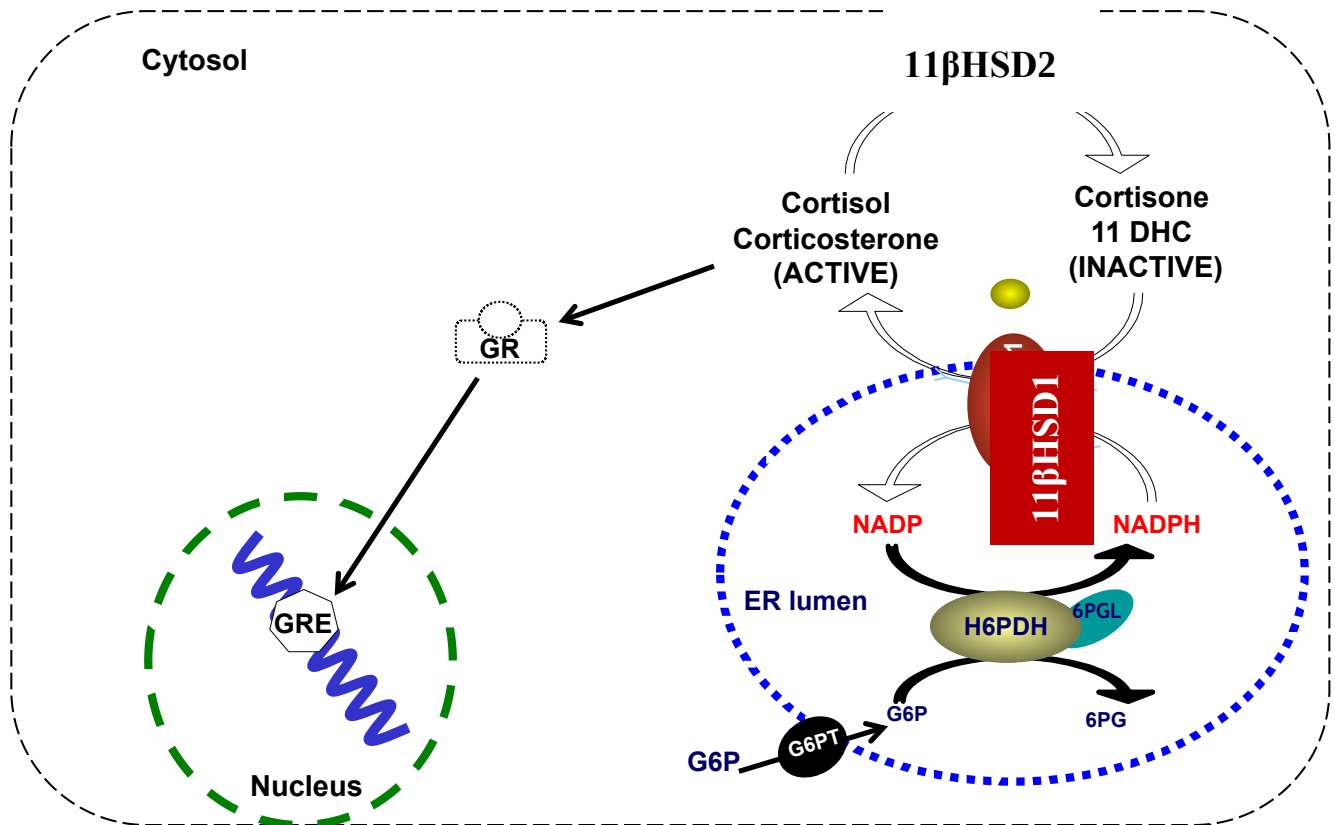
The gene for 11 $\beta$ -HSD1 is on chromosome 1 (1q32.2-41), has 6 exons (182, 130, 111, 185, 143 and 617bp respectively) and 5 introns (776, 767, 120, 25,300 and 1,700 bp, respectively)<sup>193</sup>. The gene for 11 $\beta$ -HSD2 is located on chromosome 16q22 and contains five exons spaced over approximately 6.2 kb<sup>190</sup>. As well as the naturally occurring steroids (cortisol and cortisone) the synthetic steroids prednisolone and prednisone are also substrates for the

11  $\beta$ -HSD enzyme system<sup>194,195</sup>. The main substrate for 11  $\beta$ -HSD1 in humans is cortisone and in rodents 11-dehydrocorticosterone<sup>193</sup>. 11  $\beta$ -HSD1 also has roles in biotransformation of several carbonyl group compounds xenobiotics, drugs, insecticides, carcinogens<sup>193</sup>. 9 $\alpha$ -fluorinated steroids such as dexamethasone are metabolised by 11  $\beta$ -HSD2<sup>196</sup> but may also be regenerated by 11  $\beta$ -HSD1<sup>197</sup>.

Importantly, there are species and tissue differences in enzyme kinetics and dynamics for both enzymes<sup>190,193</sup>. Therefore, any studies assessing these enzymes in rodent models need to take account of these species differences and may not be generalisable to humans.

Within colonic tissue, 11 $\beta$ -HSD2 is expressed only in the epithelial cells lining the crypts<sup>187</sup>. Exposure to inflammatory stimuli results in increased tissue expression of 11 $\beta$ -HSD1 and reduced expression of 11 $\beta$ -HSD2 and subsequently results in increased local tissue cortisol production as well as reducing local cortisol inactivation.

**Figure 4.1. Schema representing the tissue specific regulation of glucocorticoid action by 11  $\beta$ -HSD1 and 2.**



(G6P = glucose-6-phosphate, 6PG = 6 phosphogluconate, ER = endoplasmic reticulum, GR = glucocorticoid receptor, GRE = glucocorticoid response elements, G6PT = glucose-6-phosphate transporter)

#### 4.3 11 $\beta$ -HSD enzyme system and other autoimmune conditions

The role of the 11 $\beta$ -HSD enzyme system has been demonstrated to play a role in an array of autoimmune conditions in the past. For example, Terao et al. demonstrated increased 11 $\beta$ -HSD1 expression in keratinocytes and it appeared to play a role in the regulation of inflammation and keratinocyte proliferation in patients with Dermatitis<sup>198</sup>. Similarly, Hardy et al

demonstrated increased 11 $\beta$ -HSD1 in matched fibroblasts derived from various tissues (synovium, bone marrow and skin) obtained from patients with rheumatoid arthritis<sup>199</sup>.

In addition, Orsida et al. demonstrated reduced 11 $\beta$ -HSD2 expression in patients with asthma and an inverse relationship between inhaled corticosteroid dose requirements and the extent of epithelial 11 $\beta$ -HSD2 staining in lung tissue from asthmatic patients compared with controls<sup>200</sup>

Few studies to date have suggested that altered expression of this particular enzyme system, secondary to excessive exposure to pro inflammatory cytokines affects the balance between levels of active and inactive glucocorticoids at the intracellular level in these inflammatory conditions<sup>190,201,202</sup>. The pathophysiological significance of this in IBD has been minimally explored to date.

#### **4.4 11 $\beta$ -HSD enzyme system and IBD, current knowledge**

Currently there are limited studies available examining the role of the 11 $\beta$ -HSD enzyme system in IBD. In a previous study conducted by Bryndova et al, in both human and rat specimens there was an up-regulation in expression of colonic 11 $\beta$ -HSD1 mRNA and down regulation of 11 $\beta$ -HSD2 mRNA in tissue with active colitis<sup>188</sup>.

Similarly, in a study by Zbankova et al., both human and rat specimens with colitis were examined. Inflamed tissue from human colonic samples demonstrated an up-regulation of the expression of 11 $\beta$ HSD1 mRNA and down-regulation 11 $\beta$ HSD2 mRNA when compared. A similar pattern was observed at the level of local metabolism of corticosterone in rats. Oxidation of corticosterone to 11-dehydrocorticosterone was decreased and reduction of 11-dehydrocorticosterone to corticosterone was increased in colonic tissue of rats with DSS-colitis<sup>203</sup>. The overall conclusion from this study was that colitis induces local glucocorticoid activation from 11-oxo steroids and decreases glucocorticoid inactivation, i.e. inflammation increases local tissue ratio of active to inactive glucocorticoids. Another study conducted by Stegk et al in 2009 demonstrated that expression of 11 $\beta$ -HSD1 was significantly elevated in inflamed tissue compared with non-inflamed tissue in both Crohn's disease (2.7-fold) and Ulcerative Colitis (3.8-fold) whereas 11 $\beta$ -HSD2 expression was decreased in the same samples<sup>204</sup>

The results indicate that the changes in local metabolism of glucocorticoids could contribute to alterations in the inflammatory processes in the colon. In contrast to the studies above, Ahmed et al recently demonstrated reduced tissue 11 $\beta$ -HSD1 expression in paediatric IBD patients compared with controls and suggested that defective local reactivation of glucocorticoids

could result in lower 11 $\beta$ -HSD1 and contribute to the overall pathogenesis of IBD<sup>205</sup>

The exact role and interplay of cytokines and other regulatory cofactors of the HSD enzyme system in IBD has yet to be fully defined. One *in vitro* study by Ergang et al. showed tumour necrosis factor  $\alpha$  exhibited an upregulation of 11 $\beta$ -HSD1 mRNA whereas interleukin-1 $\beta$  downregulated 11 $\beta$ -HSD2 mRNA in rats with experimental induced colitis<sup>206</sup>. The interplay between inflammatory cytokines and the HSD enzyme system in patients with IBD could represent a novel insight into cortisol metabolism in this patient group. To date, none of these studies have explored the effect of 11 $\beta$ HSD enzyme system on steroid responsiveness or the specific regulators (including cytokines) of 11 $\beta$ HSD pathway in IBD.

## **4.5 Conclusions**

To date the role of 11  $\beta$  -HSD enzyme system in patients with IBD has been minimally explored and its role in steroid resistance has yet to be defined. We hypothesize that there are alterations in the 11  $\beta$  -HSD enzyme system in the gastrointestinal tissue of patients with steroid resistant IBD leading to a decrease in tissue exposure to active glucocorticoids and thus a decrease in the therapeutic effects of glucocorticoids. Our aims therefore are to expand further on the role of the 11 $\beta$ -HSD enzyme system in IBD as well as

outline the role of potential key regulators of that system and explore the interplay of inflammatory cytokines.



## **Chapter 5: The role and regulation of the 11 beta hydroxysteroid dehydrogenase enzyme system in patients with Inflammatory Bowel Disease**

### **5.1 Introduction**

Inflammatory bowel disease (IBD) is characterized by a dysregulated immune response which results in chronic inflammation of the digestive tract. Cortisol, the main glucocorticoid (GC) in humans, is secreted under the tight control of the hypothalamic-pituitary-adrenal (HPA) axis. Pro-inflammatory cytokines such as Tumor Necrosis Factor (TNF)- $\alpha$  and Interleukin-1  $\beta$  (IL- $\beta$ ) have been shown to stimulate glucocorticoid production via the HPA axis during an inflammatory event. 11 $\beta$ -HSD1 acts as an oxoreductase converting inactive cortisone into active cortisol, whilst 11 $\beta$ -HSD2 acts as a dehydrogenase converting active cortisol to inactive cortisone. Hexose-6 phosphate dehydrogenase (H6PDH) is a key regulator of 11 $\beta$ -HSD1 activity via its generation of NADPH. Variations in the 11 $\beta$ -HSD enzyme system in relation to levels of expression and regulation may have a role in IBD.

The studies to date exploring the role of the 11 $\beta$ -HSD in IBD have been cross-sectional studies, not prospective and there have been no separation of data relative to rates of steroid responsiveness. Also, there has been no

assessment of the relative amounts of 11  $\beta$ -HSD1:11  $\beta$ -HSD2 within a single individual which we feel may be a novel way of assessing the overall balance of activation: deactivation of steroid within colonic tissue.

In summary, while there have been significant advances in the management of IBD with disease modifying agents and biologic agents <sup>207,208</sup>, steroids remain a mainstay of therapy and there is still no way of predicting in which patients' steroids will be effective. This study will investigate tissue cortisol metabolism in IBD in the first instance and potentially later provide an update on mechanisms of local resistance.

## **5.2 Study Aims and Endpoints:**

The overall study aim was to provide an understanding of the role and regulation of the 11 $\beta$ HSD enzyme system in IBD and to examine the interplay of key inflammatory cytokines in relation to the HSD system

### **Endpoints**

- To determine 11 $\beta$ HSD1 colonic expression in IBD patients
- To determine 11 $\beta$ HSD2 colonic expression in IBD patients
- To examine 11 $\beta$ HSD1 cofactor, H6PDH, expression in IBD patients
- To determine the role of GR  $\alpha$  expression in IBD patients

- To examine the degree of colonic inflammation as determined by clinical assessment (Harvey-Bradshaw Index, Partial Mayo score which are simple clinical measures of activity based on key symptom assessment.), biochemical (serum C-reactive protein (crp)) and Histological parameters.
- To measure expression of inflammatory cytokines TNF  $\alpha$ , IL-1 $\beta$ , Interleukin-6 and Rela (subunit for Nuclear Factor Kappa B (NF $\kappa$ B))

## 5.3 Materials and Methods

### 5.3.1 Patient Selection

Ethical approval was sought from the Joint Research ethics committee of Trinity College Dublin, The Adelaide and Meath Hospital (AMNCH) and St. James Hospital. Following informed consent, IBD patients undergoing a colonoscopy for disease assessment aged 18-80 years were prospectively recruited. Exclusion criteria included the following: steroid therapy within 6 weeks including inhaled GCS, known Cushing's syndrome or primary hyperaldosteronism, significant comorbidity, or pregnancy. Patient demographics, clinical disease activity scores (using a Harvey-Bradshaw Index (HBI) or Mayo Score (MS)), histological and endoscopic grade of disease activity were all recorded. Disease activity was determined as mild,

moderate or severe based primarily on the global severity of disease determined endoscopically using the Mayo score or CDEIS. At the time of endoscopy, a serum C - reactive protein (CRP) level was also taken and processed in the hospital's biochemistry laboratory. A CRP > 5 mg/L determined active disease. Subjects with a normal colonoscopy without a history of IBD were also recruited and their biopsies were confirmed as histologically normal by a consultant histopathologist. Two biopsies were obtained from inflamed and non-inflamed colonic tissue in IBD patients where available, whereas a single biopsy from the left colon was obtained in controls. Standard biopsies were processed as normal in the hospitals histopathology lab and reviewed by 2 pathologists with a special interest in gastroenterology.

### ***5.3.2 Processing of biopsies***

The biopsies were transferred immediately into a tissue storage reagent RNAlater and stored at -80°C. They were later analysed in batch, using Quantitative real time RT-PCR (Taqman) and commercially available probes using 18 s as a reference gene. Initially relative transcript levels of 11 $\beta$ -HSD1 and 2 were compared amongst IBD patients and controls. Amongst IBD patients levels were compared based on levels of disease activity endoscopically and histologically.

Following these results, we later went on to measure relative transcript levels of key determinants of GC action, H6PDH, and the GR $\alpha$ . We then proceeded to further evaluate gene expression of key cytokines such as TNF  $\alpha$ , IL-1 $\beta$ , Interleukin-6 and Rela (subunit for Nuclear Factor Kappa B (NFKB)) in the same patients.

### ***5.3.3 Reverse Transcriptase PCR***

Gene expression signals were quantified with real-time PCR (Taqman) on the colonic samples described above. Probes and Primers were obtained from Applied Biosystems as Assays-on-Demand Gene Expression Assays (11 $\beta$ -HSD1; Hs00194153\_m1, 11 $\beta$ -HSD2;Hs00388669\_m1, 18s; Hs99999901\_s1, GR  $\alpha$ ; Hs00353740\_m1, H6PDH; Hs00188728\_m1, IL-1 $\beta$ ; Hs00174097\_m1, IL-6;Hs00985639\_m1, TNF $\alpha$ ;Hs01113624\_g1, Rela; Hs00153294\_m1).

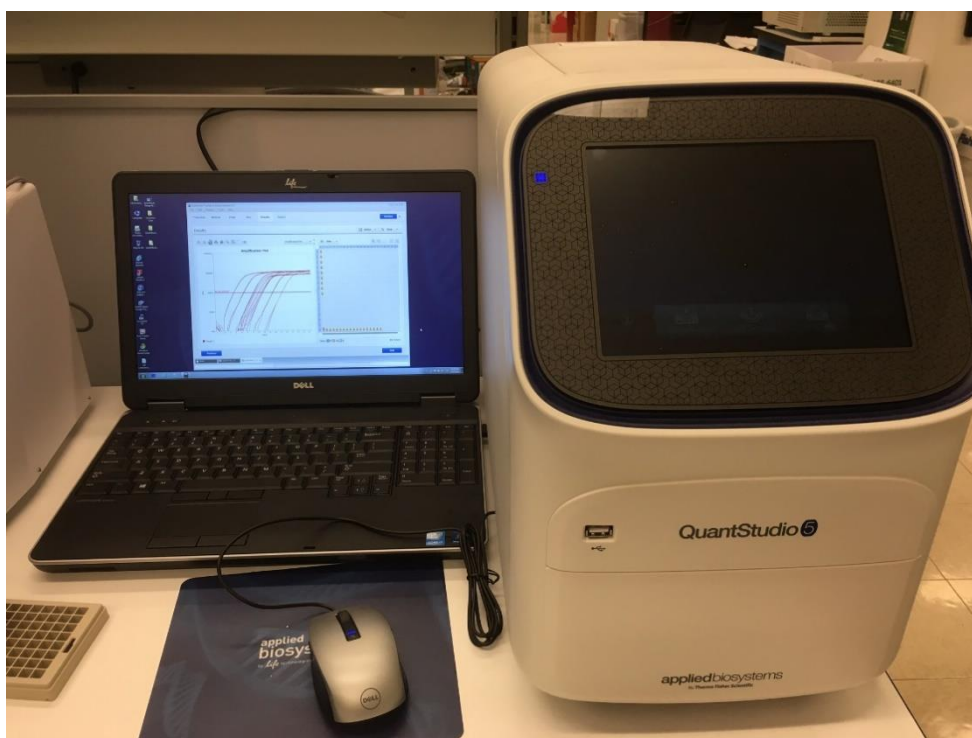
Tissue samples were mechanically disrupted using the Tissueruptor (Qiagen, Germany) and total RNA was isolated using the RNeasy kit. RNA concentrations were determined spectrophotometrically by using the Nanodrop 2000 device (Thermo Scientific, United States). Reactions were performed in 20ul volumes on 96-well plates in reaction buffer containing 2ul TaqMan Universal PCR Master mix (Applied Biosystems, Foster City, CA). RNA was reverse transcribed to cDNA following standard protocols

using first strand cDNA synthesis kit (Thermo-Scientific) and stored at -20°C.

Reactions were carried out on the Quantstudio 5 RT PCR system and relative transcript levels were determined using 18s as a reference gene.

***Figure 5.1 Reverse Transcriptase PCR***





#### **5.3.4 Statistical Analysis**

Results and patient demographics were expressed as a mean and compared between IBD patients and controls using the Student t test or Mann-Whitney test as appropriate, with a difference of  $p < 0.05$  considered significant. Results were controlled for disease activity according to clinical (HBI/Mayo score), biochemical (CRP), histological and endoscopic parameters. All analyses were performed using Graphpad version 7 (GraphPad Software, Inc. San Diego, California). Pearson's correlation analysis was completed to determine the relationship between expression of 11 $\beta$ -HSD genes and those of various cytokines

## 5.4 Results

### 5.4.1 Population

To date, 72 patients have been recruited, 48 IBD (19 CD and 29 UC) patients and 24 controls. Of these patients, matched inflamed and non-inflamed biopsies were obtained in 17 patients. IBD and control cohorts were demographically similar amongst IBD and control cohorts, 56% (n=27) and 67% (n=16) were male with a median age of 45 years (range 20-67) and 53 years (25-83), respectively, **Table 5.1**. In all, 35% (n=17) of this IBD cohort had mild, 38 % (n=18) moderate and 27% (n=13) severe disease histologically. The median HBI was 7 (range 0-21) and the median Mayo Score was 7 (range 0-12). The median CRP in the overall IBD group was 34.5mg/l (1-192.5mg/l) and for active patients the median CRP was 37mg/l (8-192.5 mg/l) vs. 2.0mg/l (1-5mg/l) for those with inactive disease, (p=.04).



**Table 5.1: Demographic and clinical data of patients with IBD and control subjects**

| Characteristic             | IBD (N=48)         | Controls (N=24)      |
|----------------------------|--------------------|----------------------|
| No. of Patients            | N=19 CD, N=29 UC   | N=24                 |
| Median Patient age (range) | 45 yrs. (17-67yrs) | 53 yrs. (19-83 yrs.) |
| Males n= (%)               | 27 (56%)           | 12 (50%)             |
| Median CRP (mg/l)          | 35.4 (1-192.5)     |                      |
| Median HBI score           | 7 (0-21)           |                      |
| Median Mayo score          | 7 (0-12)           |                      |
| <u>Disease Severity</u>    |                    |                      |
| Mild n= (%)                | 17 (35%)           |                      |
| Moderate n= (%)            | 18 (38%)           |                      |
| Severe n= (%)              | 13 (27%)           |                      |

#### ***5.4.2 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 expression***

There was a significant downregulation of 11 $\beta$ -HSD2 in IBD patients (13.8 AU  $\pm$  17.1) compared with controls (318.4 AU  $\pm$  521.1) (p=0.01) as well as compared with inflamed (50  $\pm$  94 AU) and non-inflamed samples (74  $\pm$  109 AU, p=0.03). Overall there was no statistically significant difference seen in levels of 11 $\beta$ -HSD1 across the groups; controls (390.1  $\pm$  538.7 AU), IBD

overall ( $301.3 \pm 788.5$  AU), although trends suggested higher levels in inflamed ( $422.1 \pm 944$  AU) vs. non-inflamed tissue ( $102.2 \pm 103.1$  AU) ( $p=0.09$ ) **Table 5.2**. Levels of 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 did not vary according to disease subtype (i.e CD compared to UC; **Table 5.3**. In addition, there was no significant difference in 11 $\beta$ -HSD1 based on histological or biochemical activity. Consequently, the ratio of 11 $\beta$ -HSD1 expression to 11 $\beta$ -HSD2 was increased in IBD patients; 81:1 vs Controls; 1.5:1, ( $p=0.006$ , 95% CI 57.7-78.5).

As non-inflamed tissue samples were similar to control tissue samples for both 11 $\beta$ -HSD 1 and 2 and ratio expression, for further analyses we looked at only inflamed and control samples to assess key regulators.

#### ***5.4.3 Expression of GR $\alpha$ , H6PDH and inflammatory cytokines in inflammatory bowel disease***

When comparing inflamed IBD tissue and control tissue, there was no difference in GR $\alpha$  expression ( $4.5 \pm 10.2$  AU vs  $2.1 \pm 3.6$  AU,  $p=0.4$ ) or H6PDH ( $5.2 \pm 4.2$  AU vs.  $3.9 \pm 4.2$  AU,  $p=0.2$ ) **Table 5.2**. Additionally, GR $\alpha$  expression did not appear to alter according to levels of disease activity as defined clinically, endoscopically, histologically or biochemically, **Table 5.3**.

**Table 5.2: Mean mRNA Expression of 11 $\beta$ HSD 1 and 2, GR  $\alpha$  and H6PDH in patients with IBD and healthy Controls.**

| Mean<br>AU                    | IBD<br>N=48 | Controls<br>N=24 | P<br>Value | Inflamed<br>N=31 | Non<br>Inflamed<br>N=17 | P<br>Value | Active<br>N=37 | Inactive<br>N=7 | P<br>Value |
|-------------------------------|-------------|------------------|------------|------------------|-------------------------|------------|----------------|-----------------|------------|
| <b>HSD 1</b>                  | 301.3       | 390.1            | NS         | 422              | 102.2                   | NS         | 415.9          | 166.0           | NS         |
| <b>HSD 2</b>                  | 13.8        | 318.4            | 0.01       | 50               | 74                      | NS         | 4.8            | 12.2            | 0.01       |
| <b>HSD<br/>1:2</b>            | 77.7        | 1.4              | 0.01       | 45               | 3.7                     | 0.04       | 80.9           | 79.2            | NS         |
| <b>GR-<math>\alpha</math></b> | 4.5         | 2.1              | NS         |                  |                         |            | 7.1            | 1.5             | NS         |
| <b>H6PDH</b>                  | 3.4         | 3.9              | NS         |                  |                         |            | 7.8            | 2.9             | NS         |

**\*NS=not significant**

Levels of IL-1 $\beta$ , IL-6, TNF $\alpha$  and Rela were all significantly higher amongst inflamed IBD tissue samples vs. controls **Table 5.4**. All cytokines were

statistically upregulated in response to worsening disease activity and this was accompanied by a significant downregulation of 11 $\beta$ -HSD2 (active  $4.8 \pm 5.9$  AU vs. inactive  $12.1 \pm 22.3$  AU,  $p = 0.01$ ) in response to inflammation.

**Table 5.3: Mean mRNA Expression of 11 $\beta$ HSD 1 and 2, GR  $\alpha$  and H6PDH in IBD patients based on disease subtype.**

| Mean AU      | Crohn's Disease<br>N=19 | Ulcerative Colitis N=29 | P value |
|--------------|-------------------------|-------------------------|---------|
| HSD 1        | 179                     | 390                     | 0.6     |
| HSD 2        | 14.1                    | 9.8                     | 0.5     |
| GR- $\alpha$ | 3.4                     | 5.2                     | 0.6     |
| H6PDH        | 5.6                     | 9.8                     | 0.3     |

**Table 5.4: Mean Relative expression of 11BHSD2 and cytokines overall and according to disease activity. In addition, Pearson's correlation coefficient between 11BHSD2 and cytokines overall**

| Mean Relative Expression (au)  | IBD<br>N=24 | Controls<br>N=12 | P<br>value | Active<br>N=14 | Inactive<br>N=10 | P<br>value | R<br>value |
|--------------------------------|-------------|------------------|------------|----------------|------------------|------------|------------|
| <b>11BHSD 2</b>                | 13.8        | 314.4            | 0.01       | 4.8            | 12.1             | 0.01       |            |
| <b>IL-6</b>                    | 18.2        | 0.6              | 0.02       | 25.5           | 5.2              | 0.03       | 0.4        |
| <b>TNF <math>\alpha</math></b> | 6.0         | 1.3              | 0.02       | 7.9            | 2.6              | 0.04       | 0.5        |
| <b>IL-1Beta</b>                | 13.1        | 0.2              | 0.02       | 17.9           | 5.3              | 0.04       | 0.6        |
| <b>Rela (NFKB)</b>             | 3.1         | 1.0              | 0.03       | 4.0            | 1.6              | 0.05       | 0.5        |

## 5.5 Discussion

The hallmark of active inflammatory bowel disease is a pronounced infiltration into the lamina propria of innate immune cells (neutrophils, macrophages, dendritic cells, and natural killer T cells) and adaptive immune cells (B cells and T cells). Increased numbers and activation of these cells in the intestinal mucosa elevate local levels of tumour necrosis factor  $\alpha$ , interleukin- $1\beta$ , interferon- $\gamma$ , and other cytokines of the interleukin-23–Th17 pathway<sup>209</sup>. Both endogenous and exogenous glucocorticoids play a pivotal role in counteracting inflammation. They exert their anti-inflammatory effect by binding to glucocorticoid receptor and interacting with potent

transcription factors such as NF $\kappa$ B which inhibits the production of cytokines and other mediators of the inflammatory cascade<sup>210</sup>. While the HPA axis is central to glucocorticoid control, tissue levels of 11 $\beta$ -HSD enzymes are integral to the pre-receptor modulation of GC action. Local alterations in their activity and regulation might be expected to influence the efficacy of endogenous and exogenous GC and may further enlighten understanding of GC resistance in IBD patients.

In addition, better understanding of 11 $\beta$ -HSD enzyme pathway may allow clinicians select exogenous GC less influenced by this enzyme pathway. For example, prednisone and its inactive metabolite prednisolone share a similar metabolism to that of cortisol and cortisone<sup>211</sup>. In contrast, dexamethasone does not undergo significant metabolism by 11 $\beta$ -HSD enzymes which may prove to be therapeutically advantageous. Previous studies report that dexamethasone itself is not metabolised to any great degree by 11 $\beta$ -HSD2 compared with other steroids<sup>212</sup>

In our study, 11 $\beta$ -HSD 2 was significantly downregulated in response to inflammation in IBD patients compared with controls. There was also a significant downregulation in inflamed mucosa when compared to histologically non-inflamed mucosa and controls, and levels appeared to lower in response to worsening disease activity. This is in keeping with findings observed in other animal and human studies which showed a

downregulation of 11 $\beta$ -HSD 2 in the setting of colitis<sup>190,203,209</sup>. Takashashi et al.<sup>213</sup> also observed a significant downregulation of 11 $\beta$ -HSD 2 in UC patients, although their findings, unlike ours were not observed in CD patients. There were no disease specific differences observed in 11 $\beta$ -HSD2 expression in our cohort of patients. Downregulation of 11 $\beta$ -HSD2 gene expression in IBD tissue was associated with an upregulation in the gene expression of key cytokines TNF  $\alpha$ , IL-1  $\beta$ , IL-6 and NF $\kappa$ B, which were significantly more elevated in cases of more severe disease activity.

Our findings in a human cohort, support those observed by some cell culture *in vitro* animal studies which showed potent cytokines such as TNF  $\alpha$  and IL-1  $\beta$  result in a down regulation of 11 $\beta$ -HSD2<sup>186,190,210</sup>. Other recognized cytokines common to the IBD pathway were also included in our study, IL-6 and Rela (subunit for NF Kappa B)), and they too were associated with a downregulation of 11 $\beta$ -HSD2. At present, these are associations and further work is required to assess if there is a mechanistic link or just a link to severity of inflammation.

In contrast, there was no significant elevation in 11 $\beta$ -HSD1 in our IBD patients compared with controls which contrasts with some of the previously published animal and human studies to date. Zbankova et al. demonstrated an upregulation of 11 $\beta$ -HSD 1 mRNA in rat and human specimens with colitis versus control subjects<sup>203</sup>. Additionally, Stegk et al. demonstrated an

upregulation of 11 $\beta$ -HSD1 in inflamed colonic tissue compared with non-inflamed tissue in patients with IBD without reference to controls<sup>204</sup>. Similar to our study however, Ahmed et al. did report lower levels than expected of 11 $\beta$ -HSD1 in a paediatric IBD cohort and hypothesised that this may play a role in IBD pathogenesis via imbalances in pro and anti-inflammatory proteins<sup>205</sup>.

Our study does however show trends towards higher levels of 11 $\beta$ -HSD 1 in inflamed compared to non-inflamed matched samples although this did not reach statistical significance (422.2 vs. 102.2 au, P=0.09). This may be explained by the fact that we only had matched inflamed and non-inflamed samples in only 35% (n=17) of our IBD subjects based on disease extent.

Overall, there was no difference seen in IBD vs. control subjects (301.3 vs. 390 au). This may be due to sampling given that 35% (n=17) of our patients had mild disease and 40% of patients had moderate disease with the potential for patchy disease distribution.

To our knowledge the role of H6PDH, a key cofactor required for 11 $\beta$ -HSD 1 activity has not been examined in IBD patients to date. This study showed no significant differences in levels of H6PDH amongst IBD patients compared with controls. This is not entirely unexpected given that levels of 11 $\beta$ -HSD 1 were not significantly different across the groups. Previous studies have implicated alterations in GR $\alpha$  expression in steroid



resistance<sup>133</sup>, however we did not observe any significant differences in GR $\alpha$  expression in our IBD group overall. Further work will be required to compare samples from steroid responsive and resistant groups, respectively.

## **Conclusion 5.6**

Overall, our data confirm down regulation of 11 $\beta$ -HSD2 gene expression in patients with IBD and this may be a factor involved in regulating colonic GC metabolism with an increased 11 $\beta$ -HSD 1:2 ratio correlating with inflammatory activity in IBD patients. In addition, our study highlights a possible role of cytokines as potential regulators of this pathway in subjects with IBD.

Dysregulation of this pathway could potentially explain exogenous glucocorticoid resistance in clinical practice and further prospective work is warranted on identification of an at-risk phenotype based on 11 $\beta$ -HSD2 levels. The decrease in 11 $\beta$ -HSD2 expression in inflamed colonic tissue in IBD patients may be a protective mechanism as a decrease in 11 $\beta$ -HSD2 activity leads to a decrease in deactivation of cortisol to cortisone with resultant increased tissue cortisol levels which act as an anti-inflammatory agent. An interesting area which needs further work is whether there are differences in the ability of the colon to suppress 11 $\beta$ -HSD2 expression in

patients who are steroid responsive compared to patients who are steroid resistant.

This may also allow for trials of alternative GC treatments, such as dexamethasone, which is not significantly metabolized by the 11 $\beta$ -HSD enzyme pathway.

# **Chapter 6: Comparing 11 $\beta$ -HSD enzyme system amongst GCS responders and non-responders.**

## **6.1 Introduction**

As outlined in previous chapters, steroid responsiveness remains an important therapeutic goal in IBD. However steroid resistance and dependence remains a real concern, resulting in up to 50% of CD and 30% of UC patients requiring surgery throughout their lifetime<sup>146</sup>. Significant variability in individual response rates occur when GCS are administered to IBD patients as well as those with other inflammatory disorders. Ensuring the efficacy and safety of GCS treatment involves the limitation of their use as well as early recognition of GCS failure to avoid complications as well as improve overall patient outcomes. This particularly applies to patients who show no response to GCS but may be subjected to repeated courses and potentially suffer considerable adverse events associated with their use.

Most of the published research relating to steroid responsiveness and resistance in IBD has largely centred around the early identification of predictive clinical and serological markers of resistant cohorts. Previous studies like our own have attempted to identify clinical factors associated with increased rates of steroid resistance. These can include disease subtype,

presence of a fever, persistent stool frequency, blood transfusion requirement. Indeed, our own earlier retrospective study highlighted a high CRP, anaemia, hypoalbuminemia on day 3, as well as severe disease & pancolitis, all predictive of GCS resistance. In addition, further studies describe other biochemical predictors of steroid failure including low haematocrit rates, hypokalaemia, significant thrombocytosis<sup>214</sup>.

The precise mechanisms behind steroid resistance in inflammatory conditions remains unclear, but several have been proposed which are outlined in chapter 3. Studies specific to exploring mechanisms of steroid resistance in IBD are lacking, however. Some small studies have suggested alterations in cytokine profiling to be associated with increased rates of resistance in IBD patients. For example, TNF- $\alpha$  which has been reported to reduce GCS sensitivity in monocytes by downregulation of the glucocorticoid receptor, has been shown to be higher in steroid-resistant UC patients<sup>177,215</sup>. Furthermore, Creed et al. examined a large panel of cytokines and noted their levels of expression correlated with steroid response rates. For example, high IL-10 expression significantly enhanced steroid action, while IL-2 appeared to inhibit steroid response<sup>216</sup>.

Interestingly in 2009, Flood et al. examined response rates to GCS in 28 UC patients and correlated their response rates with levels of GR and NF $\kappa$ B. They noted responders had significantly higher levels of GR in colorectal

mucosa after one week of treatment with GCS compared with non-responders and significantly higher levels of GR were found in responders in remission, compared to levels prior to treatment. They also noted NFκB levels did not differ between the groups at their initial review however increasing levels were noted only in responders as remission was achieved<sup>217</sup>.

The role of the 11β-HSD enzyme system in regulating the tissue specific control of GCS has been outlined in previous chapters and specifically its role in relation to patients with IBD has been outlined in our own study as well as other published works. Given the important role that this enzyme pathway plays in regulating tissue cortisol levels at the level of the GR as well as the interplay of inflammatory cytokines, we hypothesised that alterations in this pathway may exist in GCS resistant IBD groups. We hypothesize that there are alterations in the local levels of 11 β-HSD enzyme system in colonic tissue of patients with steroid resistant IBD.

## **6.2 Aims and objectives:**

- To prospectively report on rates of GCS resistance in a tertiary IBD centre
- To compare levels of key regulators of the 11 β-HSD enzyme system in both steroid resistant and responder cohorts

## **Study Endpoints:**

- 11 $\beta$ HSD1 colonic expression expressed in arbitrary units (au) in GCS responders and non-responders.
- 11 $\beta$ HSD2 colonic expression expressed in au in GCS responders and non-responders.
- H6PDH expression expressed in au in GCS responders and non-responders.
- GR  $\alpha$  expression expressed in au in GCS responders and non-responders.
- Cytokine expression expressed in au in GCS responders and non-responders including TNF  $\alpha$ , IL-1 $\beta$ , Interleukin-6 and Rela (subunit for Nuclear Factor Kappa B (NFKB))

## **6.3 Materials and Methods**

Ethical approval was sought from the Joint Research ethics committee of Trinity College Dublin, Tallaght University Hospital and St. James Hospital to cover all hospital sites in the study. Patients over the age of 18 years who had presented with suspected active colitis in the Inflammatory Bowel Disease clinic requiring endoscopic assessment were invited to participate in the study. Participants were excluded if they fulfilled any of the following

criteria that could impact their ability to give informed consent or interfere with the study results:

- (a) Are less than 18 years old or greater than 80 years old.
- (b) Are unable to give informed consent.
- (c) Have taken steroids in the last 6 weeks via any route (oral, inhaled etc) in advance of colonoscopy.
- (d) Were pregnant or breastfeeding.
- (e) Have a significant co-existing illness.
- (f) Coagulopathy or any contraindication to undergo a colonoscopy.
- (g) Have an underlying disorder which may affect endogenous levels of glucocorticoids including Cushing's syndrome, Cushing's disease and Conn's syndrome.

Informed written consent was obtained from all participants. A serum blood test was taken on the day of the colonoscopy for levels of C-reactive protein on all patients. Clinical assessments using a Harvey-Bradshaw Index and Mayo score were also performed. Baseline characteristics were also noted including disease subtype, duration, extent, severity, concomitant therapies, age and gender. Disease severity was determined using mayo score and HBI scores where applicable.

The colonoscopies were performed as per standard practice and as well as standard biopsies, two additional biopsies were obtained from inflamed colonic mucosa. The additional study biopsies were transferred immediately into a tissue storage reagent RNAlater and stored at -80°C. They were later analysed in batch, using Quantitative real time RT-PCR (Taqman) and commercially available probes using 18 s as a reference gene.

Severity of endoscopic inflammation was recorded using endoscopic mayo score and CDEIS scores where applicable. Biopsies were also examined for histological levels of inflammation by a consultant histopathologist as per standard practice.

In the event of active inflammation requiring treatment, these patients received a standard tapering dose of 40mg of oral prednisolone as per standard clinical practice. If the patients required hospitalization for intravenous steroids a dose of 100mg of Hydrocortisone four times daily was prescribed and tapered thereafter as per best clinical practice. Inpatients requiring IV steroids were reassessed at day 3 of admission for levels of response. Outpatients treated with steroids were then followed up in the IBD clinic 4 weeks later for re-evaluation of their clinical status and their response to steroid treatment was recorded. Their clinical parameters were documented as before and compared. A 3-point reduction from their index mayo and HBI scores determined a clinical response. A CRP level was also



repeated to objectively support if a patient was deemed a steroid responder or a non-responder. An improvement from baseline CRP was considered supportive of a response to therapy.

Patients not requiring steroid treatment were followed up in a specialist IBD clinic as per standard IBD care and were not included in the final analysis of this study.

Tissue samples from this IBD Biobank were examined and colonic mRNA expression of 11- $\beta$ HSD1 &2, GR  $\alpha$ , H6PD, and cytokines TNF  $\alpha$ , IL-1 $\beta$ , Interleukin-6 and Rela (subunit for Nuclear Factor Kappa B (NF $\kappa$ B)), were quantified by quantified using Real-Time PCR (Taqman) as per the method outlined in chapter 5. Their levels were then compared amongst all groups including those with mild, moderate, and severe disease as well as responders and non-responders.

Results and patient demographics were expressed as a mean and compared between responders and non-responders using the Student t test or Mann-Whitney test as appropriate, with a difference of  $p < 0.05$  considered significant. Results were controlled for disease activity according to clinical (HBI/Mayo score), biochemical (CRP), histological and endoscopic parameters. All analyses were performed using Graphpad version 7 (GraphPad Software, Inc. San Diego, California). Mann-Whitney U test and the Student T test were used where applicable, to compare levels 11 $\beta$ -HSD

1 and 2, GR $\alpha$ , H6PD and those of various cytokines amongst responders and non-responders.

## **6.4 Results**

### **6.4.1 *Population***

To date, 40 IBD patients requiring GCS have been recruited. Overall, 14 patients had CD and 26 UC. In all, 48% were male (n=21) with a median age of 37 years (range 18-73). Amongst the UC patients 42% (n=11) had left sided disease (E2), 39% had pan-colonic disease (E3) (n=10) and 19% (n=5) had distal disease (E1). Amongst CD patients, 14% (n=2) had ileal (L1), 57% (n=8) ileo-colonic (L3) and 28% (n=4) colonic (L2) disease, respectively. Amongst these CD patients, 21 % (n=3) had stricturing disease (B2) and 7% (n=1) had penetrating and perianal disease (B3p). Overall, the mean disease duration was 7 years (range 1-14 years). In terms on concomitant therapies, 45% (n=18) were on a 5 ASA, 43% (n=17) an immunomodulator and 37% (n=15) were on a biologic agent at the time of initial review.

The biologics in use included adalimumab (n=8), infliximab (n=5), vedolizumab (n=1) and ustekinumab (n=1). In addition, 23% (n=9) were on a combination of a biologic agent and an immunomodulator.

The median HBI score for CD patients was 9 (range 7-16) and the median mayo score for UC was 7 (range 6-12). Based on clinical scores, the majority, 83% (n=33) had moderate disease, 12% severe (n=5) and 5% (=2) had mild disease. The median CRP for the overall group was 41mg/l (range 0-156mg/l). At the time of colonoscopy 20% (n=8) were deemed to have severe, 70% (n=28) moderate and 10% (n=4) mild disease activity endoscopically. Overall, 18% (n=7) of patients required hospitalisation for IV Hydrocortisone whilst the remaining patients were prescribed a course of systemic oral prednisolone with a tapering dose of 5 mg weekly.

Amongst the hospitalised patients, the majority, 86% (n=6) had UC. The median admission Mayo Score for hospitalised patients was 10 (range 6-12) and the single CD patient had a HBI score of 10. Patients treated with oral prednisolone had an initial median HBI and mayo score of 7 (6-12) and 8 (6-12) respectively. Overall patients managed in the outpatients had a statistically lower CRP (14 mg/l vs 45 mg/l) then those requiring hospitalisation,  $p \leq 0.0066$ , 95% CI 9.23 to 53.18.

**Table 6.1 Study Population**

|                              |                    |
|------------------------------|--------------------|
| Total No. of Participants    | N=40               |
| % UC                         | 65% (n=26)         |
| Median Age                   | 37 yrs (18-73)     |
| % Males                      | 48% (n=21)         |
| Median CRP                   | 37mg/l (0-131mg/l) |
| Median HBI                   | 9 (7-16)           |
| Median Mayo score            | 7 (6-12)           |
| Median Disease Duration      | 7 (1-14yrs)        |
| <u>Disease Severity</u>      |                    |
| Mild (%)                     | 5% (n=2)           |
| Moderate                     | 83% (n=33)         |
| Severe                       | 12% (n=5)          |
| <u>Disease Extent</u>        |                    |
| E1                           | 12% (n=5)          |
| E2                           | 28% (n=11)         |
| E3                           | 25% (n=10)         |
| L1                           | 5% (n=2)           |
| L2                           | 10% (n=4)          |
| L3                           | 20% (n=8)          |
| <u>Maintenance Treatment</u> |                    |
| 5ASA                         | 45% (n=18)         |
| Immunomodulator              | 43% (n=17)         |
| Biologic                     | 37% (n=15)         |

### 6.4.2 Responders and Non-responders

Overall, amongst the group steroid response rate was 78% (n=31).

Demographic data was similar across both groups. Amongst non-responders

66% (n=6) had UC compared with 64% (n=20) of non-responders. **Table:**

**6.2** The median CRP on day 3 for hospitalised patients and week 4 outpatients appeared predictive of response amongst responders and non-responders (30 mg/l vs. 63 mg/l,  $P \leq 0.04$ , 95% CI 0.79 to 67.37). Non-responders understandably appeared to have a higher HBI and Mayo score

compared with responders at the time of re-review, (UC median 10 vs. 5  $p \leq 0.002$ , CD 12 vs. 5,  $p \leq 0.03$ ). Patients with more severe disease endoscopically also appeared to have higher rates of non-response, 56% (n=5) vs. 10% (n=3),  $p \leq 0.001$ , 95% CI 0.18-0.73. More responders were taking an immunomodulator (predominantly azathioprine) compared with non-responders, 45% (n=14) vs. 33% (n=3), however this did not reach statistical significance,  $p=0.5$ . Biologic use appeared to be highest amongst non-responders 56% (n=5) compared with responders 25% (n=10), however this too did not reach statistical significance ( $p=0.2$ ).

**Table 6.2 Baseline characteristics of responders and non-responders**

| <b>Total No of Pts (n=40)</b>  | <b>Responders (n=31)</b> | <b>Non-Responders (N=9)</b> | <b>P value</b> |
|--------------------------------|--------------------------|-----------------------------|----------------|
| <b>% UC</b>                    | 64% (n=20)               | 66% (n=6)                   | 0.2            |
| <b>Median Age</b>              | 48yrs                    | 39yrs                       | 0.2            |
| <b>Median Disease Duration</b> | 12yrs                    | 9yrs                        | 0.4            |
| <b>Median CRP</b>              | 30mg/l                   | 63mg/l                      | 0.04           |
| <b>Median HBI</b>              | 5                        | 12                          | 0.0002         |
| <b>Median Mayo Score</b>       | 5                        | 10                          | 0.03           |
| <b>% Severe Disease</b>        | 10% (n=3)                | 56% (n=5)                   | 0.002          |
| <b>Immunomodulator Use</b>     | 45% (n=14)               | 33% (n=3)                   | 0.5            |
| <b>Biologic Use</b>            | 25% (n=10)               | 56% (n=5)                   | 0.2            |

### ***6.4.3 11 $\beta$ -HSD1, 11 $\beta$ -HSD2, GR $\alpha$ , H6PDH and cytokine expression***

Overall median levels of 11 $\beta$ -HSD1 were higher amongst responders (171au) compared with non-responders (23au) ( $p \leq 0.05$ ) but levels of 11 $\beta$ -HSD2 did not appear to differ amongst the two groups, (19au vs. 16au,  $p = 0.9$ ). In addition, mean levels of H6PDH also appeared to be higher amongst responders than non-responders, 9.6au vs 1.2 au,  $p \leq 0.02$ . Like our previous data, levels of 11 $\beta$ -HSD1, 11 $\beta$ -HSD2 and H6PDH did not vary according to disease subtype, extent, or disease severity.

Interestingly significantly lower levels of the GR- $\alpha$  were noted amongst non-responders compared with responders, 0.35au vs. 3.45 au,  $p \leq 0.006$ . Although levels of GR- $\alpha$  appeared to be lower amongst resistant patients with more severe disease 0.7 vs 3.8, this did not reach statistical significance,  $p = 0.3$ .

**Table 6.3 Levels of 11 $\beta$ -HSD1, 11 $\beta$ -HSD2, H6PDH and GR- $\alpha$  amongst responders and non-responders**

| <b>Total No. (n=40)</b>          | <b>Responders (n=31)</b> | <b>Non-Responders (n=9)</b> | <b>P value</b> |
|----------------------------------|--------------------------|-----------------------------|----------------|
| <b>11<math>\beta</math>-HSD1</b> | 171 au                   | 23 au                       | 0.05           |
| <b>11<math>\beta</math>-HSD2</b> | 19 au                    | 16 au                       | 0.9            |
| <b>H6PDH</b>                     | 9.6 au                   | 1.2 au                      | 0.002          |
| <b>GR-<math>\alpha</math></b>    | 3.45 au                  | 0.35 au                     | 0.006          |

Overall, all cytokines appeared to be upregulated in response to inflammation but TNF- $\alpha$ , Rela and IL-6 appeared to be statistically more elevated in non-responders compared with responders, **Table 6.4**

**Table 6.4: Mean Relative expression of cytokines overall and according to steroid response.**

| <b>Mean Relative Expression (au)</b> | <b>Responders N=31</b> | <b>Non-Responders N=9</b> | <b>P value</b> |
|--------------------------------------|------------------------|---------------------------|----------------|
| <b>IL-6</b>                          | 15.2.                  | 28                        | 0.01           |
| <b>TNF-<math>\alpha</math></b>       | 6.0                    | 11                        | 0.02           |
| <b>IL-1Beta</b>                      | 13.1                   | 15.2                      | 0.1            |
| <b>Rela (NFKB)</b>                   | 3.1                    | 8.2                       | 0.04           |

## 6.5 Discussion

To our knowledge this is the first study to examine the role of the 11 $\beta$ -HSD enzyme system and tissue cortisol metabolism amongst GCS responders and non-responders. In line with previous studies , the overall GCS resistance rate amongst this cohort was 22%<sup>130,218</sup>. Like our own earlier study and previous published works, disease severity (56% vs. 10%) and persistently raised CRP (63mg/l vs. 30mg/l) appeared predictive of response. Immunomodulator use again demonstrated a trend towards improved steroid response rate but this did not quite reach statistical significance. Trends suggested use of a biologic agent was associated with increased rates of non-response (56% vs. 25%). This is perhaps a surrogate marker of more severe disease however, as amongst the 5 non-responders taking a biologic agent, 60% (n=3) had severe disease compared with 20% (n=2) of non-responders taking a biologic agent with severe disease.

In general, during an inflammatory state, upregulation of 11 $\beta$ -HSD1 and downregulation of 11 $\beta$ -HSD2 at inflamed tissue sites is considered secondary to the local release of the pro-inflammatory cytokines. Changes in these co-enzymes during inflammation is felt to maximise levels of endogenous cortisol to downregulate the overall inflammatory response.



In this study however, 11 $\beta$ -HSD1 appeared to be lower in patients with resistant disease (23au vs 171au,  $p \leq 0.05$ ) suggesting a potential breakdown in cortisol upregulation in resistant cohorts. Previous studies have suggested that potential inhibitions or deficiencies in 11 $\beta$ -HSD1 could attenuate local glucocorticoid production and thus worsen acute inflammation<sup>219</sup>. This is indeed what is seen in previous 11 $\beta$ -HSD1-deficient mouse studies, increased severe LPS-induced (lipopolysaccharide ) endotoxemia, an earlier onset of inflammation in the serum transfer model of inflammatory arthritis and more inflammatory cells in these deficient mice<sup>220,221</sup>. 11 $\beta$ -HSD2 levels did not appear to differ across the two groups perhaps as all groups had active inflammatory disease and reduced levels 11 $\beta$ -HSD2 expression would result in increased cortisol availability.

Given the interplay between 11 $\beta$ -HSD1 and H6PDH, it's not surprising that there was a significant down regulation in H6PDH in non-responders (1.2au) compared with responders (9.6au), in response to reduced levels of 11 $\beta$ -HSD1 ( $p \leq 0.02$ ). Exactly why deficient levels of 11 $\beta$ -HSD1 happen in these cohorts needs further work and potential mechanisms to overcome this pathway needs exploration and may be useful in overcoming resistance.

The effects of glucocorticoids are mediated by the Glucocorticoid Receptor, which is a ligand regulated nuclear receptor<sup>157</sup>. Previous studies suggest that the number of GR in a cell positively correlate with the magnitude of

response to GCS within a cell<sup>222-224</sup>. Limited studies exist exploring the role of the GR in GCS resistance amongst IBD cohorts. Flood et al. reported that higher levels of GR were noted in colonic mucosa of GCS responders compared with non-responders<sup>217</sup>. Similarly, this study also demonstrates significantly reduced GR levels amongst both UC and CD non-responders, 0.35au vs. 3.45 au,  $p \leq 0.006$ . It has been hypothesised that the lack of response to GCS in these patients may be due to reduced metabolic levels of GR or due to a defective up-regulation of GR expression in effector cells<sup>217</sup>. Potentially increased levels of GR may induce treatment efficacy and improve overall inflammatory responses to GR. These patients may benefit from alternative pharmacological approach which may be less dependent on the GR to achieve GCS response or potential mechanisms of increasing basal levels of GR should be explored.

In addition, rates of inflammatory cytokine expression appeared higher in resistant cohorts compared with responders with increased levels of IL-6 (28au vs 15.2), TNF-  $\alpha$  and Rela(NFkB) (8.2au vs 3.1au). Franchimont et al. previously noted that increased rates of TNF-  $\alpha$  reduced response rates to dexamethasone<sup>215</sup>. Whether increased cytokine expression is a potential marker for more aggressive disease or whether it plays a direct role in interrupting GCS response rate remains unclear and further larger studies are

warranted to examine their interplay with the GR and the 11 $\beta$ -HSD enzyme system and overall response to GCS.

## **6.6 Conclusions:**

Overall, our data suggests that the 11 $\beta$ -HSD enzyme system would appear to play a role in tissue GCS resistance amongst IBD patients with reduced levels of 11 $\beta$ -HSD1, H6PDH and GR-  $\alpha$  amongst GCS non-responders. This appears to be accompanied by an upregulation of inflammatory cytokines in these resistant cohorts. Further work on means of GR upregulation and increased 11 $\beta$ -HSD1 expression may provide a potential therapeutic pathway to overcome GCS resistance.

## **Chapter 7: Conclusions and Future Directions**

Glucocorticoids played an integral role in the management of multiple inflammatory conditions and remain a vital therapy in managing patients with acute presentations of IBD. This dissertation has aimed to provide a better understanding of steroid metabolism in IBD patients overall and in particular, to contribute to a better understanding of steroid resistance amongst IBD patients. Steroid resistance remains significant at 22% and we have accurately identified and reaffirmed clinical predictors of GCS failure including an elevated CRP, significant anaemia, hypoalbuminaemia, severe endoscopic disease and extensive disease. Early identification of these critical clinical indicators of GCS failure could potentially avoid prolonged GCS exposure and allow for earlier introduction of rescue therapies and improve overall outcomes for the patients. Clinicians should be aware of these early predictors of GCS failure in an effort to optimise outcomes for patients.

The potential mechanisms behind GCS resistance in the literature to date have been explored and the relevance of published exploratory data relating to specific GCS resistance amongst IBD subjects have been outlined. Alterations relative to tissue cortisol metabolism including alterations in the

GR, reduced affinity of the ligand for the GR, reduced affinity of the GR to bind DNA and altered expression of transcription factors and/ or cytokines have all been examined in detail. In addition, the literature relating to the 11 $\beta$ -HSD enzyme system and IBD has been reviewed and attempts have been made to build upon the understanding of the role and regulation of the 11 $\beta$ -HSD enzyme system and cortisol metabolism amongst IBD subjects compared with healthy controls.

Our initial data concluded that 11 $\beta$ -HSD2 gene expression was downregulated in patients with IBD with an increased 11 $\beta$ -HSD 1:2 ratio correlating with inflammatory activity in IBD patients. In addition, our initial study hypothesised a potential role of cytokines as key regulators of this pathway in subjects with IBD. We hypothesised that potential dysregulation in this pathway may contribute to our understanding of GCS resistance amongst IBD patients.

We later went on to examine the role 11 $\beta$ -HSD enzyme system specifically amongst steroid responders and non-responders. We identified that resistant subjects demonstrated reduced levels of 11 $\beta$ -HSD1, H6PDH and GR with an apparent upregulation of inflammatory cytokines compared with responders. Our results suggest a potential role for alternative GCS use amongst IBD patients such as dexamethasone whose metabolism is less influenced by the 11 $\beta$ -HSD enzyme system. From a clinical translational

point of view, GCS selection may have a role in a effort to reduce rates of resistance.

Further work on mechanisms of GR upregulation and increased 11 $\beta$ -HSD1 expression may provide a potential therapeutic pathway to overcome GCS resistance and potentially reduce rates of colectomy and alternative surgery in patients with IBD.

Alternatively, basal measurement of 11 $\beta$ -HSD1, GR and cytokines amongst IBD subjects could enable earlier identification of patients likely to fail GCS, potentially avoiding cumulative serious side-effects for patients and allow for earlier escalation of rescue therapies.

## References:

1. Colombel JF, Reinisch W, Mantzaris GJ, et al. Randomised clinical trial: deep remission in biologic and immunomodulator naïve patients with Crohn's disease - a SONIC post hoc analysis. *Aliment Pharmacol Ther.* 2015;41(8):734-746.
2. Panaccione R. Mechanisms of inflammatory bowel disease. *Gastroenterol Hepatol (N Y).* 2013;9(8):529-532.
3. Levine JS, Burakoff R. Extraintestinal manifestations of inflammatory bowel disease. *Gastroenterol Hepatol (N Y).* 2011;7(4):235-241.
4. Ng SC, Shi HY, Hamidi N, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet.* 2018;390(10114):2769-2778.
5. The global, regional, and national burden of inflammatory bowel disease in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol.* 2020;5(1):17-30.
6. Coughlan A, Wylde R, Lafferty L, et al. A rising incidence and poorer male outcomes characterise early onset paediatric inflammatory bowel disease. *Aliment Pharmacol Ther.* 2017;45(12):1534-1541.
7. Satsangi J, JD, Rosenberg WM, Bell JI. Genetics of inflammatory bowel disease. *Gut.* 1994;696-700.
8. Jostins L, Ripke S, Weersma RK, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature.* 2012;491(7422):119-124.
9. Baumgart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. *Lancet.* 2007;369(9573):1627-1640.
10. Guariso G, Gasparetto M, Visonà Dalla Pozza L, et al. Inflammatory bowel disease developing in paediatric and adult age. *J Pediatr Gastroenterol Nutr.* 2010;51(6):698-707.
11. Laass MW, Roggenbuck D, Conrad K. Diagnosis and classification of Crohn's disease. *Autoimmun Rev.* 2014;13(4-5):467-471.
12. Sandborn WJ, Feagan BG, Fedorak RN, et al. A randomized trial of Ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with moderate-to-severe Crohn's disease. *Gastroenterology.* 2008;135(4):1130-1141.
13. Williams IR. Chemokine receptors and leukocyte trafficking in the mucosal immune system. *Immunologic Research.* 2004;29(1):283-291.
14. Molodecky NA, Kaplan GG. Environmental risk factors for inflammatory bowel disease. *Gastroenterol Hepatol (N Y).* 2010;6(5):339-346.
15. Boyapati R, Satsangi J, Ho G-T. Pathogenesis of Crohn's disease. *F1000Prime Rep.* 2015;7:44-44.
16. Morson BC. Pathology of Crohn's disease. *Ann R Coll Surg Engl.* 1990;72(3):150-151.

17. Lamb CA, Kennedy NA, Raine T, et al. British Society of Gastroenterology consensus guidelines on the management of inflammatory bowel disease in adults. *Gut*. 2019;68(Suppl 3):s1-s106.
18. Höie O, Wolters F, Riis L, et al. Ulcerative colitis: patient characteristics may predict 10-yr disease recurrence in a European-wide population-based cohort. *Am J Gastroenterol*. 2007;102(8):1692-1701.
19. Langholz E, Munkholm P, Davidsen M, et al. Course of ulcerative colitis: analysis of changes in disease activity over years. *Gastroenterology*. 1994;107(1):3-11.
20. Satsangi J, Silverberg MS, Vermeire S, Colombel JF. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. *Gut*. 2006;55(6):749-753.
21. Levine A, Griffiths A, Markowitz J, et al. Pediatric modification of the Montreal classification for inflammatory bowel disease: the Paris classification. *Inflamm Bowel Dis*. 2011;17(6):1314-1321.
22. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. *N Engl J Med*. 1987;317(26):1625-1629.
23. Lewis JD, Chuai S, Nessel L, Lichtenstein GR, et al. Use of the noninvasive components of the Mayo score to assess clinical response in ulcerative colitis. *Inflammatory bowel diseases*. 2008;14(12):1660-1666.
24. Harvey RF, Bradshaw JM. A simple index of Crohn's-disease activity. *Lancet*. 1980;1(8167):514.
25. Mary JY, Modigliani R. Development and validation of an endoscopic index of the severity for Crohn's disease: a prospective multicentre study. Groupe d'Etudes Thérapeutiques des Affections Inflammatoires du Tube Digestif (GETAID). *Gut*. 1989;30(7):983-989.
26. Daperno M, D'Haens G, Van Assche G, et al. Development and validation of a new, simplified endoscopic activity score for Crohn's disease: the SES-CD. *Gastrointest Endosc*. 2004;60(4):505-512.
27. Solem CA, Loftus EV, Jr., Tremaine WJ, et al. Correlation of C-reactive protein with clinical, endoscopic, histologic, and radiographic activity in inflammatory bowel disease. *Inflamm Bowel Dis*. 2005;11(8):707-712.
28. Brandse JF, Mathôt RA, van der Kleij D, et al. Pharmacokinetic Features and Presence of Antidrug Antibodies Associate With Response to Infliximab Induction Therapy in Patients With Moderate to Severe Ulcerative Colitis. *Clin Gastroenterol Hepatol*. 2016;14(2):251-258.e251-252.
29. Menees SB, Powell C, Kurlander J, et al. A meta-analysis of the utility of C-reactive protein, erythrocyte sedimentation rate, fecal calprotectin, and fecal lactoferrin to exclude inflammatory bowel disease in adults with IBS. *Am J Gastroenterol*. 2015;110(3):444-454.
30. Wright EK, Kamm MA, De Cruz P, et al. Measurement of fecal calprotectin improves monitoring and detection of recurrence of Crohn's disease after surgery. *Gastroenterology*. 2015;148(5):938-947.e931.



31. Mowat C, Arnott I, Cahill A, et al. Mercaptopurine versus placebo to prevent recurrence of Crohn's disease after surgical resection (TOPPIC): a multicentre, double-blind, randomised controlled trial. *Lancet Gastroenterol Hepatol*. 2016;1(4):273-282.
32. Cassinotti A, Massari A, Carmagnola S, et al. P473 How to monitor the withdrawal of maintenance treatment with azathioprine in IBD patients with deep remission: results from a prospective study on multiple non invasive tests. *Journal of Crohn's and Colitis*. 2017;11(suppl\_1):S319-S320.
33. Sohrabpour AA, Malekzadeh R, Keshavarzian A. Current therapeutic approaches in inflammatory bowel disease. *Curr Pharm Des*. 2010;16(33):3668-3683.
34. Agrawal M, Colombel JF. Treat-to-Target in Inflammatory Bowel Diseases, What Is the Target and How Do We Treat? *Gastrointest Endosc Clin N Am*. 2019;29(3):421-436.
35. Drescher H, Lissos T, Hajisafari E, et al. Treat-to-Target Approach in Inflammatory Bowel Disease: The Role of Advanced Practice Providers. *The Journal for Nurse Practitioners*. 2019;15(9):676-681.
36. Mowat C, Cole A, Windsor A, et al. Guidelines for the management of inflammatory bowel disease in adults. *Gut*. 2011;60(5):571-607.
37. Ooi CJ, Hilmi I, Banerjee R, et al. Best practices on immunomodulators and biologic agents for ulcerative colitis and Crohn's disease in Asia. *Intest Res*. 2019;17(3):285-310.
38. Fumery M, Singh S, Dulai PS, et al. Natural History of Adult Ulcerative Colitis in Population-based Cohorts: A Systematic Review. *Clin Gastroenterol Hepatol*. 2018;16(3):343-356.e343.
39. Neurath MF, Travis SP. Mucosal healing in inflammatory bowel diseases: a systematic review. *Gut*. 2012;61(11):1619-1635.
40. Christensen B, Rubin DT. Understanding Endoscopic Disease Activity in IBD: How to Incorporate It into Practice. *Curr Gastroenterol Rep*. 2016;18(1):5.
41. Ardizzone S, Cassinotti A, Duca P, et al. Mucosal healing predicts late outcomes after the first course of corticosteroids for newly diagnosed ulcerative colitis. *Clin Gastroenterol Hepatol*. 2011;9(6):483-489.e483.
42. Frøslie KF, Jahnsen J, Moum BA, et al. Mucosal healing in inflammatory bowel disease: results from a Norwegian population-based cohort. *Gastroenterology*. 2007;133(2):412-422.
43. Walsh A, Palmer R, Travis S. Mucosal healing as a target of therapy for colonic inflammatory bowel disease and methods to score disease activity. *Gastrointest Endosc Clin N Am*. 2014;24(3):367-378.
44. Colombel JF, Rutgeerts P, Reinisch W, et al. Early mucosal healing with infliximab is associated with improved long-term clinical outcomes in ulcerative colitis. *Gastroenterology*. 2011;141(4):1194-1201.
45. Jess T, Loftus EV, Jr., Velayos FS, et al. Risk of intestinal cancer in inflammatory bowel disease: a population-based study from olmsted county, Minnesota. *Gastroenterology*. 2006;130(4):1039-1046.

46. Saxena AP, Limdi JK, Farraye FA. Zeroing in on endoscopic and histologic mucosal healing to reduce the risk of colorectal neoplasia in inflammatory bowel disease. *Gastrointest Endosc.* 2017;86(6):1012-1014.
47. Feagan BG, Macdonald JK. Oral 5-aminosalicylic acid for induction of remission in ulcerative colitis. *Cochrane Database Syst Rev.* 2012;10:Cd000543.
48. Ford AC, Kane SV, Khan KJ, et al. Efficacy of 5-aminosalicylates in Crohn's disease: systematic review and meta-analysis. *Am J Gastroenterol.* 2011;106(4):617-629.
49. Wang Y, Parker CE, Bhanji T, et al. Oral 5-aminosalicylic acid for induction of remission in ulcerative colitis. *Cochrane Database Syst Rev.* 2016;4(4):Cd000543.
50. Friedman LS, Richter JM, Kirkham SE, DeMonaco HJ, May RJ. 5-Aminosalicylic acid enemas in refractory distal ulcerative colitis: a randomized, controlled trial. *Am J Gastroenterol.* 1986;81(6):412-418.
51. Akobeng AK, Zhang D, Gordon M, et al. Oral 5-aminosalicylic acid for maintenance of medically-induced remission in Crohn's disease. *Cochrane Database Syst Rev.* 2016;9(9):Cd003715.
52. Hanauer SB, Sandborn WJ, Dallaire C, et al. Delayed-release oral mesalamine 4.8 g/day (800 mg tablets) compared to 2.4 g/day (400 mg tablets) for the treatment of mildly to moderately active ulcerative colitis: The ASCEND I trial. *Can J Gastroenterol.* 2007;21(12):827-834.
53. Hanauer SB, Sandborn WJ, Kornbluth A, et al. Delayed-release oral mesalamine at 4.8 g/day (800 mg tablet) for the treatment of moderately active ulcerative colitis: the ASCEND II trial. *Am J Gastroenterol.* 2005;100(11):2478-2485.
54. Sandborn WJ, Regula J, Feagan BG, et al. Delayed-release oral mesalamine 4.8 g/day (800-mg tablet) is effective for patients with moderately active ulcerative colitis. *Gastroenterology.* 2009;137(6):1934-1943.e1931-1933.
55. Pruitt R, Hanson J, Safdi M, et al. Balsalazide is superior to mesalamine in the time to improvement of signs and symptoms of acute mild-to-moderate ulcerative colitis. *Am J Gastroenterol.* 2002;97(12):3078-3086.
56. Levine DS, Riff DS, Pruitt R, et al. A randomized, double blind, dose-response comparison of balsalazide (6.75 g), balsalazide (2.25 g), and mesalamine (2.4 g) in the treatment of active, mild-to-moderate ulcerative colitis. *Am J Gastroenterol.* 2002;97(6):1398-1407.
57. Lichtenstein GR, Kamm MA, Boddu P, et al. Effect of once- or twice-daily MMX mesalamine (SPD476) for the induction of remission of mild to moderately active ulcerative colitis. *Clin Gastroenterol Hepatol.* 2007;5(1):95-102.
58. Mansfield JC, Giaffer MH, Cann PA, et al. A double-blind comparison of balsalazide, 6.75 g, and sulfasalazine, 3 g, as sole therapy in the management of ulcerative colitis. *Aliment Pharmacol Ther.* 2002;16(1):69-77.
59. Probert CS, Dignass AU, Lindgren S, et al. Combined oral and rectal mesalazine for the treatment of mild-to-moderately active ulcerative colitis: rapid symptom resolution and improvements in quality of life. *J Crohns Colitis.* 2014;8(3):200-207.

60. Lyakhovich A, Gasche C. Systematic review: molecular chemoprevention of colorectal malignancy by mesalazine. *Aliment Pharmacol Ther.* 2010;31(2):202-209.
61. Bonovas S, Fiorino G, Lytras T, et al. Systematic review with meta-analysis: use of 5-aminosalicylates and risk of colorectal neoplasia in patients with inflammatory bowel disease. *Aliment Pharmacol Ther.* 2017;45(9):1179-1192.
62. Fraser AG, Orchard TR, Jewell DP. The efficacy of azathioprine for the treatment of inflammatory bowel disease: a 30 year review. *Gut.* 2002;50(4):485-489.
63. Candy S, Wright J, Gerber M, et al. A controlled double blind study of azathioprine in the management of Crohn's disease. *Gut.* 1995;37(5):674-678.
64. Timmer A, Patton PH, Chande N, McDonald JW, et al. Azathioprine and 6-mercaptopurine for maintenance of remission in ulcerative colitis. *Cochrane Database Syst Rev.* 2016;2016(5):Cd000478.
65. Chande N, Tsoulis DJ, MacDonald JK. Azathioprine or 6-mercaptopurine for induction of remission in Crohn's disease. *Cochrane Database Syst Rev.* 2013(4):Cd000545.
66. Colombel JF, Sandborn WJ, Reinisch W, et al. Infliximab, Azathioprine, or Combination Therapy for Crohn's Disease. *New England Journal of Medicine.* 2010;362(15):1383-1395.
67. Peyrin-Biroulet L, Deltenre P, Ardizzone S, et al. Azathioprine and 6-mercaptopurine for the prevention of postoperative recurrence in Crohn's disease: a meta-analysis. *Am J Gastroenterol.* 2009;104(8):2089-2096.
68. Feagan BG, Rochon J, Fedorak RN, et al. Methotrexate for the treatment of Crohn's disease. The North American Crohn's Study Group Investigators. *N Engl J Med.* 1995;332(5):292-297.
69. Kopylov U, Katsanos KH, van der Woude CJ, et al. European experience with methotrexate treatment in Crohn's disease: a multicenter retrospective analysis. *Eur J Gastroenterol Hepatol.* 2016;28(7):802-806.
70. Pagnini C, Pizarro TT, Cominelli F. Novel Pharmacological Therapy in Inflammatory Bowel Diseases: Beyond Anti-Tumor Necrosis Factor. *Frontiers in Pharmacology.* 2019;10(671).
71. Murch SH, Braegger CP, Walker-Smith JA, et al. Location of tumour necrosis factor alpha by immunohistochemistry in chronic inflammatory bowel disease. *Gut.* 1993;34(12):1705-1709.
72. Braegger CP, Nicholls S, Murch SH, et al. Tumour necrosis factor alpha in stool as a marker of intestinal inflammation. *Lancet.* 1992;339(8785):89-91.
73. Gibson DJ, Heetun ZS, Redmond CE, et al. An accelerated infliximab induction regimen reduces the need for early colectomy in patients with acute severe ulcerative colitis. *Clin Gastroenterol Hepatol.* 2015;13(2):330-335.e331.
74. Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med.* 2005;353(23):2462-2476.
75. Armuzzi A, Pugliese D, Danese S, et al. Infliximab in steroid-dependent ulcerative colitis: effectiveness and predictors of clinical and endoscopic remission. *Inflamm Bowel Dis.* 2013;19(5):1065-1072.

76. Armuzzi A, Pugliese D, Danese S, et al. Long-term combination therapy with infliximab plus azathioprine predicts sustained steroid-free clinical benefit in steroid-dependent ulcerative colitis. *Inflamm Bowel Dis*. 2014;20(8):1368-1374.
77. Hanauer SB, Feagan BG, Lichtenstein GR, et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. *Lancet*. 2002;359(9317):1541-1549.
78. Reinisch W, Sandborn WJ, Hommes DW, et al. Adalimumab for induction of clinical remission in moderately to severely active ulcerative colitis: results of a randomised controlled trial. *Gut*. 2011;60(6):780-787.
79. Sandborn WJ, van Assche G, Reinisch W, et al. Adalimumab induces and maintains clinical remission in patients with moderate-to-severe ulcerative colitis. *Gastroenterology*. 2012;142(2):257-265.e251-253.
80. Adalimumab in the Treatment of Moderate-to-Severe Ulcerative Colitis: ULTRA 2 Trial Results. *Gastroenterol Hepatol (N Y)*. 2013;9(5):317-320.
81. Colombel JF, Sandborn WJ, Ghosh S, et al. Four-year maintenance treatment with adalimumab in patients with moderately to severely active ulcerative colitis: Data from ULTRA 1, 2, and 3. *Am J Gastroenterol*. 2014;109(11):1771-1780.
82. Hanauer SB, Sandborn WJ, Rutgeerts P, et al. Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial. *Gastroenterology*. 2006;130(2):323-333; quiz 591.
83. Colombel JF, Sandborn WJ, Rutgeerts P, et al. Adalimumab for maintenance of clinical response and remission in patients with Crohn's disease: the CHARM trial. *Gastroenterology*. 2007;132(1):52-65.
84. Sandborn WJ, Feagan BG, Marano C, et al. Subcutaneous golimumab induces clinical response and remission in patients with moderate-to-severe ulcerative colitis. *Gastroenterology*. 2014;146(1):85-95; quiz e14-85.
85. Sandborn WJ, Feagan BG, Marano C, et al. Subcutaneous Golimumab Maintains Clinical Response in Patients With Moderate-to-Severe Ulcerative Colitis. *Gastroenterology*. 2014;146(1):96-109.e101.
86. Haines ML, Ajlouni Y, Irving PM, et al. Clinical usefulness of therapeutic drug monitoring of thiopurines in patients with inadequately controlled inflammatory bowel disease. *Inflamm Bowel Dis*. 2011;17(6):1301-1307.
87. Feagan BG, Sandborn WJ, Gasink C, et al. Ustekinumab as Induction and Maintenance Therapy for Crohn's Disease. *N Engl J Med*. 2016;375(20):1946-1960.
88. Sandborn WJ SB, Panaccione R, et al. . Efficacy and safety of ustekinumab as maintenance therapy in ulcerative colitis: Week 44 results from UNIFI. *J Crohns Colitis* 2019;13:S025–026. .
89. Singh H, Grewal N, Arora E, et al. Vedolizumab: A novel anti-integrin drug for treatment of inflammatory bowel disease. *J Nat Sci Biol Med*. 2016;7(1):4-9.
90. Feagan BG, Rutgeerts P, Sands BE, et al. Vedolizumab as induction and maintenance therapy for ulcerative colitis. *N Engl J Med*. 2013;369(8):699-710.
91. Feagan BG, Rubin DT, Danese S, et al. Efficacy of Vedolizumab Induction and Maintenance Therapy in Patients With Ulcerative Colitis, Regardless of Prior Exposure

- to Tumor Necrosis Factor Antagonists. *Clin Gastroenterol Hepatol*. 2017;15(2):229-239.e225.
92. Sandborn WJ, Feagan BG, Rutgeerts P, et al. Vedolizumab as induction and maintenance therapy for Crohn's disease. *N Engl J Med*. 2013;369(8):711-721.
  93. Sands BE, Sandborn WJ, Van Assche G, et al. Vedolizumab as Induction and Maintenance Therapy for Crohn's Disease in Patients Naïve to or Who Have Failed Tumor Necrosis Factor Antagonist Therapy. *Inflamm Bowel Dis*. 2017;23(1):97-106.
  94. Hodge JA, Kawabata TT, Krishnaswami S, et al. The mechanism of action of tofacitinib - an oral Janus kinase inhibitor for the treatment of rheumatoid arthritis. *Clin Exp Rheumatol*. 2016;34(2):318-328.
  95. Sandborn WJ, Su C, Sands BE, et al. Tofacitinib as Induction and Maintenance Therapy for Ulcerative Colitis. *N Engl J Med*. 2017;376(18):1723-1736.
  96. Weisshof R, Aharoni Golan M, Sossenheimer PH, et al. Real-World Experience with Tofacitinib in IBD at a Tertiary Center. *Dig Dis Sci*. 2019;64(7):1945-1951.
  97. Van Assche G, D'Haens G, Noman M, et al. Randomized, double-blind comparison of 4 mg/kg versus 2 mg/kg intravenous cyclosporine in severe ulcerative colitis. *Gastroenterology*. 2003;125(4):1025-1031.
  98. Moskovitz DN, Van Assche G, Maenhout B, et al. Incidence of colectomy during long-term follow-up after cyclosporine-induced remission of severe ulcerative colitis. *Clin Gastroenterol Hepatol*. 2006;4(6):760-765.
  99. Williams JG, Alam MF, Alrubaiy L, et al. Infliximab versus ciclosporin for steroid-resistant acute severe ulcerative colitis (CONSTRUCT): a mixed methods, open-label, pragmatic randomised trial. *Lancet Gastroenterol Hepatol*. 2016;1(1):15-24.
  100. Campbell S, Travis S, Jewell D. Ciclosporin use in acute ulcerative colitis: a long-term experience. *Eur J Gastroenterol Hepatol*. 2005;17(1):79-84.
  101. Sternthal MB, Murphy SJ, George J, et al. Adverse events associated with the use of cyclosporine in patients with inflammatory bowel disease. *Am J Gastroenterol*. 2008;103(4):937-943.
  102. Barrett K, Saxena S, Pollok R. Using corticosteroids appropriately in inflammatory bowel disease: a guide for primary care. *Br J Gen Pract*. 2018;68(675):497-498.
  103. Lennard-Jones JE, Longmore AJ, Newell AC, et al. An assessment of prednisone, salazopyrin, and topical hydrocortisone hemisuccinate used as out-patient treatment for ulcerative colitis. *Gut*. 1960;1(3):217-222.
  104. Truelove SC, Watkinson G, Draper G. Comparison of corticosteroid and sulphasalazine therapy in ulcerative colitis. *Br Med J*. 1962;2(5321):1708-1711.
  105. Dubois-Camacho K, Ottum PA, Franco-Muñoz D, et al. Glucocorticosteroid therapy in inflammatory bowel diseases: From clinical practice to molecular biology. *World J Gastroenterol*. 2017;23(36):6628-6638.
  106. Ford AC, Bernstein CN, Khan KJ, et al. Glucocorticosteroid therapy in inflammatory bowel disease: systematic review and meta-analysis. *Am J Gastroenterol*. 2011;106(4):590-599; quiz 600.

107. Baron JH, Connell AM, Kanaghinis TG, et al. Out-patient treatment of ulcerative colitis. Comparison between three doses of oral prednisone. *Br Med J*. 1962;2(5302):441-443.
108. Sherlock ME, MacDonald JK, Griffiths AM, et al. Oral budesonide for induction of remission in ulcerative colitis. *Cochrane Database Syst Rev*. 2015(10):Cd007698.
109. Kruis W, Neshta V, Pesegova M, et al. Budesonide Suppositories Are Effective and Safe for Treating Acute Ulcerative Proctitis. *Clin Gastroenterol Hepatol*. 2019;17(1):98-106.e104.
110. Bar-Meir S, Chowers Y, Lavy A, et al. Budesonide versus prednisone in the treatment of active Crohn's disease. The Israeli Budesonide Study Group. *Gastroenterology*. 1998;115(4):835-840.
111. Benchimol EI, Seow CH, Steinhardt AH, et al. Traditional corticosteroids for induction of remission in Crohn's disease. *Cochrane Database Syst Rev*. 2008;2008(2):Cd006792.
112. Summers RW, Switz DM, Sessions JT, Jr., et al. National Cooperative Crohn's Disease Study: results of drug treatment. *Gastroenterology*. 1979;77(4 Pt 2):847-869.
113. Malchow H, Ewe K, Brandes JW, et al. European Cooperative Crohn's Disease Study (ECCDS): results of drug treatment. *Gastroenterology*. 1984;86(2):249-266.
114. Truelove SC, Witts LJ. Cortisone in ulcerative colitis; final report on a therapeutic trial. *Br Med J*. 1955;2(4947):1041-1048.
115. Seah D, De Cruz P. Review article: the practical management of acute severe ulcerative colitis. *Aliment Pharmacol Ther*. 2016;43(4):482-513.
116. Turner D, Walsh CM, Steinhardt AH, et al. Response to corticosteroids in severe ulcerative colitis: a systematic review of the literature and a meta-regression. *Clin Gastroenterol Hepatol*. 2007;5(1):103-110.
117. Roberts SE, Williams JG, Yeates D, et al. Mortality in patients with and without colectomy admitted to hospital for ulcerative colitis and Crohn's disease: record linkage studies. *Bmj*. 2007;335(7628):1033.
118. Randall J, Singh B, Warren BF, et al. Delayed surgery for acute severe colitis is associated with increased risk of postoperative complications. *Br J Surg*. 2010;97(3):404-409.
119. Schmidt KJ, Müller N, Dignass A, et al. Long-term Outcomes in Steroid-refractory Ulcerative Colitis Treated with Tacrolimus Alone or in Combination with Purine Analogues. *Journal of Crohn's and Colitis*. 2015;10(1):31-37.
120. Khan N, Abbas A, Williamson A, et al. Prevalence of corticosteroids use and disease course after initial steroid exposure in ulcerative colitis. *Dig Dis Sci*. 2013;58(10):2963-2969.
121. Selinger CP, Parkes GC, Bassi A, et al. A multi-centre audit of excess steroid use in 1176 patients with inflammatory bowel disease. *Aliment Pharmacol Ther*. 2017;46(10):964-973.
122. Lichtiger S, Present DH, Kornbluth A, et al. Cyclosporine in severe ulcerative colitis refractory to steroid therapy. *N Engl J Med*. 1994;330(26):1841-1845.

123. Rice JB, White AG, Scarpati LM, et al. Long-term Systemic Corticosteroid Exposure: A Systematic Literature Review. *Clin Ther.* 2017;39(11):2216-2229.
124. Manson SC, Brown RE, Cerulli A, et al. The cumulative burden of oral corticosteroid side effects and the economic implications of steroid use. *Respir Med.* 2009;103(7):975-994.
125. Travis SP, Farrant JM, Ricketts C, et al. Predicting outcome in severe ulcerative colitis. *Gut.* 1996;38(6):905-910.
126. Ho GT, Mowat C, Goddard CJ, et al. Predicting the outcome of severe ulcerative colitis: development of a novel risk score to aid early selection of patients for second-line medical therapy or surgery. *Aliment Pharmacol Ther.* 2004;19(10):1079-1087.
127. Lindgren SC, Flood LM, Kilander AF, et al. Early predictors of glucocorticosteroid treatment failure in severe and moderately severe attacks of ulcerative colitis. *Eur J Gastroenterol Hepatol.* 1998;10(10):831-835.
128. Gibson DJ, Hartery K, Doherty J, et al. CRP/Albumin Ratio: An Early Predictor of Steroid Responsiveness in Acute Severe Ulcerative Colitis. *J Clin Gastroenterol.* 2018;52(6):e48-e52.
129. Lynch RW, Churchhouse AM, Protheroe A, et al. Predicting outcome in acute severe ulcerative colitis: comparison of the Travis and Ho scores using UK IBD audit data. *Aliment Pharmacol Ther.* 2016;43(11):1132-1141.
130. Faubion WA, Jr., Loftus EV, Jr., Harmsen WS, et al. The natural history of corticosteroid therapy for inflammatory bowel disease: a population-based study. *Gastroenterology.* 2001;121(2):255-260.
131. Munkholm P, Langholz E, Davidsen M, et al. Frequency of glucocorticoid resistance and dependency in Crohn's disease. *Gut.* 1994;35(3):360-362.
132. Kedia S, Ahuja V, Tandon R. Management of acute severe ulcerative colitis. *World J Gastrointest Pathophysiol.* 2014;5(4):579-588.
133. CREED TJ, PROBERT CSJ. Review article: steroid resistance in inflammatory bowel disease – mechanisms and therapeutic strategies. *Alimentary Pharmacology & Therapeutics.* 2007;25(2):111-122.
134. Truelove SC, Willoughby CP, Lee EG, et al. Further experience in the treatment of severe attacks of ulcerative colitis. *Lancet (London, England).* 1978;2(8099):1086-1088.
135. Dignass A, Eliakim R, Magro F, et al. [Second European evidence-based Consensus on the diagnosis and management of ulcerative colitis Part 1: Definitions and diagnosis (Spanish version)]. *Rev Gastroenterol Mex.* 2014;79(4):263-289.
136. Ananthakrishnan AN, McGinley EL, Binion DG, Saeian K. Simple score to identify colectomy risk in ulcerative colitis hospitalizations. *Inflamm Bowel Dis.* 2010;16(9):1532-1540.
137. Kim DH, Cheon JH, Park JJ, et al. Clinical outcomes and predictive factors for response after the first course of corticosteroid therapy in patients with Crohn's disease. *Gut Liver.* 2013;7(1):58-65.

138. CHOW DKL, SUNG JJY, TSOI KKF, et al. Predictors of corticosteroid-dependent and corticosteroid-refractory inflammatory bowel disease: analysis of a Chinese cohort study. *Alimentary Pharmacology & Therapeutics*. 2009;29(8):843-854.
139. Daperno M, Sostegni R, Scaglione N, et al. Outcome of a conservative approach in severe ulcerative colitis. *Dig Liver Dis*. 2004;36(1):21-28.
140. Cacheux W, Seksik P, Lemann M, et al. Predictive factors of response to cyclosporine in steroid-refractory ulcerative colitis. *Am J Gastroenterol*. 2008;103(3):637-642.
141. Monterubbianesi R, Aratari A, Armuzzi A, et al. Infliximab three-dose induction regimen in severe corticosteroid-refractory ulcerative colitis: early and late outcome and predictors of colectomy. *J Crohns Colitis*. 2014;8(8):852-858.
142. Corte C, Fernandopulle N, Catuneanu AM, et al. Association between the ulcerative colitis endoscopic index of severity (UCEIS) and outcomes in acute severe ulcerative colitis. *J Crohns Colitis*. 2015;9(5):376-381.
143. Kaspers GJ, Pieters R, Van Zantwijk CH, et al. Prednisolone resistance in childhood acute lymphoblastic leukemia: vitro-vivo correlations and cross-resistance to other drugs. *Blood*. 1998;92(1):259-266.
144. Boumpas DT, Chrousos GP, Wilder RL, et al. Glucocorticoid therapy for immune-mediated diseases: basic and clinical correlates. *Ann Intern Med*. 1993;119(12):1198-1208.
145. Barnes PJ, Adcock I. Anti-inflammatory actions of steroids: molecular mechanisms. *Trends Pharmacol Sci*. 1993;14(12):436-441.
146. Farrell RJ, Kelleher D. Glucocorticoid resistance in inflammatory bowel disease. *J Endocrinol*. 2003;178(3):339-346.
147. de Vries F, Pouwels S, Lammers JWJ, et al. Use of inhaled and oral glucocorticoids, severity of inflammatory disease and risk of hip/femur fracture: a population-based case-control study. *J Intern Med*. 2007;261(2):170-177.
148. Souverein PC, Berard A, Van Staa TP, et al. Use of oral glucocorticoids and risk of cardiovascular and cerebrovascular disease in a population based case-control study. *Heart*. 2004;90(8):859-865.
149. Vegiopoulos A, Herzig S. Glucocorticoids, metabolism and metabolic diseases. *Mol Cell Endocrinol*. 2007;275(1-2):43-61.
150. Steinhart AH, Ewe K, Griffiths AM, et al. Corticosteroids for maintenance of remission in Crohn's disease. *Cochrane Database Syst Rev*. 2003(4):Cd000301.
151. Reinisch W, Gasché C, Wyatt J, et al. Steroid dependency in Crohn's disease. *Lancet*. 1995;345(8953):859.
152. Corrigan CJ, Brown PH, Barnes NC, et al. Glucocorticoid resistance in chronic asthma. Glucocorticoid pharmacokinetics, glucocorticoid receptor characteristics, and inhibition of peripheral blood T cell proliferation by glucocorticoids in vitro. *Am Rev Respir Dis*. 1991;144(5):1016-1025.
153. Seki M, Ushiyama C, Seta N, et al. Apoptosis of lymphocytes induced by glucocorticoids and relationship to therapeutic efficacy in patients with systemic lupus erythematosus. *Arthritis & Rheumatism*. 1998;41(5):823-830.



154. Cuppen BV, Pardali K, Kraan MC, et al. Polymorphisms in the multidrug-resistance 1 gene related to glucocorticoid response in rheumatoid arthritis treatment. *Rheumatol Int.* 2017;37(4):531-536.
155. Chakravarty BJ. Predictors and the rate of medical treatment failure in ulcerative colitis. *Am J Gastroenterol.* 1993;88(6):852-855.
156. Beato M, Sánchez-Pacheco A. Interaction of steroid hormone receptors with the transcription initiation complex. *Endocr Rev.* 1996;17(6):587-609.
157. Arlt W, Stewart PM. Adrenal corticosteroid biosynthesis, metabolism, and action. *Endocrinol Metab Clin North Am.* 2005;34(2):293-313, viii.
158. Scheinman RI, Cogswell PC, Lofquist AK, et al. Role of transcriptional activation of I kappa B alpha in mediation of immunosuppression by glucocorticoids. *Science.* 1995;270(5234):283-286.
159. Kino T. Stress, Glucocorticoid Hormones and Hippocampal Neural Progenitor Cells: Implications to Mood Disorders. *Frontiers in physiology.* 2015;6:230.
160. IIDA S, GOMI M, MORIWAKI K, et al. Primary Cortisol Resistance Accompanied by a Reduction in Glucocorticoid Receptors in Two Members of the Same Family\*. *The Journal of Clinical Endocrinology & Metabolism.* 1985;60(5):967-971.
161. Lane SJ, Lee TH. Glucocorticoid Receptor Characteristics in Monocytes of Patients with Corticosteroid-Resistant Bronchial Asthma. *American Review of Respiratory Disease.* 1991;143(5\_pt\_1):1020-1024.
162. Lane SJ, Atkinson BA, Swaminathan R, et al. Hypothalamic-pituitary-adrenal axis in corticosteroid-resistant bronchial asthma. *Am J Respir Crit Care Med.* 1996;153(2):557-560.
163. Flood L, Löfberg R, Stierna P, et al. Glucocorticoid receptor mRNA in patients with ulcerative colitis: a study of responders and nonresponders to glucocorticosteroid therapy. *Inflamm Bowel Dis.* 2001;7(3):202-209.
164. Raddatz D, Middel P, Bockemühl M, et al. Glucocorticoid receptor expression in inflammatory bowel disease: evidence for a mucosal down-regulation in steroid-unresponsive ulcerative colitis. *Alimentary Pharmacology & Therapeutics.* 2004;19(1):47-61.
165. Hagendorf A, Koper JW, de Jong FH, et al. Expression of the human glucocorticoid receptor splice variants alpha, beta, and P in peripheral blood mononuclear leukocytes in healthy controls and in patients with hyper- and hypocortisolism. *J Clin Endocrinol Metab.* 2005;90(11):6237-6243.
166. Lewis-Tuffin LJ, Cidlowski JA. The physiology of human glucocorticoid receptor beta (hGRbeta) and glucocorticoid resistance. *Ann N Y Acad Sci.* 2006;1069:1-9.
167. Charmandari E, Chrousos GP, Ichijo T, et al. The human glucocorticoid receptor (hGR) beta isoform suppresses the transcriptional activity of hGRalpha by interfering with formation of active coactivator complexes. *Mol Endocrinol.* 2005;19(1):52-64.
168. Charmandari E, Raji A, Kino T, et al. A Novel Point Mutation in the Ligand-Binding Domain (LBD) of the Human Glucocorticoid Receptor (hGR) Causing Generalized Glucocorticoid Resistance: The Importance of the C Terminus of hGR LBD in Conferring

Transactivational Activity. *The Journal of Clinical Endocrinology & Metabolism*. 2005;90(6):3696-3705.

169. Oakley RH, Sar M, Cidlowski JA. The human glucocorticoid receptor beta isoform. Expression, biochemical properties, and putative function. *J Biol Chem*. 1996;271(16):9550-9559.
170. Oakley RH, Jewell CM, Yudt MR, et al. The dominant negative activity of the human glucocorticoid receptor beta isoform. Specificity and mechanisms of action. *J Biol Chem*. 1999;274(39):27857-27866.
171. Honda M, Orii F, Ayabe T, et al. Expression of glucocorticoid receptor beta in lymphocytes of patients with glucocorticoid-resistant ulcerative colitis. *Gastroenterology*. 2000;118(5):859-866.
172. Cho YJ, Lee KE. Decreased glucocorticoid binding affinity to glucocorticoid receptor is important in the poor response to steroid therapy of older-aged patients with severe bronchial asthma. *Allergy Asthma Proc*. 2003;24(5):353-358.
173. Kam JC, Szeffler SJ, Surs W, et al. Combination IL-2 and IL-4 reduces glucocorticoid receptor-binding affinity and T cell response to glucocorticoids. *J Immunol*. 1993;151(7):3460-3466.
174. Bioque G, Crusius JB, Koutroubakis I, et al. Allelic polymorphism in IL-1 beta and IL-1 receptor antagonist (IL-1Ra) genes in inflammatory bowel disease. *Clin Exp Immunol*. 1995;102(2):379-383.
175. Cantagrel A, Navaux F, Loubet-Lescoulié P, et al. Interleukin-1beta, interleukin-1 receptor antagonist, interleukin-4, and interleukin-10 gene polymorphisms: relationship to occurrence and severity of rheumatoid arthritis. *Arthritis Rheum*. 1999;42(6):1093-1100.
176. Bantel H, Domschke W, Schulze-Osthoff K, et al. Abnormal Activation of Transcription Factor Nf-kB Involved in Steroid Resistance in Chronic Inflammatory Bowel Disease. *Official journal of the American College of Gastroenterology | ACG*. 2000;95(7):1845-1846.
177. Ishiguro Y. Mucosal proinflammatory cytokine production correlates with endoscopic activity of ulcerative colitis. *J Gastroenterol*. 1999;34(1):66-74.
178. Wine E, Mack DR, Hyams J, et al. Interleukin-6 is associated with steroid resistance and reflects disease activity in severe pediatric ulcerative colitis. *J Crohns Colitis*. 2013;7(11):916-922.
179. Santaolalla R, Mañé J, Pedrosa E, et al. Apoptosis resistance of mucosal lymphocytes and IL-10 deficiency in patients with steroid-refractory Crohn's disease. *Inflammatory Bowel Diseases*. 2010;17(7):1490-1500.
180. Hoffmeyer S, Burk O, von Richter O, et al. Functional polymorphisms of the human multidrug-resistance gene: Multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity *in vivo*. *Proceedings of the National Academy of Sciences*. 2000;97(7):3473-3478.
181. Farrell RJ, Menconi MJ, Keates AC, et al. P-Glycoprotein-170 inhibition significantly reduces cortisol and ciclosporin efflux from human intestinal epithelial cells and T lymphocytes. *Alimentary Pharmacology & Therapeutics*. 2002;16(5):1021-1031.

182. Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev.* 2000;21(1):55-89.
183. Oakley RH, Cidlowski JA. The biology of the glucocorticoid receptor: new signaling mechanisms in health and disease. *J Allergy Clin Immunol.* 2013;132(5):1033-1044.
184. Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids--new mechanisms for old drugs. *N Engl J Med.* 2005;353(16):1711-1723.
185. Turnbull AV, Rivier CL. Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. *Physiol Rev.* 1999;79(1):1-71.
186. Smith RE, Maguire JA, Stein-Oakley AN, et al. Localization of 11 beta-hydroxysteroid dehydrogenase type II in human epithelial tissues. *The Journal of Clinical Endocrinology & Metabolism.* 1996;81(9):3244-3248.
187. Whorwood CB, Ricketts ML, Stewart PM. Epithelial cell localization of type 2 11 beta-hydroxysteroid dehydrogenase in rat and human colon. *Endocrinology.* 1994;135(6):2533-2541.
188. Bryndová J, Žbáňková Š, Kment M, et al. Colitis up-regulates local glucocorticoid activation and down-regulates inactivation in colonic tissue. *Scandinavian Journal of Gastroenterology.* 2004;39(6):549-553.
189. Lavery GG, Walker EA, Tiganescu A, et al. Steroid biomarkers and genetic studies reveal inactivating mutations in hexose-6-phosphate dehydrogenase in patients with cortisone reductase deficiency. *The Journal of clinical endocrinology and metabolism.* 2008;93(10):3827-3832.
190. White PC, Mune T, Agarwal AK. 11 beta-Hydroxysteroid dehydrogenase and the syndrome of apparent mineralocorticoid excess. *Endocr Rev.* 1997;18(1):135-156.
191. Mune T, Rogerson FM, Nikkilä H, et al. Human hypertension caused by mutations in the kidney isozyme of 11 beta-hydroxysteroid dehydrogenase. *Nat Genet.* 1995;10(4):394-399.
192. Krozowski Z, Maguire J, Stein-Oakley A, et al. Immunohistochemical localization of the 11 beta-hydroxysteroid dehydrogenase type II enzyme in human kidney and placenta. *The Journal of clinical endocrinology and metabolism.* 1995;80 7:2203-2209.
193. Tomlinson JW, Walker EA, Bujalska IJ, et al. 11beta-hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response. *Endocr Rev.* 2004;25(5):831-866.
194. Frey FJ. Kinetics and dynamics of prednisolone. *Endocr Rev.* 1987;8(4):453-473.
195. Murphy BE. Specificity of human 11 beta-hydroxysteroid dehydrogenase. *J Steroid Biochem.* 1981;14(8):807-809.
196. Ferrari P, Smith RE, Funder JW, et al. Substrate and inhibitor specificity of the cloned human 11 beta-hydroxysteroid dehydrogenase type 2 isoform. *Am J Physiol.* 1996;270(5 Pt 1):E900-904.
197. Diederich S, Eigendorff E, Burkhardt P, et al. 11beta-hydroxysteroid dehydrogenase types 1 and 2: an important pharmacokinetic determinant for the activity of synthetic mineralo- and glucocorticoids. *J Clin Endocrinol Metab.* 2002;87(12):5695-5701.

198. Terao M, Itoi S, Matsumura S, et al. Local Glucocorticoid Activation by 11 $\beta$ -Hydroxysteroid Dehydrogenase 1 in Keratinocytes: The Role in Hapten-Induced Dermatitis. *Am J Pathol*. 2016;186(6):1499-1510.
199. Hardy RS, Filer A, Cooper MS, et al. Differential expression, function and response to inflammatory stimuli of 11 $\beta$ -hydroxysteroid dehydrogenase type 1 in human fibroblasts: a mechanism for tissue-specific regulation of inflammation. *Arthritis Research & Therapy*. 2006;8(4):R108.
200. Orsida BE, Krozowski ZS, Walters EH. Clinical relevance of airway 11beta-hydroxysteroid dehydrogenase type II enzyme in asthma. *Am J Respir Crit Care Med*. 2002;165(7):1010-1014.
201. Hardy R, Rabbitt EH, Filer A, et al. Local and systemic glucocorticoid metabolism in inflammatory arthritis. *Ann Rheum Dis*. 2008;67(9):1204-1210.
202. Lee SH. Mechanisms of Glucocorticoid Action in Chronic Rhinosinusitis. *Allergy Asthma Immunol Res*. 2015;7(6):534-537.
203. Žbáňková Sá, Bryndová J, Leden P, et al. 11 $\beta$ -hydroxysteroid dehydrogenase 1 and 2 expression in colon from patients with ulcerative colitis. *Journal of Gastroenterology and Hepatology*. 2007;22(7):1019-1023.
204. Stegk JP, Ebert B, Martin HJ, et al. Expression profiles of human 11beta-hydroxysteroid dehydrogenases type 1 and type 2 in inflammatory bowel diseases. *Mol Cell Endocrinol*. 2009;301(1-2):104-108.
205. Ahmed A, Schwaderer J, Hantusch A, et al. Intestinal glucocorticoid synthesis enzymes in pediatric inflammatory bowel disease patients. *Genes Immun*. 2019;20(7):566-576.
206. Ergang P, Vytáčková K, Svec J, et al. Upregulation of 11 $\beta$ -hydroxysteroid dehydrogenase 1 in lymphoid organs during inflammation in the rat. *J Steroid Biochem Mol Biol*. 2011;126(1-2):19-25.
207. Baumgart DC, Sandborn WJ. Crohn's disease. *Lancet*. 2012;380(9853):1590-1605.
208. Ordas I, Eckmann L, Talamini M, et al. Ulcerative colitis. *Lancet*. 2012;380(9853):1606-1619.
209. Abraham C, Cho JH. Inflammatory bowel disease. *N Engl J Med*. 2009;361(21):2066-2078.
210. McKay LI, Cidlowski JA. Molecular control of immune/inflammatory responses: interactions between nuclear factor-kappa B and steroid receptor-signaling pathways. *Endocr Rev*. 1999;20(4):435-459.
211. Cooper MS, Bujalska I, Rabbitt E, et al. Modulation of 11beta-hydroxysteroid dehydrogenase isozymes by proinflammatory cytokines in osteoblasts: an autocrine switch from glucocorticoid inactivation to activation. *J Bone Miner Res*. 2001;16(6):1037-1044.
212. Draper N, Stewart PM. 11beta-hydroxysteroid dehydrogenase and the pre-receptor regulation of corticosteroid hormone action. *J Endocrinol*. 2005;186(2):251-271.
213. Takahashi KI, Fukushima K, Sasano H, et al. Type II 11beta-hydroxysteroid dehydrogenase expression in human colonic epithelial cells of inflammatory bowel disease. *Dig Dis Sci*. 1999;44(12):2516-2522.

214. JEMMALI C, Labidi A, Jouini MA, et al. P593 Predictors of steroid-resistance in severe colitis of inflammatory bowel disease. *Journal of Crohn's and Colitis*. 2020;14(Supplement\_1):S494-S495.
215. Franchimont D, Martens H, Hagelstein MT, et al. Tumor necrosis factor alpha decreases, and interleukin-10 increases, the sensitivity of human monocytes to dexamethasone: potential regulation of the glucocorticoid receptor. *J Clin Endocrinol Metab*. 1999;84(8):2834-2839.
216. Creed TJ, Lee RW, Newcomb PV, et al. The effects of cytokines on suppression of lymphocyte proliferation by dexamethasone. *J Immunol*. 2009;183(1):164-171.
217. Flood L, Innala E, Löfberg R, et al. Patients with ulcerative colitis responding to steroid treatment up-regulate glucocorticoid receptor levels in colorectal mucosa. *Journal of Crohn's and Colitis*. 2008;2(2):123-130.
218. Manz M, Vavricka SR, Wanner R, et al. Therapy of steroid-resistant inflammatory bowel disease. *Digestion*. 2012;86 Suppl 1:11-15.
219. Chapman KE, Coutinho AE, Zhang Z, et al. Changing glucocorticoid action: 11 $\beta$ -Hydroxysteroid dehydrogenase type 1 in acute and chronic inflammation. *The Journal of Steroid Biochemistry and Molecular Biology*. 2013;137:82-92.
220. Bertini R, Bianchi M, Ghezzi P. Adrenalectomy sensitizes mice to the lethal effects of interleukin 1 and tumor necrosis factor. *Journal of Experimental Medicine*. 1988;167(5):1708-1712.
221. Bhattacharyya S, Brown DE, Brewer JA, et al. Macrophage glucocorticoid receptors regulate Toll-like receptor 4-mediated inflammatory responses by selective inhibition of p38 MAP kinase. *Blood*. 2007;109(10):4313-4319.
222. Vanderbilt JN, Miesfeld R, Maler BA, et al. Intracellular receptor concentration limits glucocorticoid-dependent enhancer activity. *Mol Endocrinol*. 1987;1(1):68-74.
223. Silva CM, Powell-Oliver FE, Jewell CM, et al. Regulation of the human glucocorticoid receptor by long-term and chronic treatment with glucocorticoid. *Steroids*. 1994;59(7):436-442.
224. Alexandrová M, Mascuchová D, Tatár P. Comparison of the biopotency of corticosterone and dexamethasone acetate in glucocorticoid receptor down regulation in rat liver. *J Steroid Biochem*. 1989;32(4):531-535.