Biomarkers in Inflammatory Bowel Diseases

Nik Sheng Ding • Peter De Cruz
Editors

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Foreword

When I was asked by the Editors of *Biomarkers in Inflammatory Bowel Disease* to write a foreword for their book, my first thought was: in this day and age do we need another book on Inflammatory Bowel Disease (IBD), and will it just stay as a decoration on my shelves for the next years as many others? Then I started to read the different chapters, starting with those of most interest to me, and eventually completed my lecture with a different opinion. So let me tell you why you need to read this book! It is actually meeting the challenge to be of interest for everybody with an interest in IBD and other immune-mediated diseases. Even though the biomarker story is the thread, this book is actually covering almost all aspects of IBD. If you are a clinician, there is a lot to learn here about optimal management of specific challenging conditions from perianal Crohn's disease or post-operative care to acute severe ulcerative colitis and pouchitis. If you are a fellow with possible interest in developing a career in IBD, you should especially read to stimulate your interest in the comprehensive reviews on epidemiology, genetics or nutrition. And there is also a lot to learn for more advanced practitioners in IBD from state-of-the-art chapters on "omes" such as exposome, metabonome and cutting-edge science on big data and systems biology. What makes also this book a great book is that the chapters are well-written by experts in their field and tell a good story. On a more sobering note, I had a good time perusing one of my old IBD textbooks (have still very good ones!) after removing the accumulated dust comparing what was known or suspected 20 years ago with what is described here. My overall conclusion is that the technologies have evolved tremendously but the key questions remain, and it is, for instance, striking that almost no progress has been made regarding the environmental risk factors of IBD or even more strikingly what explains the key macroscopic features of IBD that are under our nose every day in the endoscopy and operating room! Finally I almost forgot to mention another reason of growing importance to me: several studies (not quoted here!) are showing that reading by engaging the brain may keep it active enough to prevent cognitive decline!

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Nik Sheng Ding:
I would first like to thank all the coauthors who have graciously given their time to share their expert knowledge and breathed life into this textbook. Secondly, to the editors, Springer Nature, and Evgenia, thank you for believing in us to bring this book together. I would like to also acknowledge and thank my mentors from St Vincent's Hospital, Melbourne and St Mark's Hospital, London along with students who have taught me so much and continue to inspire me. I am grateful to Dr. Peter De Cruz who has been a dear friend and mentor over many years and has provided such wise council. This book is dedicated to my wife, Natalie, whose love continues to lift me to new heights, and to my daughters, Emily and Grace, who help me realize the power of child-like inquisition and humility.

Peter De Cruz:
I would like to thank all of the contributors for sharing their wealth of knowledge which has made this textbook on biomarkers in IBD so unique. I am grateful for the mentorship that I have received from the world's leading experts in IBD who have shaped my approach to clinical practice and research. Thank you to my coeditor, Dr. Nik Sheng Ding, for inviting me to work on this textbook with him. It has been a great couple of years and wonderful adventure. I would like to thank my wife, Maree, and daughters, Annabel, Maya, and Ella, for cherishing me with unconditional love and affection. I would like to express my sincere thanks to the many people with IBD whose journey through life I have had the privilege to share and who remain the source of my inspiration and passion!

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Introduction

1

James Rickard, Nik Sheng Ding, and Peter De Cruz

Inflammatory bowel disease (IBD), comprised largely of ulcerative colitis (UC) and Crohn's disease (CD), is a complex, polygenic, heterogeneous group of diseases with great variation in phenotypic expression. Due to this variation, opportunities for personalised medicine, also known as 'precision medicine', are afforded at practically every step of IBD management, including diagnosis, risk stratification, drug selection and optimisation and prediction and management of IBD-related complications. Central to translating the promise of personalised medicine into improved IBD management are biomarkers (Fig. 1.1). As measurable or detectable markers of biological processes, biomarkers allow the characterisation and quantification of genetic predisposition, drug metabolism and response, disease activity and adverse drug effect monitoring. Ideally, biomarkers are noninvasive, safe, cost-effective and easily used, important factors for IBD patients where endoscopy and radiological imaging are often relied upon with significant risk, expense and inconvenience. Despite their clear utility, optimally selecting biomarkers from a practically infinite number of possibilities is challenging. Rapidly evolving scientific platforms offer rich opportunities for biomarker discovery, but the associated analysis of vast data readouts can be unwieldly. This introductory section will broadly outline various approaches to biomarker discovery, with a particular view to the translation of such efforts into improved personalised medicine for IBD patients. The structure of the book will also be outlined.

The move towards personalised medicine in IBD is founded upon the premise that this will improve patient outcomes. One of the clearest indications for personalised medicine is to accurately stratify disease activity and avoid the pitfalls of under- or overtreating disease. A personalised treat-to-target approach (T2T), incorporating the targeting of endoscopic, histological and biochemical remission, is now seen to be superior to symptom-based reactive care geared towards clinical remission [1]. Further, the traditional 'step-up' approach to care may be inappropriate in patients at high risk of aggressive disease and complications, whereas an early 'top-down' approach may temper the natural history of their disease. Conversely, in patients predicted to have mild disease, a 'top-down' strategy may result in unacceptable drug toxicity for little or no additional benefit. Disease prognostication to optimise treatment is important

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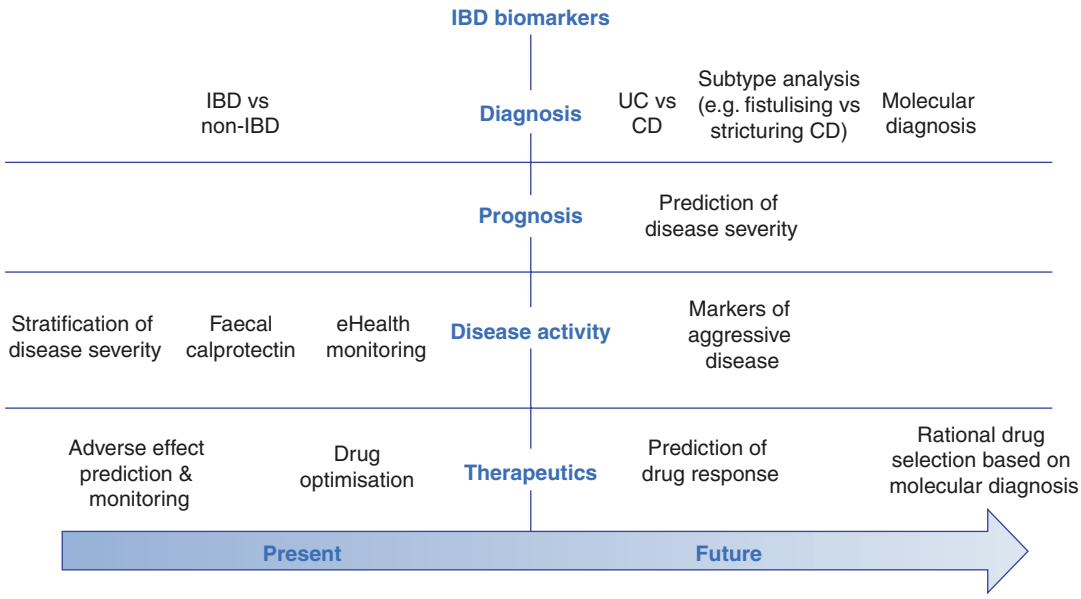

Fig. 1.1 Examples of presently used IBD biomarkers in key domains, shown alongside possible areas for future biomarker development. There is still considerable refinement and development needed in many areas where biomarkers are currently utilised

not only at diagnosis but on an ongoing basis, particularly following key events such as disease flares, phenotypic changes such as altered disease distribution or surgery. Indeed, intensified monitoring schedules in high-risk patients reduce the risk of recurrence in CD patients' postsurgical resection [2]. Personalised medicine is the standard of care for thiopurine use, where thiopurine methyltransferase (TPMT) genotyping allows for identification of patients at risk of bone marrow toxicity from decreased TPMT activity [3]. Thiopurine dose titration is based on body weight and thiopurine metabolite testing, further highlighting the utility of personalised medicine. A further example is provided with tumour necrosis factor inhibitor (TNFi) therapy, also a mainstay of moderate to severe IBD management, where drug levels and immunogenicity can be assessed to facilitate dose optimisation [4].

An inappropriate and exaggerated pro-inflammatory immune response to the commensal intestinal microbiota appears to drive IBD, and current trends indicate the future of personalised medicine for IBD patients may centre on manipulation of the microbiome in addition to the host immune response [5]. Indeed, microbiome manipulation, such as with dietary measures, supplementation or faecal transplantation, may ultimately be tailored on an individual basis. Rationally targeting immunological pathways in IBD has already proven successful, such as with biologics directed against the interleukin (IL)-23 driven T_H17 pathway implicated in both UC and CD by genome-wide association studies (GWAS) [6]. However, the practical ability to target immunological pathways on a more individual level is still lacking. Despite the marked success of biologics targeting both the TNF and IL-23 pathways in IBD, there is substantial variability in response to these agents. This may be due in part to differing levels of activation of these immunological pathways in IBD patients. GWAS have revealed incredible complexity in the genetic architecture of both UC and CD, with in excess

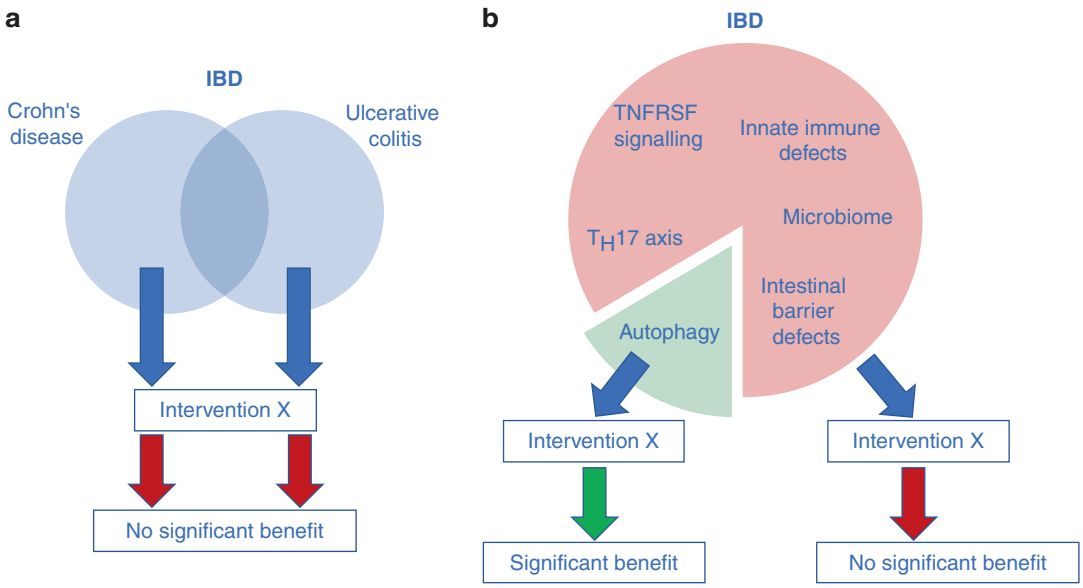

Fig. 1.2 Using biomarker-defined IBD subpopulations to more effectively research and implement therapeutic interventions. (**a**) UC and CD are typically viewed as two conditions, albeit with significant overlap. This leads to the testing or treatment of heterogeneous patient populations which can mask the effects of a given intervention of only a small subgroup benefits; (**b**) testing interventions in discrete biomarker-defined IBD subpopulations are more likely to show such a benefit. Note the proportion represented in the figure is not to scale

of 200 implicated genes [7]. This suggests that molecular phenotyping of IBD patients may lead to personalised rationalisation of therapy with greater success than with broad targeting of CD and UC cohorts (Fig. 1.2). Systems biology approaches indicate that the effects of many gene variants or mutation converge on common immunological pathways, so the challenge will likely be predicting downstream effects of a patient's genotype rather than developing an exhaustive array of therapeutics [7]. The future of personalised medicine in IBD may be akin to personalised cancer therapy, where molecular cancer characterisation to guide therapy is proving a quantum advance over anatomical-based therapy.

If personalised medicine, arguably the future of IBD care, is only as powerful as the biomarkers at its disposal, then a key question is how to optimise biomarker discovery. Publically funded, small-group, hypothesis-driven research conducted in academia has historically been the main force in medical discovery. However, large-scale non-hypothesis-driven, 'goal-oriented' research (also termed 'discovery science') has been a dramatic deviation from this model, sometimes yielding spectacular results with far-reaching utility, such as with the complete sequencing of the human genome [8, 9]. Amidst increasing pressure on the healthcare dollar, the relative yields of hypothesis-driven versus goal-oriented research become an increasingly important issue. A major benefit of hypothesis-driven research is the promotion of creativity in experimental design and interpretation, based on a careful critique of available evidence. It allows important knowledge gaps to be targeted and for efforts to be streamlined. Yet, in IBD research, considerable insight has also been gained from the application of new platform technologies derived from goal-oriented research such as with global microbiome profiling using DNA fingerprinting techniques or GWAS. This has greatly informed

on the complexity and individual variation of the microbiome and of a significant shared genetic underpinning of both CD and UC. However, the acquisition of such data is costly and its output sometimes unwieldy. Arguably, the key to optimising IBD biomarker discovery will be to effectively integrate goal-oriented findings with hypothesis-driven research. Goal-oriented research should be seen as an enabler and facilitator of hypotheses generation, providing a base level of data and technology that can be used both to generate targeted hypotheses and as a vehicle for experiments [8, 10, 11]. Global microbiome profiling has, for instance, greatly enhanced our ability to interrogate the role of the microbiome in IBD. Similarly, GWAS have paved the way for the functional characterisation of genes implicated in IBD, drawing upon and advancing our current understanding of the relevant biological pathways. Without the biological context etched out using hypothesis-driven research, the impact of 'big data' such as GWAS is greatly diminished. So, rather than seeing hypothesis-driven and goal-oriented approaches as dichotomous entities vying for the research dollar, the real challenge probably lies in achieving the most synergistic balance of the two [8].

Effective integration of hypothesis-driven and goal-oriented research is dependent on multiple factors including the structure and culture of research centres and researcher access to discovery platforms and expertise. Geographically positioning smaller research groups, including both clinicians and scientists, with easy access to discovery science platforms, projects and expertise, such as with research precincts or 'hubs', is one way to synergise the different approaches. This promotes not only the translation of discovery science [8] but also the refinement of the platforms for new or more specific uses as needed by smaller hypothesis-driven research groups. This feedback may ultimately be the catalyst for developing new platform technologies (Fig. 1.3). The explosion of basic science that has fuelled the development of powerful discovery platforms has forced a scientific language that is increasingly subspecialised and divergent with that of clinical medicine, often driving a wedge between discoveries at the bench and the patients they are intended to benefit. Open cross-discipline communication is needed to overcome this, and this too would be facilitated by collaborative research hubs and potentially with funding schemes that incentivise relevant multidisciplinary collaboration.

With exponentially more efficient technology, time and cost pose less of a barrier than ever to accessing big data. Next-generation sequencing illustrates this, with whole genome sequencing now available for approximately $1000 [12]. Yet, if biomarker discovery is akin to 'finding needles in a haystack', big data in itself risks merely creating a larger haystack with more needles. Improvements in analytical methods used to decipher big data will facilitate 'needle identification' and biomarker discovery. Recent elegant work by Peters et al. [7] that attempts to delineate the role of GWAS identified IBD susceptibility loci exemplifies exactly this. By using predictive modelling founded upon functional genomics to compare three distinct IBD subpopulations, including an advanced disease cohort, untreated paediatric and TNFi refractory patients, 'key driver genes' predicted to have the greatest influence on various inflammatory pathways were identified and validated. If we are to make sense of big data, then work such as this that moves to create a deep and rich blueprint of the biological processes underlying IBD will be invaluable. Ultimately, the more we understand the underlying biology, the more targeted and less serendipitous biomarker discovery will be. As research increasingly moves towards understanding the coordinated and dynamic nature of inflammatory pathways rather than single-gene or protein-focused investigations, our ability to predict biomarker utility in wider ranging real-world scenarios, and stratify their use accordingly, will be enhanced. Undoubtedly, systems biology and advanced informatics are pivotal to translating

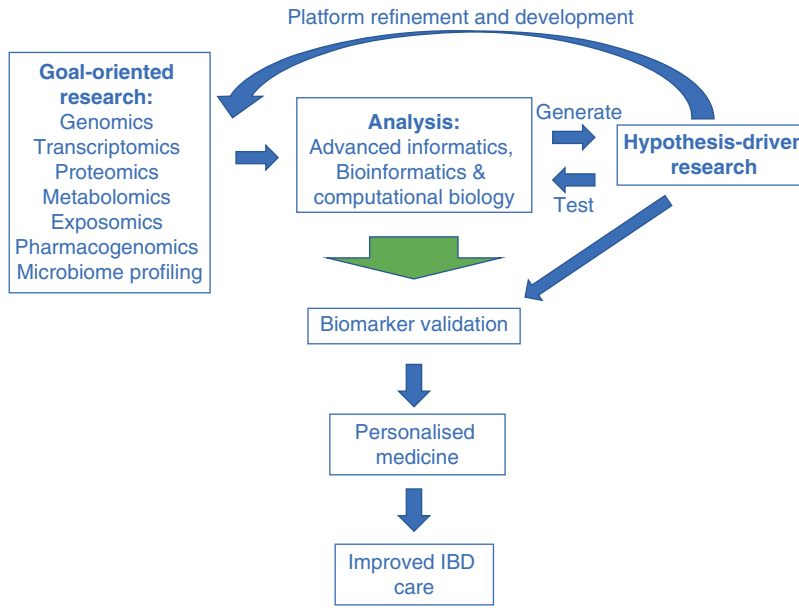

Fig. 1.3 Effective integration of goal-oriented and hypothesis-driven research. Biomarker discovery stands to benefit from the integration of both goal-oriented and hypothesis-driven research

big data into IBD biomarkers, as is facilitating widespread researcher access to this expertise [13]. If this can be achieved, we should see correspondingly greater integration of big data into hypothesis-driven research experimental design, which should further drive effective biomarker discovery.

It is intended that this conceptual overview of some contemporary issues facing both the use of biomarkers in IBD, and approaches to their discovery, has paved the way for the chapters that follow, which are broadly divided into three parts. The first part will address in detail many overarching concepts applicable to IBD biomarkers, including biomarker categorisation and utility, personalised medicine and the state of the art of biomarker discovery. The second part will focus on specific areas of unmet needs in IBD that stand to benefit from suitable biomarkers. The final part of the book will focus on the acquisition and use of big data in relation to biomarker discovery and the role of systems biology approaches in harnessing the staggering potential on offer. Each chapter will discuss and summarise current knowledge and its limitations and provide key learning points, recommendations and future directions. We hope this book lays a comprehensive framework for understanding the central role of biomarkers in improving the lives of IBD patients, amidst what is an exciting and revolutionary time for research and care into these enigmatic and potentially disabling diseases.

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Part I

Background to Biomarkers

2 Clinical Risk Factors: Lessons from Epidemiology

Zsuzsanna Kurti, Zsuzsanna Vegh, Lorant Gonczi, and Peter L. Lakatos

Abstract

Current therapeutic goals of inflammatory bowel diseases (IBD) have evolved from symptomatic remission to a complex remission defined by clinical, biochemical, and endoscopic remission, with ultimate outcomes involving prevention of disease progression, surgery, or hospitalization. Thus, risk assessment and prediction of expected disease course by clinical, biochemical, and endoscopic markers has become important in patient stratification, management, and therapy optimization and prediction of the aforementioned outcomes. This chapter highlights the importance of epidemiological studies. The role of clinical factors in the prediction of disease course has been studied in both population-based and referral cohorts. In the majority of papers, negative disease outcomes were defined as progression of disease behavior from inflammatory to complicated (penetrating/stricturing) phenotype and surgical intervention in CD, compared with proximal disease extension, hospitalization, and colectomy in UC. Age at onset, disease phenotype characteristics (early disease course, behavior and localization/disease extension), smoking status, and accelerated treatment algorithms in early disease were reported to be important in the prediction of the disease course in both CD and UC. An important challenge of the future is the harmonization of definitions of disease progression and disabling disease. In addition, the predictive potential of some factors needs to be addressed since most studies report associations rather than focusing on prediction. These require further elucidation with prospective studies. Despite all these limitations and heterogeneity of the definitions, the importance of clinical factors in predicting disease outcomes is unequivocal.

2.1 Introduction

Inflammatory bowel diseases (IBD) are chronic inflammatory disorders of the gastrointestinal tract that are thought to arise due to an interplay between genetic predisposition, environmental factors, intestinal microbial flora, and the immune system. During the disease course, complications develop in a significant proportion of patients with Crohn's disease (CD) and ulcerative colitis (UC), leading to repeated hospitalization, surgeries, colectomy, and disability.

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In the last decade, a significant change in patient management has been observed. The current therapeutic goals comprise not only symptomatic control but also long-term clinical, biochemical, and endoscopic remission, prevention of surgery, or hospitalization with the ultimate aim of changing the natural history of disease. Early introduction of intensive therapies (including immunomodulators and/or biological therapies) may be justified in patients at risk of progression to complicated disease. Therefore, risk assessment and prediction of expected disease course using clinical, biochemical, and endoscopic markers has become important in patient stratification, management, therapeutic optimization, and prediction of the outcome and side effects of medical therapy.

2.2 Current Epidemiological Data on Inflammatory Bowel Disease

In the past decade, IBD has emerged as a public health challenge worldwide. Recent systematic epidemiological reviews have reported that the incidence has reached a plateau in some of the "western" countries in the late twentieth century. The prevalence of IBD exceeds 0.3% in most European and North American countries, and it is constantly growing due to the stable incidence, the chronic nature of the disease, and improved life expectancy [1]. Additionally, newly industrialized countries in Asia, South America, and Africa are facing rising incidence analogous to trends seen in the western world during the last decades of the twentieth century, and the peak of the incidence is yet to be reached [1]. Thus, developing countries will also have to prepare for managing this complex and expensive disease. Research should concurrently focus on the identification of current environmental risk factors seen during the early stage of industrialization to identify factors that may possibly prevent the development of IBD.

Epidemiological data provides important insights into the natural history and outcomes of IBD. Most of the previous epidemiological studies are population-based studies from Europe, North America, and Australia with more recent publications from China and South Korea [2–6]. A change in the natural history of IBD has been suggested, partly owing to improvements in disease management, accelerated approaches to therapeutic strategies, and earlier diagnosis with advanced diagnostic procedures. A decline in hospitalization rates and reduced steroid exposure have been demonstrated in most studies [3]. Nevertheless, the impact of early and accelerated treatment with biologicals on disease course including surgical outcomes is still conflicting according to the available epidemiological studies [3, 7, 8].

2.3 Crohn's Disease

2.3.1 Clinical Prognostic Factors and Their Impact on Disease Course in Patients with Crohn's Disease (Table 2.1)

Several authors have aimed to identify possible clinical predictors of disease progression, complications, hospitalizations, and surgical outcomes in CD. Of note, definitions of disease progression have not been uniform across studies; therefore, extrapolation of the results and conclusions for clinical practice are often challenging. In most available papers, progression of disease behavior from inflammatory to complicated (penetrating/structuring) phenotype and surgical intervention have been defined as unfavorable disease outcomes [9–11]. However, some papers have used other definitions. Beaugerie et al. defined "disabling disease" arbitrarily, based on different clinical scenarios [12, 13]. Some of these can be regarded as therapeutic decisions (e.g., the need for immunosuppressives) rather than as disabling outcomes. In other studies, markers of unfavorable CD course have been defined as either a single event (first clinical recurrence or surgical operation) or the presence of one or multiple elements of a list of scenarios defined as "disabling disease" [14, 15].

Table 2.1 Possible predictors and their impact on the disease course in patients with Crohn's disease

Prognostic factor			Outcome parameter		
			Disease behaviour and progression	Hospitalization	Surgery
Clinical	Age at diagnosis	Young age at diagnosis (< 40 years)	Disabling CD		Increased risk of surgery
			Intestinal failure		
		Pediatric onset	Extensive disease		
			Upper GI location (L4)		
	Early disease course and behaviour	Need for steroids at diagnosis	Disabling CD		
		Complicated disease behaviour at diagnosis (B2 or B3 or B4)		Need for hospitalitation	Increased risk of surgery
	Disease location	Ileal or ileocolonic disease (L1 or L3)	Disabling CD	Short time to first hospitalization	Increased risk of surgery
			Complicated disease behaviour		
			Progression of disease behaviour		
		Colonic CD (L2)	Non-stricturing non-penetrating disease behaviour	lower hospitalization rates	Decreased risk of surgery
					Permanent stoma (severe rectal disease, rectal resection)
		Upper GI disease (L4)	Complicated disease behaviour	Need for hospitalitation	Multiple surgeries
		Perianal disease	Disabling CD		Permanent stoma (refractory perianal disease, anal canal stricture)
	Other factors	Smoking	Complicated disease behaviour and disease progression		Increased risk of surgery and postoperative recurrence
			Need for more aggressive therapy		
		Weight loss	Progression of disease behaviour		
		Early IS or biological th (protective)	Decreased risk of disease progression		Decreased risk of surgery
		Specialist care (protective)			Decreased risk of surgery
Laboratory		CRP, ESR, Calprotectin	Clinical flares (short term)		
Serological	Positive antimicrobial markers	ASCA, anti-ompC, anti-Cbir1, pANCA)	Complicated disease behaviour		Increased risk of surgery
Genetic		NOD2 mutations	Complicated disease behaviour		Increased risk of surgery
			Ileal disease location		
Endoscopy		Deep ulceration at index colonoscopy	Penetrating complications		Increased risk of surgery
		Complete or partial MH (protective)	Clinical flares and surgery		

Hospitalization rates have varied across geographic areas and countries based on differences in healthcare systems and reimbursement policies; therefore, it may be challenging to directly compare results from different locations or levels of care (e.g., community vs. referral centers). Moreover, in several clinical situations, surgery can be considered as a proactive therapeutic decision and not necessarily a negative outcome [16, 17]. Interestingly, despite all above limitations and heterogeneity of the definitions, some factors (e.g., age below 40 years or presence of perianal disease at diagnosis) have been identified as negative predictors of subsequent disease course in most published cohorts.

2.3.1.1 Age at Onset

IBD has become more widespread, and now all age groups are affected by the rise in the incidence especially early childhood and adolescence as well as the elderly population. Earlier studies have suggested that the phenotype and natural history of the disease may be different depending on age of onset.

Younger Age at Onset in Adults

Younger age at diagnosis (<40 years) is an important risk factor for disabling CD, intestinal failure, and surgery. However, it is important to note that the majority of patients with CD are diagnosed at an age of less than 40 years; thus this factor alone cannot be used to stratify high-risk CD patients. Age less than 40 years at diagnosis has been associated with progression to disabling disease and has been identified as an independent predictor for surgery in the majority of the population-based cohorts [12, 15, 18, 19]. In a systematic review by Torres et al., disabling CD course, need for multiple surgeries, and increased risk of intestinal failure were associated with younger age of onset in adults [20].

Elderly-Onset CD

Due to the aging population and rising incidence of IBD, the rate of elderly-onset IBD is expected to increase worldwide. Although limited evidence is available for elderly-onset CD, available data indicate that elderly-onset CD patients (>60 years) are more likely to have colonic disease, less frequently have ileocolonic disease, and have a similar frequency of ileal or upper gastrointestinal involvement as younger-onset disease. Disease behavior has been reported to be more inflammatory, with similar likelihood of stricturing disease with less penetrating disease or perianal involvement compared to younger age groups. The need for surgical intervention was similar, despite the lower use of immunomodulatory and biologicals in elderly-onset CD patients [21–25]. The interpretation of these data may be conflicting: these surgical trends do not imply a more benign natural history, despite fewer having complicated disease and lower use of intensive medical therapies. Nevertheless, another interpretation may be that physicians have preferred surgery instead of immunosuppressive or biological therapies in elderly patients, especially in those with stricturing disease. Thus, surgery may be regarded as a therapeutic decision rather than a negative outcome in at least a proportion of the elderly-onset CD population [25].

Pediatric-Onset CD

There exists an ongoing debate regarding whether pediatric-onset disease represents a different entity and a more severe phenotype compared to adult-onset disease. It is almost universally reported that pediatric-onset CD patients have more extensive disease with higher rates of ileocolonic disease location and higher frequency of upper GI involvement [26, 27]. However, some authors suggested that these higher rates of upper GI involvement in children may represent at least partially a lower threshold for performing upper endoscopies [28]. The indication for gastroscopies is broader in the adult setting, and an over-interpretation of minute lesions by pediatric gastroenterologists may at least partly contribute to the observed differences.

Moreover, pediatric disease onset may be predictive for more surgeries and disabling disease [29]. Some studies have suggested frequent progression of disease behavior toward complicated forms, high prevalence of extraintestinal manifestations (EIMs), and early and frequent need for corticosteroids [30], while other

population-based cohorts reported no significant differences in the evolution of disease behavior between pediatric and adult onset CD [31]. The therapeutic strategy seems to be more aggressive, with earlier and more frequent use of immunomodulators in children, and up to one-third of children have been reported to receive biological treatments early in their disease course [29, 32, 33]. Nevertheless, surgical rates in the pediatric-onset cohorts have paralleled those of adult-onset CD cohorts in the population-based studies including both pediatric- and adult-onset CD [31, 34–36].

Additionally, very young pediatric patients (usually <10 years [37]) may have a milder disease course, with isolated colonic disease and lower risk of surgical resection [20, 38]. The progression of phenotypes occurred mainly during adulthood, and perianal disease was associated with older age at diagnosis in a recent study from Israel [39]. In addition, a diverse spectrum of rare genetic disorders can present or mimic IBD (e.g., interleukin-10 signaling disorders, genetic mutations of phagocytic NADPH oxidase, complex defects in T- and B-cell function) particularly in children with very early onset IBD (defined as <6 years, [40, 41]). Several of these monogenic conditions do not respond to conventional therapy and are associated with high morbidity and mortality [41].

IBD in the Family: Is It Worse?

Disease severity and characteristics of familial vs. sporadic IBD cases are controversial. Early disease onset is often found in children of parents with IBD that can be explained by genetic anticipation or sometimes as observational bias. In studies, where positive family history was defined as having a first-degree relative with IBD, there was no difference between sporadic and familial CD patients with regard to disease characteristics (location, behavior) or surgical outcomes [42–44], even after matching sporadic and familial patients for sex, location of disease at onset, date of birth, and date of diagnosis [43]. In contrast, in a recent registry-based study using a different definition of positive family anamnesis, defining patients with a second- or third-degree relative with IBD as familial cases (app. 40% of cases were defined as familial), familial IBD cases presented with an earlier disease onset, more EIMs, higher prevalence of penetrating behavior, and perianal disease at the time of diagnosis, although surgical outcomes were not different among the familial and sporadic cases with a median follow-up of 92 months [45].

2.3.1.2 Disease Phenotype at Diagnosis

The phenotypic classification of CD is important when determining patient management and treatment strategy, and some of the variables may assist in predicting clinical disease course. Multiple efforts have been made to classify IBD [37, 46, 47]. While clinical classifications have provided a useful approach to the risk assessment of a given patient at a given time point, significant changes in disease behavior can occur over time, whereas disease location remains relatively stable.

Disease location has been associated with disease behavior at diagnosis, disease progression to complicated behavior, and with time to progression toward complicated disease in epidemiological studies. Disease located in the small bowel is associated with a higher probability of complicated disease phenotype and carries a higher risk for surgery compared to isolated colonic disease [10, 15, 18, 19, 31, 48, 49]. Similarly, the probability of a change in disease behavior from inflammatory to complicated phenotype is significantly higher in patients with ileal involvement and perianal disease [31]. Patients with ileal CD have a high probability of stricturing disease and ileal resection [15]. Nevertheless, patients with colonic disease are more likely to develop penetrating complications in the rectum and/or perineal regions, particularly with stricturing and complex fistulizing disease, and are at risk of requiring a permanent stoma. Interestingly, the long-term prognosis of CD is not related to anatomical characteristics of the disease, except for patients with rectal involvement with a permanent stoma [50].

In relation to disease behavior, penetrating and stricturing phenotypes at diagnosis are

independent risk factors for surgery. Patients with penetrating disease are at further increased risk of postoperative recurrence [20, 51]. The majority of patients have an inflammatory phenotype at diagnosis, and almost half of these patients may develop a penetrating and/or stricturing phenotype over time [10, 15]. These complications may occur in the first 5 years of disease or after 10 years [48].

One of the strongest predictors of disabling course of CD is perianal disease [20, 31]. The typical course for patients with perianal CD includes frequent relapses and long periods of active disease with draining fistulas. The cumulative risk of developing a perianal fistula is ~10% at 1 year and 20% at 10 years [18, 52]. Complex perianal disease is also a risk factor for a permanent stoma.

Extraintestinal manifestations can also impact the patients' quality of life, leading to disability in several cases. EIMs develop in about one-third of CD patients [53, 54]. The majority of these manifestations are already present at diagnosis or develop during the early years of the disease. The probability of having at least one EIM varies from 22% at diagnosis to 40%, 10 years after diagnosis [54]. Patients with colonic involvement seem to be more susceptible to developing EIMs, and patients with EIMs have a higher risk of a more severe clinical course [55], which is associated with a greater need for steroids [53].

The long-term disease course and prognosis has been investigated in a few population-based cohorts [15, 50]. A large proportion of patients have reported a decrease in the severity of bowel symptoms during 10 years of their follow-up (43%) or were in clinical remission during the last 5 years of follow-up (44%), but the cumulative relapse rate was 90% in the IBSEN cohort from the prebiological era [15]. In the Saint-Antoine cohort, non-severe evolution was defined as clinically inactive disease for greater than 12 years and less than one intestinal resection without permanent stoma [50]. Factors independently associated with a non-severe 15-year clinical course were non-smoking status, rectal sparing, high educational level, older age at onset, and longer disease duration.

Multiple clinical prediction models for unfavorable disease course or CD-related surgery have been created using referral centers' data, population-based cohorts, and most recently, clinical trial data from community gastroenterology practices [12, 56–59]. These models all include clinical variables, yet their use in clinical practice may be challenging, either due to the complexity or nature of these models.

2.3.1.3 Treatment as an Indicator of Severe Disease

Early steroid exposure or the need for steroids for treating the first flare has been reported as a predictor of poor outcomes in most studies [12, 13, 49]. Of note, only a small proportion of patients starting as mild disease do not need steroids; thus this factor alone cannot discriminate accurately between moderate and/or severe patients [60, 61]. In contrast, early steroid requirement should be regarded as a disease severity indicator rather than a predictor for disabling outcome. Similarly, early azathioprine (AZA) or biological therapy can be used as an indicator of IBD severity, and in some, but not all studies, early AZA use has been associated with reduced need for surgery in population-based cohorts [9, 62, 63]. Of note, the benefit of early aggressive therapy including anti-TNFs could not be proven in all studies, e.g., the very recent 5-year follow-up of the ECCO-EpiCom cohort failed to show a surgical benefit for Western European compared to Eastern European CD patients, who received significantly less biological therapies [8].

2.3.1.4 Smoking

Multiple studies have reported significant associations between cigarette smoking and the risk of IBD. Current smoking has been associated with a higher risk of CD but a lower risk of UC. The reason why smoking has opposite effects on these two diseases that share so many similarities remains unknown. During Crohn's disease course, there is a strong association with detrimental effects of smoking. Smoking has

been associated with an increased need for therapy escalation, progression to complicated disease behavior, the need for surgery, and postoperative recurrence in CD [20, 64, 65]. Smoking has also been associated with a higher prevalence of small bowel disease [66, 67]. The risk of the first surgery was significantly increased in current smokers as compared to those who had never smoked, but former smokers were not at an increased risk of surgery relative to those who had never smoked [64].

2.3.2 Clinical Prediction of Postoperative Recurrence in CD

Among clinical factors, smoking and history of prior resection are the strongest predictors of symptomatic disease recurrence. Smoking status appears in most studies as a strong predictor of postoperative recurrence: the risk of clinical recurrence and reoperation is approximately doubled in smokers [68]. A history of prior resection, penetrating behavior, noncolonic location, extensive bowel resection, and prior intestinal surgery has also been identified as risk factors in retrospective cohort studies [69, 70].

2.4 Ulcerative Colitis

2.4.1 Possible Predictors and their Associated Impact on Disease Course in Patients with Ulcerative Colitis (Table 2.2)

2.4.1.1 Age at Diagnosis

Younger Age at Onset in Adults

Ulcerative colitis has two peak incidences, with the main onset peak between ages 15 and 30 years and a second smaller peak between ages 50 and 70 years. Young age at onset (usually <40 years) is associated with more disease flares and increased risk of colectomy [71].

Elderly-Onset UC

Most studies have suggested that patients with elderly-onset UC have less aggressive natural history, with less need for immunosuppressives or biological therapies. The most prevalent disease extent is left-sided colitis in the elderly population (approximately 45–65%) [23, 25, 72]. Disease extent seems to be stable in more than 80% of elderly-onset UC patients, with infrequent proximal disease extension [28]. Medical strategies have traditionally been less aggressive, with less need for systemic steroids, immunosuppressives, and biological therapy [22, 23]. However, data regarding the frequency of colectomy in elderly-onset patients have been conflicting. The IBSEN cohort reported a reduced hazard ratio (0.28) for subsequent colectomy in elderly-onset UC patients (>50 years), while colectomy was less frequent but with no significant difference compared to other age groups in a Hungarian cohort [23, 73]. In addition, Ananthakrishnan et al. suggested that elderly-onset UC patients were significantly more likely to undergo surgery [25]. However, the increased risk of surgery may represent a therapeutic decision rather than a more aggressive disease course. Interestingly, a study from the USA reported that elective colectomy seemed to be associated with improved survival relative to medical therapy among patients aged >50 years with advanced UC. Moreover, only a minority (9%) of colectomized elderly UC patients underwent construction of an ileal pouch-anal anastomosis (IPAA), while 41% had an ileorectal anastomosis and in half of the individuals definite ileostomy was performed in the EPIMAD cohort [22]. UC-associated dysplasia and cancer seems to be similar in different age groups, but with a shorter time to development in elderly-onset UC patients [20, 23]. Mortality was not different from the background population in elderly-onset UC patients [74, 75].

Pediatric-Onset UC

Pediatric UC needs tight control by physicians, as pediatric-onset UC patients have a more aggressive disease course compared to adult-onset UC patients. Pancolitis is more common at diagnosis in pediatric-onset UC compared to

Table 2.2 Possible predictors and the associated impact on the disease course in patients with ulcerative colitis

Prognostic factor	Outcome parameter				
	Proximal disease extension	Hospitalization	Colectomy	Colorectal neoplasia	Acute severe UC
Young age at diagnosis	More extensive disease (pediatric UC)		Increased risk of colectomy	Increased risk of colorectal neoplasia	Acute severe UC
	Proximal disease extension				
Refractory proctitis (>3 relapses per year)	Proximal disease extension				
Steroid dependence/ resistance		Higher rates of hospitalization	Increased risk of colectomy		
Extensive colitis			Increased risk of colectomy	Increased risk of colorectal neoplasia	Increased risk of acute severe UC
High histological inflammation score				Increased risk of colorectal neoplasia	
Disease duration >10 years			Increased risk of colectomy	Increased risk of colorectal neoplasia	
Smoking	Protective against proximal disease extension	Less need for hospitalization	Protective against colectomy		
Concurrent infection	Flare and hospitalization				
PSC	Proximal disease extension	Less need for hospitalization		Increased risk of colorectal neoplasia	
Family history	Proximal disease extension (family history of IBD)			Increased risk of colorectal neoplasia (if family history of CRC)	
Male sex			Increased risk of colectomy		

adult-onset cases, and disease extension is also frequent [76, 77]. According to a systematic review on pediatric population-based cohorts, half of patients had disease extension during follow-up, while about two-third of patients had pancolitis at the end of follow-up [78]. In relation to the surgical outcomes, different rates of colectomy were reported [32, 34, 77]. The cumulative risk of colectomy was about 15% and 20% at 5 and 10 years from diagnosis [77]. This is comparable to those reported from the adult cohorts. The therapeutic strategy was intensive; about two-thirds of pediatric-onset UC patients required steroid therapy, and up to 25% were steroid dependent in population-based cohorts before the biological era [76]. In a more recent study from the USA, 62% and 30% of patients received immunosuppressives and biologicals [32].

2.4.1.2 Disease Extension

Extent of colitis is an important clinical predictor in UC, as extensive colitis (E3) is associated with higher hospitalization rates, increased need for corticosteroids, increased need for colectomy, and higher risk of progression to dysplasia or colorectal cancer [20, 73]. Colectomy risk is about four times greater in extensive colitis compared to proctitis (E1) [79].

In population-based cohorts, the largest proportion of patients had left-sided colitis (E2) at

diagnosis, but proximal disease extension can occur with time [73, 80]. During follow-up, proximal disease extension was observed in one-fifth of patients with left-sided colitis to extensive colitis and in 10–28% and 6–14% of patients from proctitis to left-sided colitis and extensive colitis [73, 80]. Disease flares associated with the progression of extent usually are associated with a severe course. The risk of colectomy was higher in E1/E2 patients with proximal disease extension to E3 compared to E1/E2 non-extenders, but no significant difference or only a trend was found between patients with proximal extension to E3 and patients with extensive colitis initially [73, 80].

2.4.1.3 Early Disease Course

The clinical course of ulcerative colitis is characterized by alternating periods of remission and relapse. A large proportion of UC patients is generally most active at diagnosis with a more mild subsequent disease course [73]. Of note, about 10–15% of patients have an aggressive course, and the cumulative risk of relapse is 70–80% at 10 years [73]. Severe early course of the disease with frequent need for steroid and more than 3 relapses a year is associated with poor prognosis of UC, including proximal disease extension, hospitalization, and increased colectomy rates [20]. In contrast, mucosal healing early in the disease course (1 year) in patients with UC has been associated with a reduced risk of subsequent colectomy in population-based cohorts [81].

2.4.1.4 Disease Duration

The duration of disease is associated with the development of dysplasia and colorectal carcinoma in UC patients and is consequently associated with increased colectomy rates. Screening colonoscopies should be offered after 8 years of disease to all UC patients with surveillance thereafter based on guidelines encompassing disease extent and risk factors for dysplasia [82].

2.4.1.5 Smoking

Smoking can be considered as a protective prognostic factor, as UC affects predominantly non-smokers and former smokers [83, 84]. Disease has been documented as having a more benign course in smokers compared to non-smokers [78]. Flare-ups, hospitalization rates, need for steroids, and colectomy rates have been reported to be lower in most, but not all studies [85, 86]. In a recent meta-analysis, only former smokers had an increased risk for colectomy compared to those who never smoked or current smokers [64]. Moreover, smoking cessation increases the risk of relapse, especially in the first few years after cessation [87, 88]. Disease extent is not affected by smoking, but proximal extension has been found less frequently in current smokers [20, 89].

2.4.1.6 Other Factors

Primary sclerotizing cholangitis (PSC) is usually associated with a decreased risk of hospitalization for UC flare according to the epidemiological data. However, PSC is an important clinical risk factor for colectomy, as it is associated with extensive disease and increases the risk of colorectal carcinoma. In addition, patients with concomitant PSC are also at increased risk for cholangiocarcinoma and bile duct carcinoma is identical, and end-stage liver disease, representing substantial morbidity and mortality in that subgroup [90]. Similarly, a family history of a first-degree relative with sporadic CRC may indicate an increased risk of CRC. Therefore, UC patients with PSC or positive family history for CRC need a different endoscopic surveillance for CRC.

In addition, there are conflicting data as to whether gender-based differences are important in predicting the disease course in UC. The male sex is considered to be associated with increased risk for colectomy and the development of CRC. In contrast, female sex has been associated with a higher risk of relapses. Among females, the use of oral contraceptives has been associated with disease onset, while breastfeeding has been found to be protective against subsequent development of ulcerative colitis [91, 92]. Finally, appendectomy in children with acute appendicitis has been found to be protective for the development of ulcerative colitis [93, 94]. In a Swedish population-based cohort, appendectomy before developing UC was associated with

a lower risk of colectomy and UC-related hospitalizations, while appendectomy after established UC was associated with a worse disease course, with an increased rate of subsequent colectomy [95].

2.5 Current Limitations and Future Directions

An important need for the future is the standardization of definitions of disease progression and disabling disease used for clinical studies. In addition, the predictive potential of some factors needs to be addressed as studies thus far have reported on associations and are not focused on causation. Nevertheless, despite all these limitations and heterogeneity of the definitions, the evidence for some clinical factors seems to be unequivocal.

2.6 Conclusion and Take-Home Messages

In conclusion, age at onset, disease phenotype characteristics (early disease course, behavior, and localization/disease extension), smoking status, and the adopted treatment algorithm are important in the prediction of the disease course in both CD and UC patients. These are easy data to collect and important in everyday practice to enable clinicians to stratify patients at the time of diagnosis and facilitate the most appropriate management in terms of therapy and follow-up.

Summary Points

- The importance of clinical factors in the prediction of disease course has been extensively studied in both population-based and referral cohorts.
- A combination of clinical factors (e.g., age at onset, disease phenotype, smoking status, early disease course, and response to treatment algorithms) can support early patient stratification.
- Early stratification of patients at the time of diagnosis facilitates more appropriate management including tailored therapy and follow-up.

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Disease Modification in Crohn's Disease

3

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Abstract

Crohn's disease (CD) is a chronic inflammatory disease of the gastrointestinal tract (GIT) that evolves in a relapsing and remitting pattern. CD is a destructive disease that can result in progressive bowel damage and ultimately disability. It mainly affects young adults, causes morbidity, and impacts on quality of life. The majority of patients with CD initially present with an inflammatory phenotype at diagnosis, but over time various complications that result in tissue damage can occur such as strictures, abscesses, and fistulae, which often require surgery. The functional correlate of tissue damage is disability which may develop over time. Current management strategies therefore base their premise on "treating to target" with the aim of achieving deep and prolonged disease remission, thereby preventing tissue damage and disability. The following sections will address the identification and assessment of tissue damage and how we can evaluate disability in CD.

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3.1 Introduction

Crohn's disease (CD) is a chronic inflammatory disease of the gastrointestinal tract (GIT) that evolves in a relapsing and remitting pattern. CD is a destructive disease that can result in progressive bowel damage and ultimately disability. It mainly affects young adults, causes morbidity, and impacts on quality of life. All segments of the GIT can be affected by CD and have a predilection for the terminal ileum and colon. The majority of patients with CD initially present with an inflammatory phenotype at diagnosis, but over time various complications that result in tissue damage can occur such as strictures, abscesses, and fistulae, which often require surgery. The functional correlate of tissue damage is disability which may develop over time. Current management strategies therefore base their premise on "treating to target" with the aim of achieving deep and prolonged disease remission, thereby preventing tissue damage and disability.

The following sections will address the identification and assessment of tissue damage in CD. The evaluation of disability in CD will be discussed in addition to validated methods to assess this important outcome. In addition, recently reported clinical trials that have identified better outcomes with early aggressive disease modification as opposed to conventional management and how these approaches may

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impact on future disability in CD will be discussed.

3.2 Evaluation of Tissue Damage and Disability in Crohn's Disease

3.2.1 Bowel Damage in CD

The cumulative risk of developing stricture or penetrating complications has been found to be 34% and 51% at 5 and 20 years, respectively, based on a US population-based cohort study that evaluated CD patients who were diagnosed from 1970 to 2004 [1]. The *Lémann index* was developed to address the need to quantify tissue damage in CD based on disease location and duration [2, 3]. The digestive tract was divided into four organs and subsequently into segments, and the overall level of organ damage was calculated from the average score of segmental damage [4].

It has recently been suggested that four out of ten CD patients had bowel damage at the time of the first cross-imaging study (CT or MRI) [5]. The presence of bowel damage in early CD was associated with a worse outcome, with increased risks of surgery and hospitalization [5]. Although tissue damage was initially thought to be irreversible, the Lémann index has recently been found to reduce with anti-TNF therapy, highlighting that biologics may be able to reverse bowel damage in some CD patients [6]. To date the Lemann index has not been fully validated, and ideally this should be performed during the conduct of a drug trial of known clinical efficacy.

3.3 Why Is Crohn's Disease a Disabling Condition?

CD is known to significantly reduce the health-related quality of life (HRQOL) of patients [7]. Many previous studies have focused on the quality of life with the IBD-questionnaire (IBD-Q) [8]. However, the IBD-Q is subjective and was not developed and validated according to the Food and Drug Administration (FDA) guidance for the development of patient-reported outcomes [9]. The lack of validation of the IBD-Q according to FDA guidance has consequences for future new drug approval in IBD. Fatigue is a commonly reported symptom in CD and has been shown to reduce the HRQOL in patients [10–14]. Patients with IBD also experience frequent concomitant anxiety and depression [15], and some studies have reported that patients with CD have higher rates of anxiety and depression than UC patients [11, 16]. In a Swiss cohort study, patients with CD had temporary work disability which was associated with gender, disease duration, disease activity, C-reactive protein level, smoking, depressive symptoms, fistulas, and extraintestinal manifestations and the use of steroids/immunosuppressants [17]. In another cohort study from the IBSEN group, patients with CD had an increased relative risk for requiring a disability pension than the "normal" population in the 10 years after disease onset (RR: 2.0) [18].

Overall, disability appears to be common in CD and contributes to disease burden in patients, but a validated tool to objectively measure disability has previously been lacking.

3.4 Development and Validation of the IBD-Disability Index (IBD-DI): A WHO Initiative

The *International Classification of Functioning, Disability and Health* (*ICF*) is a common conceptual framework which describes and measures the dimensions of human functioning, disability, and health that was approved by the *World Health Organization* (WHO) in 2001 [19, 20]. In an attempt to quantify disability, the *IBD Disability Index* (IBD-DI) was developed with the World Health Organization [21, 22]. It is comprised of 14 questions, with scores ranging from 0 to 100. The latter study commenced in 2007, and the IBD-DI has recently been validated in a French population-based study [22]. The IBD-DI demonstrated high internal consistency, inter-observer reliability and construct validity, and moderate intra-observer reliability.

The responsiveness of the IBD-DI is being evaluated in ongoing prospective stud-

ies such as CURE (EudraCT Number: 2013-003199-11) and the ICARE (ClinicalTrial.gov Number: NCT 02377258) studies. The IBD-DI should be a major secondary endpoint in clinical practice and future disease modification trials, but its use requires further longitudinal evaluation [23].

Disability in general is associated with lower earnings indirectly and directly; it also has been associated with reducing access to education and can lead to social exclusion. This impact of disability on society is challenging for health promotion as disability may be preventable [24]. Disability is a major factor of disease burden in CD, and the main associations with disability of disease severity are the impact of the disease on the individual, disease burden, and the disease course. A disease severity classification including disability is being developed and validated by the IOIBD [25].

As the IBD-DI evaluates functional status, it is used mainly in the clinical trial setting. A more recent *IBD Disk* was developed which is a shortened, self-administered adaption of the validated IBD-DI. This should permit immediate visual representation of patient-reported IBD-related disability. In the preparatory phase, the *IBD CONNECT* group (agreed with consensus) included ten items in the IBD Disk including abdominal pain, body image, education and work, emotions, energy, interpersonal interactions, joint pain, regulating defecation, sexual functions, and sleep. The IBD Disk requires further validation but has the potential to be an outcome measure for use during routine clinical visits and may correlate changes in disability over time [26].

3.4.1 New Treatment Goals in CD

Unfortunately, there appears to be a lack of correlation between clinical symptoms and mucosal lesions in CD. In a post hoc analysis of the SONIC trial, half of patients that were in clinical remission had mucosal ulcerations [27].

More recently mucosal healing (MH) has emerged as a major therapeutic goal in CD [28]. Achieving MH may change the course of CD by preventing bowel damage, thus reducing the rates of hospitalization for complications and of surgery [28].

Deep remission has been defined empirically as a composite of clinical remission (a Crohn's disease activity index [CDAI] less than 150 points) plus complete MH [29]. In an exploratory study of patients with moderate to severe ileocolonic CD who received adalimumab induction and maintenance therapy, patients in deep remission at 52 weeks had better outcomes than patients not in deep remission [30]. However, the short-term outcomes of patients with deep remission versus only clinical remission were similar [30].

It has been demonstrated that deep remission is achievable with available drugs, notably with combination therapy in a high percentage of CD patients [31]. A post hoc analysis of the SONIC trial showed that 57% of patients achieved deep remission at week 26 with combination therapy, compared to 30% with infliximab monotherapy ($p = 0.017$) and 19% with azathioprine monotherapy ($p = 0.002$) [31].

In an effort to select potential treatment targets in IBD, the International Organization for the Study of Inflammatory Bowel Diseases (IOIBD) developed the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) program [32]. An expert panel evaluated potential treatment targets to be used in a treat-to-target strategy in IBD [32]. The agreed upon targets in CD patients were clinical-/patient-reported outcomes remission (defined as resolution of abdominal pain and diarrhea or altered bowel habit) and endoscopic remission (defined as resolution of ulcers at ileocolonoscopy) or resolution of findings of inflammation on cross-sectional imaging in patients who cannot be adequately assessed with ileocolonoscopy [32].

However, the definition of MH varies across studies, and no validated definition of MH or endoscopic remission currently exists for CD. Additional evidence is needed to support the concept that treating CD patients treating to the target of MH in patients who are asymptomatic leads to disease modification (as measured by a reduction of disease-related complications or surgery) [33]. To address this important issue,

prospective trials are underway, such as the REACT2 trial, which compares outcomes between a step-up treatment intensification algorithm based on the findings of ileocolonoscopy and a traditional clinical approach in which symptoms are used to intensify treatment (NCT01698307).

Tight control by means of objective markers of inflammation, such as ileocolonoscopy and cross-sectional imaging, may result in an increase in invasive procedures and cost. Given these barriers, attempts have been made to use surrogate biomarkers for MH [33]. Serum CRP and fecal calprotectin (FCP) have been considered potential candidate biomarkers for this purpose but were not selected as primary treatment targets by the STRIDE consensus panel, who instead recommended their use as adjunctive biomarkers, because of the current lack of controlled trials demonstrating their use as surrogates to endoscopy [32].

The recently reported landmark study, named the "CALM" trial, was a randomized, phase 3 trial which evaluated adult patients with active endoscopic CD (CDEIS >6 [Crohn's disease Endoscopic Index of Severity]), a CDAI 150–450, and no previous use of immunomodulators or biologics. Patients were randomly assigned to either a tight control or clinical management group. In both groups treatment was escalated in a stepwise manner, from no treatment to adalimumab induction followed by adalimumab every other week, adalimumab every week, and lastly to both weekly adalimumab and daily azathioprine. Escalation was based on treatment failure based on symptoms and biomarkers. In addition, de-escalation was possible for patients receiving weekly adalimumab and azathioprine or weekly adalimumab alone, if failure criteria were not met. The primary endpoint was mucosal healing (CDEIS < 4) with absence of deep ulcers at 48 weeks. The authors reported that in the tight control group, a higher proportion of patients achieved the primary endpoint at week 48 than those in the conventional group (46 vs 30%, respectively; $p < 0.01$). The treatment-related side effects in both groups were similar. This is the first study to demonstrate that timely escalation of anti-TNF therapy on the basis of clinical symptoms combined with biomarkers in patients with early CD results in better clinical and endoscopic outcomes than symptom-driven decisions alone [1].

3.4.1.1 Disease Modifying in CD

The concept of disease-modifying anti-Inflammatory bowel disease drugs (DMAIDs) [34] is gaining momentum. Despite their widespread use in CD, two controlled trials demonstrated that azathioprine was not effective for disease modification in CD (AZTEC and RAPID studies) [35, 36]. This result is not surprising, given the general limited efficacy of azathioprine. TNF antagonists (infliximab, adalimumab, certolizumab pegol), anti-adhesion molecules (vedolizumab), and anti-IL-23 (ustekinumab) are biologics approved for refractory CD.

The previous treatment goal for CD was to induce and maintain steroid-free clinical remission. Theoretically, deep remission might be the only way to modify the course of CD by preventing long-term complications, including surgery, hospitalizations, bowel damage, and disability [37]. At present, treatment with biologics appears to have the most promise for achieving that treatment goal.

In the recently reported Randomized Evaluation of an Algorithm for Crohn's Treatment (*REACT*) study, community gastroenterology practices from Belgium and Canada were randomly assigned to early combined immunosuppression (ECI, anti-TNF, and antimetabolite drug) or conventional management [38]. The primary outcome of clinical remission at 12 months was similar in both groups (66% vs. 62%, $p = 0.52$) [38]. However, the rates of the composite endpoint of major adverse outcomes (defined as occurrence of surgery, hospital admission, or serious disease-related complications) at 24 months were lower at ECI practices than at conventional management practices (28% vs. 35%, hazard ratio [HR]: 0.73, $p = 0.0003$) [38]. Despite initial concerns in the literature, ECI was not associated with an increased rate of serious disease-related adverse events or mortality [38].

Pending the results of future disease modification trials in patients with moderately active

CD without poor prognostic indicators and without disease complications, immunomodulators are still being used in a rapid step-up approach based on tight monitoring of objective signs of inflammation. Anti-TNF agents, preferably used in combination with either azathioprine or methotrexate, are the most effective agents. Accordingly, this combination strategy should be initiated as first-line therapy in patients with CD with existing bowel damage (stricture/fistula/abscess) and/or those with poor prognostic indicators and/or with severe disease [39–42].

3.5 Drug Withdrawal in CD

In CD, a systematic review reported that stopping immunomodulator monotherapy after a period of remission is associated with high rates of relapse (~75% of patients relapse within 5 years) [43]. However previous studies in CD patients reported that in those who discontinued their immunomodulator after combination therapy (immunomodulator and biological therapy) the rates of relapse did not differ significantly compared to those who remained on combination therapy [43]. The ongoing CURE trial will help identify patients with a very low risk or relapse and progression in the long term.

3.6 Current Limitations and Future Directions

Many of the reported trials in CD have been in patients with more established complicated disease, and furthermore many trials have not employed validated disease damage scores or measures of disability in their outcomes. The current therapeutic agents in CD have limited impact on changing the natural history of the disease, and newer more effective disease modifying agents are keenly awaited. In addition, the long-term costs and safety of these agents require further evaluation.

The recent development of the IBD Disk has the potential to be a valuable tool for use in routine clinical visits and potentially accurately assess the changes in disability in CD patients over time. Future clinical trials in CD will be directed toward targeting response and remission to ensure deep remission, with an improvement in defined (and validated) patient-reported outcomes. These trials should aim to assess the impact of disease modification on disability in a longitudinal fashion, employing the use of the Lémann index and the IBD-DI. The ultimate aims will be to prevent complications and disease progression and thereby prevent future disability.

3.7 Conclusions

At present disease modification in CD has not been fully evaluated but does show some promise. Future disease modification trials in CD should consider the inclusion of patients with early CD and the use of the Lémann index and the IBD-DI as primary or secondary endpoints [37] and stratify patients according to disease severity [25, 44].

Disease modification trials are required to prospectively evaluate novel therapeutic strategies in CD, based on tight control of objective signs of inflammation into change disease course and patients' lives by ameliorating inflammatory disease or ultimately preventing the occurrence of bowel damage.

Summary Points

- Disability appears to be prevalent in CD, but previously validated tools to assess this were lacking
- Disease modification in CD with early combined immunosuppression (versus conventional management) can reduce adverse outcomes
- The validated IBD-DI should become a major endpoint in clinical trials to assess disability
- The recently developed IBD Disk tool has the potential to become a valuable outcome measure to assess disability over time

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Personalized Medicine - Dream or Reality?

4

Elizabeth A. Spencer and Marla C. Dubinsky

Abstract

Precision medicine is gaining popularity since it strives to take into account individuals' differences in genes, environment, and lifestyle choices. It has numerous applications within the IBD space. Current day applications include the idea of treating to target goals over a finite timeline and using proactive therapeutic drug monitoring. Future applications are myriad and range from alterations in the microbiome, to advanced modeling to better characterize patients, to decision support tools and to increasingly targeted therapeutics.

4.1 Personalized Medicine: Dream or Reality

Personalized medicine is the dream of modern medicine, held by doctors wanting to deliver the best care, patients enthusiastic to receive tailored care, and healthcare institutions wanting to maximize gain for cost. Biomarkers are a cornerstone of personalized medicine since they can be used to effectively group patients to tailor care, but personalized medicine describes the broader concept that care for each patient can be individualized based on their genomic, epigenomic, and environmental profile to encompass diagnosis, prognosis, treatment, and prevention (Fig. 4.1). With the rapid incorporation of cutting-edge discoveries in the various, and ever-expanding, −omics fields (genomics, transcriptomics, proteomics, and metabolomics to name a few), personalized medicine is transforming from vision to reality.

4.1.1 Rebrand: Precision Medicine

As personalized medicine has become an increasingly trendy topic in the news, there has been a movement to rebrand it as precision medicine given that it can be misinterpreted to denote treatments developed specifically for an individual patient. The new term, precision medicine, was first adopted when the US National Research Council published their 2011 report, *Toward Precision Medicine*, outlining the modernization of disease taxonomy to include genetic information. This has since been gaining momentum, and the National Council of Research currently prefers the use of the terminology precision medicine [1]. The NIH

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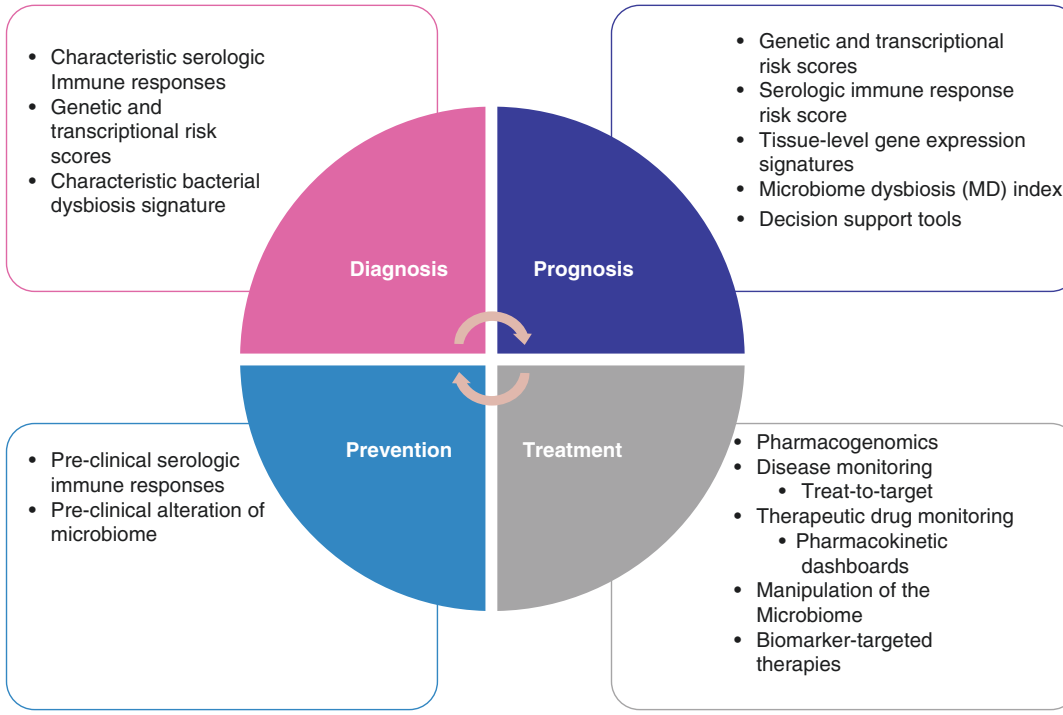

Fig. 4.1 Precision medicine in IBD

defines precision medicine as "an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person" [2]. This has taken off over the last couple of decades due to the development of large-scale biologic databases, most notably the human genome sequence, as well as the explosion of new -omic methods for characterizing patients with burgeoning inexpensive, rapid clinical assays. This goes hand in hand with a rise in and refinement of computational tools for analyzing large sets of data.

In his 2015 State of the Union address, Former President Barack Obama launched a new precision medicine initiative:

> Doctors have always recognized that every patient is unique, and doctors have always tried to tailor their treatments as best they can to individuals. You can match a blood transfusion to a blood type. That was an important discovery. What if matching a cancer cure to our genetic code was just as easy, just as standard? What if figuring out the right dose of medicine was as simple as taking our temperature? [3]

There is, thus, a call for new tools for precision medicine, and the current thrust of precision medicine is to provide "the right drug, with the right dose, at the right time to the right patient."

Precision health, in contrast, is focused on preventing disease on a population level using targeted genomic and molecular tools, and, within the field of public health, there is equal enthusiasm for this type of population precision.

4.1.2 Is Precision Medicine Feasible: Oncology as a Case Study

Despite the change in name, some continue to doubt that precision medicine is a viable strategy, thinking it will be too costly, and create therapies useful in only one or two patients. However, oncology can serve as a case study to

demonstrate that there is a place for precision medicine therapeutics. A classic example is the discovery of the Bcr-Abl gene fusion in chronic myeloid leukemia (CML). This breakthrough led to the development of an inhibitor of BCR-ABL, imatinib, which was able to be used to treat most CML since this gene fusion occurs in the vast majority of CML patients. It improved survival rates within CML greatly to over 90% at 5 years [4]. A similar success story can be found with human epidermal growth factor receptor 2 (HER2)-positive breast cancers since there are numerous drugs targeted at binding the HER2 receptor, like trastuzumab, lapatinib, pertuzumab, or ado-trastuzumab emtansine, which have all been shown to improve survival [5, 6]. There are also savings in HER2-based therapies since the cost of the HER2 screening is minute in comparison to the benefits of faster treatment times, given that treatment cost for breast cancer runs into the tens of thousands of dollars per patient year.

By its very nature, precision medicine has built in efficiencies for the research and development of new therapeutics since it provides tools to both better select drug trial participants and measure more reliable endpoints. It could lead to the elimination of nonperforming candidate drugs quickly to avoid the loss of resources in drug development. A prime example of this is the drug pembrolizumab (Keytruda, Merck & Co), a monoclonal antibody against the programmed death 1 (PD-1) receptor on lymphocytes, useful in a number of different cancers including non-small cell lung cancer (NSCLC) and melanoma. Merck used a biomarker, the overexpression of programmed death ligand 1 (PD-L1), as well as genotype, lack of epidermal growth factor receptor (*EGFR*), and anaplastic lymphoma kinase (*ALK*) mutations, to choose patients with NSCLC more likely to respond to the drug leading to a more successful trial outcome [7]. Merck was able to demonstrate improved survival in pembrolizumab's NSCLC trials, where its rival, nivolumab (Opdivo, Bristol-Meyers Squibb Co), was not, due to their use of targeting with PD-L1, EGFR, and ALK [8]. This is all over a very short timeline; the first checkpoint inhibitor (ipilimumab, an antibody against cytotoxic T-lymphocyte-associated protein 4 (anti-CTLA-4)) was approved by the US Food and Drug Administration (FDA) only in March of 2011, and pembrolizumab and nivolumab followed on its heels in 2014 [9]. These examples show that the identification and targeting of certain mutations and the use of biomarkers can be used in a cost-efficient manner to benefit a large group of patients and cut down on the costs, in both time and money, of research and development.

4.1.3 Precision Medicine in IBD

The diagnosis of IBD can be challenging since it can present with non-specific symptoms that overlap with other disorders, including common functional gastrointestinal disorders. Clinicians must undertake considerable diagnostic testing, including colonoscopy with biopsies and cross-sectional imaging, to exclude the diagnosis of IBD in those cases. The non-specific symptoms, which are more typical of Crohn's disease (CD) than ulcerative colitis (UC), can also lead to long delays in diagnosis with an attendant increase in complications. Precision medicine strives to fill this gap and find an accurate, noninvasive diagnostic tool to quickly distinguish IBD from non-IBD and, hopefully, also identify phenotype, predict prognosis, and direct therapy. We currently have many tools in the making to fulfill these goals.

4.1.4 Diagnosis

To date, there is no highly sensitive and specific, noninvasive diagnostic test in IBD. Much work has been done on identifying candidate markers within a variety of modalities (genetic, transcriptomic, serologic responses, and the microbiome). There has also been a push to combine these markers into predictive models to perhaps identify a multimodal model to fill this diagnostic role.

4.1.4.1 Genomics and Transcriptomics

The genetic predisposition of IBD was initially suggested by ethnic and familial aggregations of the disease [10]. Roughly one in five CD patients has at least one affected family member, and the Ashkenazi Jewish population carries an approximately fourfold increased risk of IBD [11, 12]. Twin studies have demonstrated that the concordance rate for IBD was higher in monozygotic twins than in dizygotic twins, 20–50% vs 4% [13–17]. Large genome-wide association studies (GWAS) have since gone on to identify hundreds of single-nucleotide polymorphisms (SNPs) associated with the risk of developing IBD [18–23].

The gene with the largest effect size, *NOD2* (nucleotide-binding oligomerization domain containing 2) on chromosome 16 (16q12), was actually identified on the initial genome-wide linkage studies [24, 25]. In the intestinal epithelium, NOD2 plays an important role in maintaining epithelial barrier integrity via Paneth cell production of α-defensins, and its expression is highest in the Paneth cells of the terminal ileum [26]. It works on a feedback loop with commensal bacteria to keep them in check, and NOD2 variants are associated with dysbiosis [27]. It has been estimated that 20–30% of CD patients carry an abnormal *NOD2* variant compared to 6–7% of controls [28]. Individuals heterozygous for NOD2 variants have a two- to fourfold increased risk of developing CD, and this rises to 20- to 40-fold for homozygotes and compound heterozygotes [29]. Given its presence in unaffected individuals and absence in 70% of CD patients, there is limited utility in using NOD2 as a diagnostic test. It has been shown to be useful in phenotyping patients given its strong association with ileal disease location (odds ratio (OR) = 1.90) [28]. Beyond NOD2, HLA alleles have been associated with colonic disease [28, 30]. NOD2 and MHC, in addition to 3p21/MST1, were the three loci that achieved significance for association with disease phenotype in a large GWAS by Cleynen and colleagues. In this study, they also developed a composite genetic risk score from the other 163 susceptibility signals, and this risk score was able to distinguish UC, colonic CD, and ileal CD [31].

The Risk Stratification and Identification of Immunogenetic and Microbial Markers of Rapid Disease Progression in Children with CD (RISK) study, a landmark study of newly diagnosed treatment-naïve pediatric CD patients [32], took this one step further to develop a transcriptional risk score (TRS) that integrated GWAS risk loci, expression quantitative trait locus data, and RNA-seq data. This score performed better than genetic risk scores in distinguishing CD from non-IBD with the TRS of those with CD being significantly higher than those without IBD ($\Delta SD = 1.46$; $p = 1 \times 10^{-13}$) [33]. With more risk alleles being discovered each year and more sophisticated usage of expression profiles, there will likely be increasing granularity in these genetic and/or transcriptional risk scores as well as expansion to more diverse (non-Caucasian) populations.

4.1.4.2 Serologic Immune Response

Serologic immune responses to enteric pathogens were identified as biomarkers with 80% of CD patients positive for at least one of these serologic markers (anti-*Saccharomyces cerevisiae* (ASCA), anti-outer membrane C (Anti-OmpC), and anti-flagellins (anti-CBir1, anti-FlaI2, and antiFlaX) [34–36]. Perinuclear anti-neutrophil antibody (pANCA), which has been proposed to be the cross-reaction of the DNA-binding domain of histone H1 with a structural domain in mycobacteria [37], has a 60% prevalence in UC patients [38–40]. Subsequent years of research concluded that these serologic immune responses are of limited clinical value in primary diagnosis since they only have moderate sensitivity and negative predictive value. For example, a panel of serologic markers, the IBD7 panel, which contains anti-OmpC, anti-CBir-1, ASCA, and ANCA, showed only a 67% sensitivity and 76% specificity in diagnosis in a retrospective study of 300 pediatric patients [41]. On the other hand, their high specificity and positive predictive value still lend them to be considered for use in precision medicine as part of a larger model. A combined serologic, genetic, and inflammatory panel

improved the discrimination of IBD from non-IBD (area under the curve (AUC) 0.87; 95% confidence interval (CI): ± 0.04) when compared to a panel of six serologic markers alone (AUC, 0.80; 95% CI, ± 0.05; $p < 0.001$) [42].

4.1.4.3 Microbiome

The intestinal microbiome plays a pivotal role in the pathogenesis of IBD. This is supported by a characteristic dysbiosis in patients with IBD [43, 44], the success of fecal stream diversion in improving disease activity [45, 46], the abundance of susceptibility loci within IBD contributing to mucosal barrier function [23], and the appearance of colitis in germ-free animals, with genetic susceptibility, after the introduction of fecal bacteria [47]. There is a well-documented reduction in the microbial biodiversity in IBD, which alters the microbiome's ability to withstand changes from environmental disturbances [48, 49]. *Faecalibacterium prausnitzii*, a member of the *Firmicutes* family that is decreased in IBD, have been shown to play a protective role through their production of metabolites that reduce the secretion of inflammatory cytokines, and, thus, their depleted presence leads to an increased propensity for inflammation [50]. Other organisms that are increased, like *Escherichia* [51] and *Fusobacterium* [52], have been shown to exacerbate inflammation. Beyond the microbiome, the virome [53, 54] and fungome [55, 56] are currently receiving more attention, and we are seeing the slow unraveling of an increasingly complicated interaction of the host with its microbiome, virome, and fungome. Gevers et al. confirmed, in the large pediatric newly diagnosed CD population of the RISK study, that there are alterations in bacteria of the gut characteristic to CD [57]. This dysbiosis could represent a disease-specific signature useful in diagnosis.

4.1.5 Prognosis

4.1.5.1 Genomics and Transcriptomics

NOD2 has a role in prognostication since it is an independent risk factor for stricturing (OR: 1.82) disease, penetrating (OR: 1.25) disease, and the need for surgery (OR: 1.73) [28]. Beyond NOD2, a large GWAS divided CD patients into two groups based on prognosis. They found four loci tied to prognosis – *FOXO3*, *XACT*, a region upstream of *IGFBP1*, and the MHC region. Interestingly, none of the 170 susceptibility variants known at the time were tied to prognosis in their study, suggesting that the determinants of susceptibility and prognosis are different [58]. In RISK, it was also noted that CD patients with NADPH oxidase gene mutations were three times more likely to have perianal disease ($p = 0.0008$) and stricturing complications ($p = 0.002$). Abdominal surgery was also more common in the patients with NADPH oxidase mutations – 31% versus 9% ($p = 0.0004$) [59]. Perianal CD has also been noted to be highly associated with variations in the JAK-STAT pathway ($p = 3.72 \times 10^{-5}$) [60]. As with diagnosis, a more comprehensive risk score is more likely to be fruitful in determining prognosis. Marigorta et al. showed that their transcriptional risk scores could be used to predict progression to complicated CD ($\Delta SD = 0.63$; $p = 5 \times 10^{-5}$) [33].

The RISK study also assessed ileal gene signatures of newly diagnosed CD patients from tissue specimens, and they found novel signatures associated with the development of disease complications. Prior to RISK, it had been shown that mucosa overlying strictures have a pro-fibrotic gene signature [61]. In RISK, they showed that this signature, when enriched in genes that regulate extracellular matrix accumulation, was found in newly diagnosed patients without current strictures who ultimately progressed to stricture. Those who developed penetrating complications had more induction of genes regulating acute inflammatory responses to microbes. There were also signatures thought to confer protection, like a mitochondrial respiratory chain gene signature found in patients noted to be at risk for complications who did not develop complications [32]. Thus, tissue-level analysis of the gene expression may be the next frontier to better characterize the biology and target therapies.

4.1.5.2 Serologic Immune Response

Vasiliauskas et al. first introduced the concept that serologic immune responses could be used for prognostication when they reported that, in CD, high ASCA levels were associated with fibrostenosing and internal-penetrating disease and surgery and pANCA was associated with a more colonic, UC-like disease [62]. Omp-C and anti-Cbir1 have also been shown to be associated with more aggressive disease with a faster progression to complications [63]. Anti-Cbir1 was also noted to be more common with younger age of diagnosis in both CD [64] and UC [65]. A meta-analysis examined the utility of ASCA, anti-OmpC, anti-I2 (anti-*Pseudomonas fluorescens*-associated sequence I2), and anti-CBir1 in prognosis, and they found that anti-OmpC was associated with the highest risk of both complications (OR = 2.61; 95% CI, 2.16–3.15) and surgery (OR = 2.93; 95% CI, 2.48–3.47) in CD patients. They also noted that any two pooled antibodies increased the odds of complications (OR = 2.93; 95% CI, 2.42–3.56) and surgery (OR = 3.39; 95% CI 2.73–4.20) more than any single antibody [66]. In UC, high titers (≥100 EU/mL) of pANCA are associated with pancolitis [38] and pouchitis following ileal pouch anal anastomosis [67]. Perianal disease has been associated with higher titers of ASCA and Omp-C [60]. Beyond associations with complications after the fact, the RISK inception cohort showed serologies can be predictive of future complications, specifically CBir1 with future penetrating and stricturing behavior and ASCA IgA with future penetrating behavior [32]. It is thought that an even larger predictive model with genomics, transcriptomics, and the microbiome will be able to truly characterize and risk stratify patients.

4.1.5.3 Microbiome

Gevers et al. also showed that the microbiome dysbiosis (MD) index, which is the log of total abundance in organisms increased in IBD over total abundance of organisms decreased in IBD, strongly correlated with clinical disease severity, and this MD index could be used in the stratification of patients [68]. The Postoperative Crohn's Endoscopic Recurrence (POCER) study showed a specific microbial signature in patients with postoperative recurrence, specifically elevated *Proteus genera* ($p = 0.008$) and reduced *Faecalibacterium* ($p < 0.001$). Model of postoperative recurrence using these two bacteria in addition to smoking status was moderately predictive (AUC = 0.740; 95% CI = 0.69–0.79) [69]. Finally, it is also possible to detect virulence factors in stool, and, in a small 2018 study, differences in virulence factors between UC, CD, and non-IBD were shown (51% of CD, 26% of UC, and 14% of healthy controls) [70]. In fact, the metatranscriptomics that reveal the functional activity of the gut microbiome have been shown to provide an increased depth of understanding into the disease course, and understanding them could be key in understanding the dynamic microbiome [71, 72].

4.1.6 Treatment

4.1.6.1 Genomics and Transcriptomics

Pharmacogenomics, or the study of how genes affect a patient's response to a drug, has long been used in IBD with thiopurines, which are commonly used immunomodulators. Thiopurines have a complex metabolism, and one of the enzymes responsible for their metabolism, the thiopurine S-methyltransferase (TPMT) enzyme, has a genetically determined activity level with variable myelosuppression [73–75]. The 3% of the population who are homozygous for $TPMT^{L}$ have very low to absent activity, and the 11% who are heterozygous, $TPMT^{L}/TPMT^{H}$, have intermediate activity; standard doses of thiopurines in the homozygous group can lead to life-threatening myelosuppression [73]. Coenen et al. showed in 2015 that an empirical dose reduction in TPMT variant carriers led to a tenfold reduction in leukopenic events (relative risk (RR), 0.11; 95% CI, 0.01–0.85) [76]. This, thus, exemplifies the precision medicine principle of predetermining the right dose to prevent drug toxicity.

Pharmacogenomics is not as straightforward in the monoclonal antibody (MAb) space. NOD2 variants have not been shown to predict response

to infliximab (IFX) [77, 78]. Genetic variants in IL-23R have been implicated in IFX response in moderate-to-severe UC at week 14 with variants in certain SNPs increasing the probability of response (rs1004819, rs10889677, rs11209032, rs2201841, and rs1495965) and another subset decreasing the probability of response (rs7517847, rs11465804, rs10489629, and rs1343151) [79]. Genetic variants in two other cytokines, IL-6 (rs10499563 associated with better response) and IL-1 (rs4251961 associated with worse response), have also been linked with response to IFX [80]. Other variants in the Fas ligand, caspase-9, and FCGR3A genotypes have all been associated with altered response to IFX in CD patients [81, 82]. An autophagy-related 16-like 1 genotype (TT genotype of rs10210302) was associated with improved response to adalimumab when compared with another genotype (CC) [83]. Studies still need to be performed to see if variants in IL-23R might alter response to anti-p40 therapies which target IL12 and IL23 or anti-p19 future therapies.

Gene expression profiles may also help to predict response to therapy. Arijs et al. found predictive gene expression profiles for anti-TNFα responders in both Crohn's colitis and UC [84, 85]. Toedter and colleagues showed that responders to IFX had a modulation in their gene expression profiles, most notably in the T_{H1}, T_{H2}, and T_{H17} pathways [86].

4.1.6.2 Therapeutic Drug Monitoring (TDM)

The two clinically measured active metabolites of thiopurines are 6-thioguanine nucleotide (6-TGN) and 6-methylmercaptopurine (6-MMP). Multiple studies have noted an improved clinical response with 6-TGN > 235–250 pmol/8 × 10^8 RBC [74, 87–91], and it has been used as a proxy for measuring therapeutic effect. 6-MMP, which can also cause leukopenia [92], has been additionally associated with hepatotoxicity [93]. These two metabolites have been used to monitor for toxicity and assess for clinical response, and they set the historical stage for the more sophisticated use of TDM in the IBD space.

The concept of TDM has been adopted to optimize anti-TNF alpha therapies. ACCENT I showed that patients who failed IFX had lower serum IFX concentrations than those who had a sustained response (1.9 versus 4.0 μg/mL; $p = 0.03$) [94]. More studies are defining these trough concentration targets for Mabs with increasing accuracy since different TDM targets may be required for different phenotypes [95, 96].

There are two primary TDM strategies being implemented in current clinical practice. First, there is reactive TDM where drug concentrations are obtained when a patient experiences symptoms to determine if the patient has lost response due to low drug concentrations and/or the development of antidrug antibodies. Second, there is proactive monitoring with an algorithmic approach to drawing levels at certain time points to predict the appropriate dose and interval of medication prior to the development of symptoms and complications. Proactive monitoring fits well within the goals of precision medicine since it could improve patient outcomes by delivering the correct dose to a patient through preemptive dose monitoring and adjustment.

The Trough level Adapted infliXImab Treatment (TAXIT) trial, a groundbreaking prospective RCT of 251 IBD patients, showed that proactive dose adjustment to maintain trough IFX concentrations between 3 and 7 μg/mL leads to decreased disease activity [97]. In a large multicenter, retrospective study, it was shown that proactive monitoring in IBD reduced the risk of treatment failure (hazard ratio (HR), 0.16; 95% CI, 0.09–0.27; $p < 0.001$), IBD-related surgery and hospitalization (HR, 0.30; 95% CI, 0.11–0.80; $p = 0.017$ and HR, 0.16; 95% CI, 0.07–0.33; $p < 0.001$, respectively), and antidrug antibody formation (HR, 0.25; 95% CI, 0.07–0.84; $p = 0.025$). The most efficacious timing of the levels is not fully defined, but there is growing evidence to support earlier measurement of levels. Singh and colleagues showed in a pediatric population that target week 14 IFX concentrations could predict persistent remission at week 54 [98]. A 2016 study of UC patients showed that therapeutic week 6 ($p = 0.025$) and 14 ($p = 0.004$)

IFX trough levels were associated with higher likelihood of short-term mucosal healing [99]. The use of TDM is still much debated, and a recent study from GETAID reported no difference between using a combination of symptoms, biomarkers, and serum drug concentrations and symptoms alone on corticosteroid-free clinical remission [100].

4.1.6.3 Microbiome

A recent study by Kolho et al. examined whether the microbiome could be used to evaluate for response to anti-TNFα therapy. They showed that, by week 6 of therapy, both microbiome diversity and similarity to a healthy control microbiome were significantly higher in the anti-TNFα responders than in nonresponders ($p < 0.01$) [101]. Beyond use in the assessment of treatment response, there is a push to manipulate the microbiome as a therapy to restore intestinal microbial homeostasis and curtail the downstream inflammatory effects [102, 103]. Fecal microbial transplant was shown to transiently change the microbiome in patients with IBD [104], with some patients returning to their baseline dysbiosis after only 4 weeks [105]. Researchers are racing to develop techniques of more permanently or persistently altering the microbiome [106]. Enteral feeding [107] and dietary changes [108] are two of the more accepted techniques to change the microbiome, but these are rudimentary methods that set a high bar on patient compliance. The hope is to use the microbiome both to more accurately characterize patients and then to alter the microbiome in a targeted way to achieve a persistent homeostasis of the interconnected bacterial, fungal, and viral species living in the gut. Yet, this work is still in its infancy given the complexity of this web of organisms.

4.1.7 The Future of Precision Medicine in IBD

4.1.7.1 Prevention: The Next Frontier of Precision Medicine

Finding a preclinical predictive biomarker panel would be transformative in IBD. There is a known increased seroprevalence of ASCA in approximately 20–25% of asymptomatic first-degree relatives of patients with IBD [34–36]. In twin studies, they noted that concentrations of anti-OmpC and anti-I2 were observed in discordant monozygotic twins but not in discordant dizygotic twin pairs with CD (anti-OmpC, intraclass correlation (ICC), 0.80 in monozygotic and ICC, −0.02 in dizygotic; anti-I2, ICC, 0.56 and 0.05, respectively) [109], reflective of the multifactorial nature with contributions from both genetics and environment. The European Prospective Investigation into Cancer and Nutrition (EPIC) study showed that patients with a positive serological score, which was a relative regression coefficient of perinuclear anti-neutrophil cytoplasmic antibody (pANCA), ASCA IgG, anti-CBir1, and anti-OmpC, had a 23-fold increase in their risk for developing CD (OR, 23.1; 95% CI, 3–177) within 2.5 years of follow-up [110]. In the Proteomic Evaluation and Discovery in an IBD Cohort of Tri-service Subjects (PREDICTS) study, 65% of patients had at least one positive antimicrobial antibody present in their serum within a median time of 6 years before diagnosis, and the numbers of positive antibodies increased steadily leading up to diagnosis [111]. Thus, serologic responses could be used to institute preventative measures and/or the early identification of disease, especially in high-risk first-degree relatives.

Manipulation of the microbiome of at risk patients may also play a role in prevention of IBD. One recent "Exploring MEChanisms Of disease traNsmission In Utero through the Microbiome" (MECONIUM) study prospectively investigated the effects of maternal IBD on the infant's microbiome [112]. The IBD mother group as a whole had lower bacterial diversity ($p = 0.001$, ANOVA), and the placental microbiome was also changed with a decrease in *Firmicutes* and expansion in *Alphaproteobacteria* and *Actinobacteria* ($p = 0.001$, PERMANOVA, unweighted). The infants showed a different stool bacterial composition ($p = 0.001$, PERMANOVA, unweighted UniFrac) as compared to babies born to control, non-IBD mothers, and these differences were persistent over time. The mode of delivery did not affect the changes observed in

the infants' microbiomes [112]. This hints that perhaps achieving maternal bacterial homeostasis at critical time points in an infant's microbiome development might be worth exploring in those with a high genetic risk, especially since the microbiome is relatively fixed after 1 to 3 years old [113].

4.1.7.2 Decision Support Tools

Decision support tools are an important component of future precision medicine. Currently, these have been piloted to help patients better visualize their risk to better understand their priorities to make more informed decisions [114]. One of these models, PROSPECT, is a validated web-based tool to predict an individual patient's risk of developing a CD complication based on clinical, serologic, and genetic variables. It was found to have a discriminatory ability of 0.73 and 0.75 for adult and pediatric validation cohorts, respectively, using Harrell's *C*-statistic [115]. These tools will only become more refined with the real-time integration of more genomic, pharmacogenomic, and proteomic data into the clinical workflow.

Pharmacokinetics play such an important role in optimizing Mab therapies due to unique clearance mechanisms being the key to overcoming therapeutic failure. In a study of dashboard-guided dosing system for IFX in a pediatric population, 44% of patients had a week 14 IFX concentration less than 3 mg/mL, and they required non-standard-of-care dosing to achieve appropriate levels. In fact, within a machine-learning forecasting model using drug levels, presence of antidrug antibody, and clinical data, the standard-of-care dosing was only recommended in 11% of all patients [116]. There is an ongoing prospective intervention trial using dosing based on the dashboard-guided dosing, and the results will help shape the future role of such dashboards in the clinic.

4.1.7.3 CRISPR/Cas9 and Organoids

One exciting but fledgling technology within therapeutics is CRISPR/Cas9, which enables controlled exchange, insertion, and deletion of DNA sequences. The first clinical trial using this technology for gene therapy recently was approved by the NIH for the treatment of myeloma, melanoma, and sarcoma. The world is watching this closely as its success could change medicine. Of interest, CRISPR-Cas9 can now target multiple genes at the same time [117], and this gives more credence to the possibility that it could be used in the complex, multigene model of IBD.

Organoids are 3D cultures made from embryonic, induced pluripotent, or leucine-rich repeat containing G protein-coupled receptor 5 (Lgr5)-positive stem cells [118]. The 3D cell cultures can more closely mimic the complexity of in vivo cellular heterogeneity [119, 120], and they offer both a new and powerful way to identify molecular targets for therapies as well as an avenue for gene therapy when they are combined with CRISPR/Cas9 [118].

4.1.7.4 Crowdsourcing the Cloud

Precision medicine is optimized by collaboration from various investigators, and there are numerous platforms being developed to aid in this collaboration that could hasten therapeutic discovery. For example, the FDA has created precisionFDA, a cloud-based platform that could allow for crowd-sourced analysis of next-generation sequencing [121]. Additionally, crowdsourcing campaigns are gaining popularity; for example, a stem cell therapeutic company launched a campaign via social media to share its proprietary biomarker with qualified respondents who agreed to test the new biomarker tool and report back findings and data points [122]. There is a hope that this collaboration and crowdsourcing can speed up the development of new drugs.

4.2 Conclusions

Precision medicine is already a reality. However, the fully realized dream of finely targeted diagnosis, prognosis, treatment, and prevention is still evolving, and precision medicine's tantalizing promise to revolutionize medicine as we know it remains, as of yet, distant. The fear of the expense of personalization of medicine is well-founded, but there are efficiencies and economies in attaining precision that could offset this cost.

Summary Points

- Current use of precision medicine in IBD can be seen in:
  - Treat-to-target goals
  - Therapeutic drug monitoring
  - Stratification via serologic responses to enteric pathogens and genetic data
- Future applications of precision medicine within IBD are myriad and aimed at all aspects of the disease from diagnosis to prognosis and treatment to even prevention.
- Development of increasingly sophisticated decision support tools will make phenotypic and prognostic recommendations based on patient-specific findings (genetic profile, protein expression at the tissue level, and microbial signatures).
- Delivery of targeted therapy based on complex and proactive dashboard modeling that includes pharmacogenomics and other patient-specific factors, instead of trial and error, to reduce exposure to ineffective medications and avoid toxicity.
- Development of preclinical markers of IBD will facilitate earlier diagnosis, prevent morbidity and facilitate initiation of preventative measures.
- Development of more sophisticated therapies will target patient-specific biology in a collaborative fashion.

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5 Clinical Trial Design to Facilitate Biomarker Discovery

Anne S. Strik, Toer W. Stevens, and Geert R. D'Haens

Abstract

The role of clinical trials to generate knowledge about IBD treatments is very important. Endpoints have shifted from mainly subjective measures such as clinical disease activity, to patient reported outcomes in combination with objective measurements such as mucosal improvement. Even though therapeutic options for treatment of IBD are increasing, with many new therapeutic antibodies and small molecules under active investigation, treatment failure and withdrawal rates in clinical trials vary from 30–50%. Aside from side effects, primary non-response and secondary loss of response are possible reasons for discontinuation. More individualized treatment based on optimized dosing strategies and phenotypic and/or biological markers will probably lead to higher success rates. To move towards more individualized treatment in IBD, identification of biomarkers that can predict response to treatment would be valuable. Data and materials collected in clinical trials are a valuable source for identification of predictive biomarkers or development of pharmacokinetic models. By using a dashboard system with an incorporated pharmacokinetic model, the exact medication dose a patient should receive and the exact date it should be given to optimize drug exposure can be calculated. Pharmacokinetic analyses are increasingly used both in daily clinical practice as well as in clinical trials to individualize and thereby optimize medical treatment.

5.1 Introduction

With a wide range of therapeutic antibodies and small molecules under investigation, future options to treat patients with inflammatory bowel disease (IBD) appear promising. Several drugs have received market authorization (anti-TNF antibodies, vedolizumab and ustekinumab) [1, 2]. The JAK inhibitor tofacitinib is the most recently approved agent for ulcerative colitis. Additionally, other agents are in development that target inflammatory cytokines (anti-IL23, anti-IL36 and others), trafficking mechanisms (anti-MadCam, anti-αEβ7 and sphingosine-1-phosphate receptors) and the intestinal microbiome. Phosphodiesterase 4 inhibitors (e.g. apremilast and roflumilast) and various other JAK inhibitors (e.g. filgotinib and upadacitinib) are promising small molecules [3–5]. Despite intensive investment in drug development for IBD, only a limited number of compounds in the

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last decade have successfully completed the processes required for approval.

With an increasing number of available therapeutics, there is a need to optimize treatment selection and as such to improve efficacy. In most clinical trials, there are considerable treatment discontinuation rates varying between 30% and 50% for all of the available agents due to side effects, primary non-response or secondary loss of response [6–9]. Suboptimal dosing strategies may lead to failure early on.

In the future, a major challenge lies in identification of the most suitable drug for the individual patient, a concept that is commonly described as 'tailored or personalized treatment'. With the development of new technologies which enable high-throughput sequencing and detailed molecular characterization, the search for biomarkers in medicine is rapidly expanding. Once predictive biomarkers have been established, the time to (mucosal) remission will be shortened significantly leading to improved long-term outcomes and minimizing unnecessary exposure to potentially harmful drugs. In oncology, several biomarkers have already been incorporated in clinical decision-making. Predictive biomarkers such as HER2, BCR-ABL, BRCA, KRAS and the recent CDX2 mutations guide physicians in optimal treatment selection [10–15]. In the field of IBD however, predictive biomarkers are still lacking.

5.2 Biomarkers in IBD

5.2.1 Biomarkers

Biomarkers are measureable substances, structures or processes that can objectively evaluate disease states and therapeutic outcome. They should ideally be non-invasive, rapidly available, convenient, inexpensive, standardized and reproducible [16]. Many of the existing biomarkers in IBD focus on diagnosis, differentiation between Crohn's disease and ulcerative colitis and disease severity assessment. To move towards personalized treatment in IBD, the identification of biomarkers that can predict disease course and complications or predict response to treatment would be invaluable. These biomarkers should ideally be derived from pretreatment biological samples as this facilitates the optimal choice of drug and minimizes the time to remission. Sequential sampling after initiation of treatment provides the opportunity to characterize on-treatment changes in the molecular profile and to identify biomarkers of success or failure of treatment.

5.2.1.1 Biomarkers for Response Prediction

Many observational studies have looked at biomarkers that determine response to anti-TNF antibodies. In Crohn's disease the presence of ulcerations on endoscopy was associated with more favourable outcome. Very active disease at start of treatment, smoking and a longer disease duration are factors associated with poorer response. Although active disease has been associated with poorer response, two different trials demonstrated an association between high serum C-reactive protein (CRP) levels at baseline and response to infliximab treatment [17, 18]. A trial performed by Jürgens and colleagues in CD patients demonstrated that response to IFX treatment was associated with higher baseline CRP levels and that early normalization of CRP levels correlated with sustained long-term response. In patients with secondary loss of response, CRP levels at time of loss of response were significantly increased and did not return to baseline levels [17]. Colombel et al. demonstrated higher corticosteroid-free remission rates at week 26 in patients with high CRP levels at baseline (≥0.8 mg per decilitre) in CD patients treated with infliximab [18].

The rates of corticosteroid-free clinical remission at week 26 in both the combination-therapy group and the infliximab group, as compared with the azathioprine group, were greater among subgroups of patients with higher baseline CRP levels (0.8 mg per decilitre or more), baseline mucosal lesions, and both higher baseline CRP levels and mucosal lesions.

Looking at factors that predict anti-TNF success, many research initiatives have been

unsuccessful. Polymorphisms related to the FC receptor, TNF signalling, apoptosis and autophagy have all been studied and found to be somewhat associated with response, but large prospective validation using objective response criteria is still lacking [19]. Perhaps the combination of several different polymorphisms in a predictive genetic model provides the best approach for response prediction [20]. Gene expression profiling of the intestinal mucosa has yielded several potential predictive biomarkers for both anti-TNF and etrolizumab [21–24].

The most attractive approach is to make use of predictive biomarkers in the peripheral blood. An interesting protein marker that merits further research is IL-22. Baseline IL-22 serum level was associated with clinical response and remission to a selective anti-IL23p19 monoclonal antibody in a phase 2a trial [25]. Finally, exploration of the faecal microbiota composition warrants further study since certain microbiome signatures have been associated with response to anti-TNF, vedolizumab and ustekinumab [26–29].

5.2.1.2 Biomarkers for Disease Prognostication

An evolving goal in clinical trials and IBD management is to alter the natural course of the disease and to prevent irreversible damage and surgery [1]. After the initial diagnosis, optimal treatment varies from a classical step-up to a more aggressive top-down approach. Accurate patient stratification is of paramount importance as prognosis between individuals may vary substantially. In moderate to severe Crohn's disease, it has been shown that treatment with anti-TNF agents at an earlier disease stage was associated with higher efficacy in terms of clinical, endoscopic and histological endpoints [30–32]. Considering the risks of early immunosuppressive treatment, it is essential to identify patients with risk factors that predict a more complicated disease course who will benefit most from such an approach. Several clinical markers are associated with an aggressive phenotype such as smoking, significant weight loss (>5 kg) at time of diagnosis, active perianal disease, young age at diagnosis, corticosteroid dependency, extensive small bowel disease and perhaps deep colonic ulcerations [33–38]. However, the accuracy of these phenotypic markers is limited.

Increasing efforts are made in the identification of molecular prognostic markers. Four single-nucleotide polymorphisms were associated with prognosis and corresponded to the candidate genes XACT, MHC, FOXO3 and IGFBP1-IGFBP3. Interestingly, none of the 170 investigated disease susceptibility loci were associated with prognosis [39]. In a pediatric CD inception cohort (n = 913), a prognostic model for disease complications was developed that comprised older age at diagnosis, African-American race, ileal disease location and anti-*Saccharomyces cerevisiae* antibodies (ASCA) and anti-flagellin (CBir1) seropositivity [40]. To date, other molecular prognostic markers are under investigation.

5.3 Trial Designs in IBD

5.3.1 Types of Designs

A randomized double-blind placebo-controlled trial is considered the most optimal design to minimize bias in testing a new treatment. However, the field of clinical trial designs has evolved, and alternative designs are being explored to investigate novel therapeutic agents (Fig. 5.1). The sample size calculation is based on a clinically relevant difference from the control treatment (often placebo). In IBD, effect sizes of 10–20% are considered clinically relevant. Sample size planning must include unforeseeable circumstances such as missing values and patients that will be lost to follow-up during the trial. In the next paragraphs, the most commonly used clinical trial designs in pharmaceutical science will be discussed.

5.3.1.1 Randomized Controlled Trials (RCTs)

Patients participating in RCTs are randomly allocated, after giving consent, to the novel compound or placebo (or another control treatment). By assigning patients to different treatment

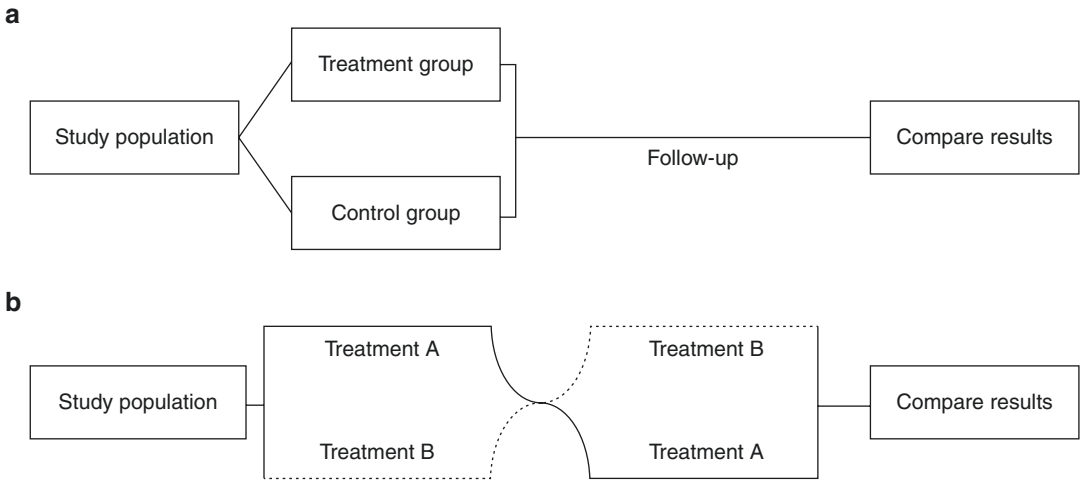

Fig. 5.1 Two frequently used clinical trial designs to investigate the treatment effect of therapeutic agents. (**a**) Study design randomized controlled trial. Patients are randomly allocated to the investigated drug (treatment group) or standard treatment/placebo (control group). (**b**) Study design crossover trial. Patients are assigned to a treatment arm and can 'crossover' to another treatment arm

groups using randomization and keeping other variables constant, treatment effect can be determined with a minimal risk of selection bias. The main reason to use randomization is to avoid baseline differences between treatment groups to ensure that differences in outcome between both groups can be attributed to the investigational product. A RCT may be blinded or non-blinded although bias is minimized in a double-blind design.

5.3.1.2 Crossover Trials

Although crossover trials can be observational or randomized, in healthcare the latter is the most frequently used design. In a crossover trial, patients are assigned to a treatment arm and can 'crossover' to another after a predetermined treatment duration. This crossover can be based on re-randomization and/or on initial response. Most crossover trials nowadays include an open-label extension phase in which treatment with the investigational product is guaranteed. By using each patient as his/her own control, the influence of possible confounders is minimal. Another advantage of this trial design is that a smaller sample size is needed compared to non-crossover designs.

5.3.1.3 Equivalence and Non-inferiority Trials

To demonstrate higher efficacy of a (new) drug over another (placebo or standard treatment), a superiority trial is necessary. However, a clinical trial can also be designed to demonstrate equivalence (i.e. new drug is neither worse nor better) or non-inferiority (i.e. new drug is not inferior) to an already existing treatment. The most optimal design to compare the efficacy of two different therapeutic agents is a head-to-head clinical trial. If no statistically significant difference is seen, the two interventions are considered equivalent provided the study is sufficiently powered to detect potential differences. Therefore, it is important to define a margin of non-inferiority or equivalence in the study protocol. If the 95% confidence interval of the difference does not cross the margin, the new drug is non-inferior or equivalent.

5.3.1.4 Post Hoc Analyses

Post hoc data analyses are common in large multicentre clinical trials. These types of analyses can be planned or unplanned, after completion/publication of the initial trial. Results from unplanned post hoc analysis can be controversial

in a way that differences discovered can be accidental (since the trial was usually not powered to address the question). Although data derived from post hoc analyses generally are of limited value, they can provide important information by which new hypotheses can be generated that can be explored in future (randomized controlled) trials.

5.3.1.5 Trial Design to Facilitate Biomarker Discovery

No gold standard trial design for biomarker discovery exists. Nevertheless, some recommendations can be made and opportunities exist for future research. Firstly, clinical trials should take advantage of the opportunity to collect biomaterials in a structured way from a large number of patients. This allows for treatment-by-biomarker interaction analysis which remains the most straightforward way to discover predictive biomarkers [41, 42]. Moreover, it allows for post hoc prediction analysis and together could promote biomarker discovery during early drug development. Examples of this are the development programmes of etrolizumab and a selective anti-IL23p19 antibody [24, 25]. Secondly, in order to obtain the required volume of data for integrative omics analysis, multicentre international collaborations are needed such as the IBD Character and the International Inflammatory Bowel Disease Genetics consortia. Finally and most importantly, healthcare providers, researchers, hospitals and the pharmaceutical industry need to collaborate. Clinically relevant biomarkers could already be present in one of the many databases that exist. Large academic hospitals should align their biobank protocols with regard to time points of sample collection, demographic data collection and outcome assessment. The development of a large research platform – including a biobank – called the IBD Plexus as an initiative from the Crohn's and Colitis Foundation of America (CCFA) exemplifies such an approach.

5.3.2 Endpoints in Clinical Trials

5.3.2.1 Need for Objective Endpoints

Clinical symptoms used to be the primary outcome in large clinical trials in IBD. This approach has been suboptimal since it led to high placebo response rates in many development programmes. In IBD (predominantly in Crohn's disease), the discrepancy between clinical disease activity and intestinal mucosal inflammation has been well documented [18, 43]. Ongoing inflammatory burden leads to an accumulation of bowel damage with increased risk of relapse, hospitalization, surgery and neoplasia over time [44–48]. Hence, in accordance with the advice of the International Organization of Inflammatory Bowel Disease in 2015, the main treatment goal has shifted towards a combination of both symptom control and healing of the intestinal mucosa [49], and clinical trials have adopted objective assessments in the primary endpoint, as well.

At the level of symptoms, composite scores that were developed by experts such as the Crohn's Disease Activity Index (CDAI) are being replaced by genuine patient-reported outcomes (PRO). So far, only the Inflammatory Bowel Disease Questionnaire [IBDQ], a disease specific quality of life instrument has been validated. Ongoing initiatives are working on new PRO's using an approach that has been recommended by the FDA. Additional questionnaires that are being used include the Short Form-36 [SF-36]), fatigue (Functional Assessment Chronic Illness Therapy-Fatigue [FACIT-F], work productivity (Work Productivity and Activity Impairment Questionnaire [WPAI]) and depression/anxiety (Hospital Anxiety and Depression scale [HADS]), but these are not IBD specific [50]. As a generic assessment of overall disease disability or overall disease control, the Inflammatory Bowel Disease Disability Index [IBD-DI] and the IBD CONTROL questionnaire have recently been developed and validated [51, 52].

At the level of objective disease assessment, mucosal healing is the most commonly used

criterion. The mucosa is usually assessed by endoscopy using validated endoscopic scorings with Simple Endoscopic Score for Crohn's disease (SES-CD) and MAYO Score for UC. The recorded videos are to be centrally read by independent gastroenterologists to provide objective, standardized grading of the mucosa without knowledge of treatment assignment.

5.3.3 The Role of Biomarkers in Clinical Trials

Biomarkers can be used to monitor patients and even optimize treatment during the course of a trial as recently demonstrated in the CALM trial [53]. In this multicentre prospective trial, Crohn's disease patients were randomly allocated to either a tight control treatment algorithm (based on the biomarkers faecal calprotectin and CRP) or a conventional treatment algorithm. A significantly higher proportion of the patients in the biomarker driven 'tight control' group compared to the clinical management group achieved mucosal healing at 48 weeks (45.9% vs 30.3%, $p = 0.01$) [53]. These findings emphasize the potential role for biomarkers in monitoring patients, identifying underlying intestinal inflammation and adjusting the treatment strategy accordingly.

Over the past few years, with the advent of stringent endoscopic endpoints, the burden on patients participating in a clinical trial has increased significantly since the trial designs have become increasingly demanding. In particular the need for repeated endoscopies is challenging and unpleasant. Surrogate markers of inflammation could potentially alleviate this burden. A promising assay that reflects mucosal healing quite accurately was recently presented by a group of international investigators in collaboration with Prometheus Laboratories. The serum assay measures 13 proteins related to tissue injury and remodelling in an algorithmic model that is associated with mucosal disease severity in Crohn's disease [54].

5.3.4 Pharmacokinetics and Pharmacodynamics in Trials with Monoclonal Antibodies

A standard randomized double-blind placebo-controlled clinical trial is still considered as the most optimal design to test the efficacy of a new drug. This type of study design is increasingly supplemented by advanced pharmacokinetic monitoring and even dose optimization (TDM or 'therapeutic drug monitoring'). Pharmacokinetic and pharmacodynamic analyses are used to identify factors which affect the clearance of the drug, to select the most optimal administration route and to develop specific dosing algorithms. Drugs with a therapeutic 'threshold' or 'window' (e.g. monoclonal antibodies, azathioprine, cyclosporine) require correct dosing to control intestinal inflammation, without under- or overdosing.

The development of monoclonal antibodies is complex and requires careful pharmacological evaluation. Firstly, pharmacokinetic and pharmacodynamic preclinical and phase 1 data are used to identify the appropriate starting dose for human trials. Extrapolation of preclinical results is often challenging since nontarget-related disposition can show large species-specific variability. Another important point to take into consideration is immunogenicity (i.e. the formation of antidrug antibodies) which also has large interindividual variability potentially determined by the HLA genotype. It also needs to be determined whether a therapeutic antibody should be dosed based on body weight or alternatively dosed using a fixed dose. A retrospective analysis performed by Wang et al. showed no difference between fixed and weight-based dosing of monoclonal antibodies at a population level [55]. Weight became only relevant in extremely under- or overweight patients. They recommended starting with fixed dosing in early drug development until data about the effect of body weight on clearance of that monoclonal antibody was available. Testing a drug in the target patient population will lead to important pharmacological insights since these data will then be derived

from patients with a wide range of disease severity. Since disease activity itself can be a covariate for drug clearance, collecting pharmacological data during phase II and III trials is essential. After gathering sufficient clinical data during trials in the target patient population, a population-based pharmacokinetic model can be developed. Several important questions such as factors influencing drug clearance, specific dosing algorithms and the necessity of an induction schedule can be addressed. Initiation of treatment with an induction phase before continuation to maintenance treatment is usually preferable for monoclonal antibodies with non-linear pharmacokinetics to saturate or clear available antigen targets.

5.3.4.1 Dashboard-Driven Dosing

A useful tool for non-pharmacologists to use population-based pharmacokinetic models is a dashboard system [56]. A dashboard system is a specific computer system with an incorporated pharmacokinetic model. By using a Bayesian approach, the dashboard system calculates the exact dosage a patient should receive and the exact date this dosage should be given to maintain a certain drug concentration in the blood. Individualization of dosing increases with increasing patient specific information about serum drug concentrations at different time points and all known covariates influencing drug clearance (e.g. patients' body weight and serum albumin). To optimize its predictive value, frequent blood sampling throughout treatment is necessary. To date, pharmacokinetic analyses are increasingly used both in daily practice as in clinical trials to individualize and thereby optimize medical treatment.

5.4 Conclusion

To date, available biomarkers for IBD lack accuracy to predict prognosis or treatment response. In the foreseeable future, a combination of signals reported in the literature may lead to prognostic models. Clinical trials as part of drug development collect patient data and biological materials (DNA, biopsies, serum, faeces, urine) in parallel. This biological material can be a source for the identification of new molecular markers. Treatment-by-biomarker interaction and post hoc analysis using initially collected material during the trial may result in new insights regarding differences in therapeutic response between patients.

Summary Points

- Clinical trials have contributed to our understanding of IBD treatment and response
- Objective measurements such as mucosal and biochemical response are important endpoints
- Advanced pharmacokinetics are increasingly used in clinical trial development
- Dashboard systems are useful tools for physicians to apply therapeutic drug monitoring
- Datasets derived from clinical trials are potentially valuable for biomarker discovery

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Part II

Clinical Algorithms Incorporating Predictive and Prognostic Biomarkers: Crohn's Disease

Luminal Crohn's Disease

6

Amanda M. Lynn and Edward V. Loftus Jr

Abstract

Crohn's disease (CD) is a chronic inflammatory disorder which can lead to progressive intestinal damage and debilitating complications over time. Therefore, obtaining an accurate assessment of disease activity is paramount for risk-stratifying patients and initiating or escalating therapy when appropriate. Within the classic management paradigm, treatment decisions have been largely predicated on patient-reported symptoms. However, more recent evidence suggests that the correlation between clinical symptoms and endoscopically active disease is poor. A significant proportion of patients in clinical remission will have unrecognized active disease; conversely, many patients with mucosal healing will continue to have symptoms. This disconnect highlights the fact that focusing solely on clinical remission will inevitably lead to either undertreating those with clinically silent disease or overtreating those with symptoms unrelated to their CD.

Therefore, in addition to assessing clinical symptoms, there is a need to incorporate more objective markers of disease activity into the management of CD. In the pursuit of providing such information, a number of tools have been assessed in their ability to risk stratify patients, predict active disease, assess risk of relapse or recurrence, and monitor response to therapy. These include serologic, fecal, radiographic, and endoscopic modalities. When used in concert with clinical symptoms, they provide a more detailed and accurate assessment of overall disease activity, in turn allowing for more informed therapeutic decision-making.

6.1 Background

Historically, treatment targets for Crohn's disease (CD) have emphasized clinical remission with symptom control. However, there is increasing evidence that symptoms correlate poorly with the level of disease activity [1, 2]. If symptoms alone are utilized to direct therapeutic decisions, patients are more likely to be undertreated, allowing cumulative mucosal damage to occur in the setting of under-recognized active inflammation. Furthermore, an important minority of patients with conditions such as bile acid malabsorption may be overtreated if symptoms alone are used to make treatment decisions. Achieving deep remission becomes important as it has been demonstrated that CD patients who achieve mucosal healing have better outcomes than their counterparts who do not [3–5].

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With this in mind, treatment endpoints are rapidly evolving to encompass both clinical and biologic remission, in the hope of altering the course of disease and preventing future complications and disability [6, 7]. This approach will necessitate the use of more objective markers of inflammation in order to more closely monitor disease activity over time, so that therapy can be escalated when appropriate – not only when symptoms develop. A number of serologic, fecal, radiographic, and endoscopic techniques have been evaluated for this purpose.

6.2 Biochemical Markers

Although objective assessment of disease activity can be achieved with radiographic and endoscopic methods, these are expensive and invasive, making biomarkers an attractive and increasingly studied method of monitoring disease activity in inflammatory bowel disease (IBD). Several serologic and fecal biomarkers have been explored to fulfill this need, though few have been shown to clearly correlate with disease activity or future risk of complications. C-reactive protein (CRP) and fecal calprotectin are exceptions, as both have been extensively studied and demonstrate correlation with disease activity and ability to risk stratify patients and to predict response to therapy and future risk of complications.

6.2.1 C-Reactive Protein (CRP)

CRP is an acute phase reactant produced by hepatocytes in response to systemic inflammation. It is a non-specific marker that becomes elevated in a variety of conditions. Of all biochemical markers in IBD, it is the most extensively studied and shown to have likely the best performance in its ability to parallel endoscopic and histologic findings of active CD [8, 9]. Its short half-life, affordability, and accessibility make it an ideal marker for objectively evaluating disease activity in real time.

Aside from providing information regarding the current level of disease activity, it may also predict response to therapy. Patients with higher pre-treatment CRP levels experience higher likelihood of response to anti-TNF therapy compared to those with normal CRP [10–12]. Moreover, normalization in CRP levels following treatment with infliximab has correlated with higher levels of sustained response compared to those with persistently elevated levels [10].

Beyond therapeutic response, a higher index CRP and/or persistently elevated CRP during therapy is associated with increased risk for hospitalizations, readmissions, and use of biologic therapies [13–15]. Reports regarding its ability to predict future CD-related surgery are variable [15–19].

Some of the variability among studies may be explained by the significant heterogeneity in CRP response based on disease location and even within individual patients. An estimated 25–33% of CD patients with active disease will have normal CRP at diagnosis, with levels remaining normal despite ongoing activity and/or subsequent flares [13, 16]. Genetic polymorphisms are postulated to play a role in this inconsistency [1, 20, 21]. For this reason, monitoring CRP is more useful in patients who have had an elevation at baseline.

In regard to disease location, several studies have noted less correlation between CRP and disease activity in ileal versus ileocolonic or colonic CD [1, 17, 22]. The explanation for this is unclear, but one theory suggested that ileal CD may affect the locoregional environment, while colonic CD creates a larger systemic response [22]. This incongruency has been suggested by a number of studies in which abnormal small bowel imaging did not positively correlate with CRP level [8] and whereby there was a lower prevalence of CRP elevation among patients with ileal versus colonic or ileocolonic CD (43.2% vs 70% and 72.6%, $P = 0.002$) [13]. However, discordant results have been published suggesting disease location has little to no effect on CRP level [16]. Therefore, further investigation is required to understand the utility of CRP as a biomarker in ileal CD.

6.2.2 Fecal Calprotectin

Fecal calprotectin (fCal) is a cytosolic protein present in granulocytes. As neutrophilic migration to the gastrointestinal mucosa occurs with inflammation, it becomes detectable in stool. The idea that levels rise in parallel with the degree of inflammation has been exploited as a means of monitoring disease activity in IBD. Proposed applications include assessing response to therapy, risk of relapse, candidacy for treatment de-escalation, and risk of recurrence postoperatively.

Several studies have established a positive correlation between fCal levels and the severity of inflammation, including Schoepfer et al. who demonstrated significant correlation with the Simple Endoscopic Score for Crohn's Disease (SES-CD) [23]. Reported sensitivity and specificity for detecting endoscopically present inflammation ranged from 70–100% to 44–100%, respectively [1, 23–25]. Variation in performance characteristics is likely explained by differences in specific cutoff levels utilized.

Once disease activity has been established and CD-directed therapy initiated, fCal can be utilized in monitoring over time. A decrease in fCal following induction therapy with anti-TNF has been shown to predict improved maintenance of remission and rates of endoscopic healing [25]. Moreover, there is evidence to suggest that fCal levels begin to rise 4–6 months prior to clinical relapse, implying that this could provide a predictive model to preemptively perform necessary diagnostic procedures and/or therapeutic intervention [26]. The overall sensitivity and specificity to predict relapse within a year is between 43–90% and 43–88%, respectively, depending on cutoff values used [27–32].

In patients who require intestinal resection, endoscopic recurrence rates are high, at up to 80% within the first year. Although endoscopy is typically employed for surveillance, there is no predefined timeframe during which this should be performed, and recurrence may be missed entirely in patients who remain asymptomatic. Therefore, fCal has been proposed as a noninvasive method of risk-stratifying and monitoring patients postoperatively and has demonstrated promising results. Results from the postoperative Crohn's endoscopic recurrence (POCER) study demonstrated that FC at 6 months can be useful in predicting patients with relapse and need for step-up therapy [74]. However, it is difficult to make any gross generalizations regarding specific cutoff values that are deemed "high risk," as those used in available studies are highly variable [34, 35].

Similar to CRP, fCal may be less reliable in assessing ileal disease [1, 23]. However, it has also been argued that these studies may be misleading, as many did not intentionally assess for isolated small bowel disease, potentially leading to underrepresentation of this population. Jensen and colleagues evaluated this specific question using full ileocolonoscopy, enterography, and capsule endoscopy, during which they found similar sensitivities for fCal to detect isolated ileal versus colonic disease [33]. Therefore, although the majority of the literature seems to suggest fCal is less reliable in isolated small bowel disease, further exploration is required.

6.2.3 Emerging Biochemical Markers

Additional biochemical modalities to monitor disease activity and predict or assess response to therapy continue to evolve. In an effort to noninvasively assess disease activity, a newer serologic test has been developed that incorporates 13 serum-based biomarkers into an algorithm to construct a validated scale, termed the Mucosal Healing Index (MHI) [79–81]. The MHI generates a score between 0 and 100 to assess mucosal healing, with a negative predictive value of 92% and positive predictive value of 87% in identifying patients with endoscopically active disease. Moreover, the performance characteristics of MHI seem to be comparable across anatomic disease locations, which theoretically could be a major advancement given that other available serology-based tests, such as CRP, are limited in the assessment of isolated ileal disease. However, the methodology behind the test is opaque and has not been independently validated by a third party, so the test's true operating characteristics remain unclear.

In addition to serologic assays, newer stool-based tests are also being explored. One such modality, proposed by Ananthakrishnan and colleagues, makes use of the fecal microbiome to predict clinical outcomes [82]. Utilizing a prospective cohort of UC and CD patients receiving gut-selective anti-integrin therapy, it was demonstrated that early changes in the fecal microbiome could predict response to therapy long term. This information was then combined with clinical data to create a more comprehensive prediction model able to accurately classify response to therapy. While currently this has only been studied in anti-integrin therapy, the hope is that similar models could be constructed for use with other IBD therapies. This could then become a potential method by which the various biologic therapies could be directed to patients in a more individualized manner, based on their anticipated responses to certain medication classes.

6.2.4 Role of Fecal and Serologic Markers in Diagnosis and Monitoring

Both serologic and fecal biomarkers provide more objective measurements of disease activity than clinical symptoms. Their use is attractive due to their cost-effectiveness, accessibility, and noninvasiveness. However, heterogeneity among studies regarding their performance, possible varied utility based on disease location, and lack of specificity for IBD complicates their use, and the frequency and methods by which they are to be used are not well-established. In practice, it is unlikely that a single biomarker would suffice as a noninvasive proxy for assessment of disease activity and prognostication. Rather, they should be used as surrogate markers to identify patients who are most likely to benefit diagnostically from further radiographic and/or endoscopic assessment.

6.3 Radiologic Modalities

While the use of endoscopy in the assessment of CD is well-established and widely available, it is limited in its ability to evaluate transmural disease, extraluminal complications, and isolated involvement of the small bowel proximal to the terminal ileum. Moreover, significant edema or fibrostenotic complications may completely preclude ileal intubation and small bowel visualization. Even when ileal intubation is possible, prior series have demonstrated that over 50% of subjects with an endoscopically normal terminal ileum will have active proximal small bowel or isolated transmural disease identified via computed tomography enterography (CTE) or magnetic resonance enterography (MRE) [36]. As many CD complications develop insidiously in the absence of symptoms, care must be taken to consider the need for radiographic studies in the evaluation of CD patients, understanding that such complications may be missed with endoscopic assessment alone.

The most utilized and well-studied radiographic modalities in CD are CTE and MRE, which have high sensitivity and specificity in evaluating small bowel inflammation [37, 38]. Beyond the identification of active inflammation, radiographic response may also be an adequate treatment target, as Deepak and colleagues reported that demonstrating radiologic response on either CTE or MRE was associated with reduced future risk of corticosteroid use, hospitalizations, and surgeries among patients with small bowel CD [39].

With the increasingly important role that cross-sectional imaging plays in the assessment and surveillance of CD, a number of scoring systems have been developed to provide a more objective measurement of disease activity and severity [40–44]. Only a few have been validated for MRE, with none having formal validation for CTE.

6.3.1 Magnetic Resonance Enterography

Though the sensitivity for detecting small bowel inflammation is similar for both MRE and CTE [38], MRE is often touted as the preferred modality given its lack of ionizing radiation exposure. This is particularly true when considering the chronic nature of CD, the generally young popu-

lation it affects, and the expanding use of cross-sectional imaging for disease monitoring.

Additional strengths of MRE include excellent soft-tissue contrast and diffusion-weighted imaging with assessment of intramural edema and wall thickness. In pregnant patients and those who have poor renal function, IV contrast can be avoided. The main downfalls are its higher cost, longer acquisition time, and decreased image quality from motion artifact when patients are unable to remain still during image acquisition. Both CTE and MRE are less accurate in patients who are unable to tolerate oral contrast. It also does not allow for tissue collection and may miss isolated mucosal inflammation. Findings shown to best correlate with active disease include bowel wall thickening and enhancement, mural edema, and ulcerations.

The use of MR has been best validated in the evaluation of terminal ileal and colonic disease [40, 41], though additional studies have assessed its use in small bowel inflammation proximal to the terminal ileum [42, 45]. There is also evidence to suggest that the ability of MR to accurately detect involvement in the very proximal small bowel may be lower, and when suspicion is high, endoscopic examination of these regions may be required [46, 47].

6.3.2 MR Scoring Systems

6.3.2.1 Magnetic Resonance Index of Activity (MaRIA) Score

The MaRIA score was developed to provide objective measurements of disease activity, allowing for classification of disease severity and monitoring of therapeutic response in the terminal ileum and colon [40, 41]. It utilizes wall thickness, relative contrast enhancement (RCE), edema, and ulcerations seen on MR enterocolonography to provide a quantitative index of disease activity. These MR findings closely paralleled the severity of endoscopic lesions graded with the Crohn's Disease Endoscopic Index of Severity (CDEIS) ($r = 0.82$, $p < 0.001$).

The initial validation studies assessed its utility against ileocolonoscopy, so there was no endoscopic comparison data to determine its utility in assessment of deeper small bowel lesions. Takenaka and colleagues [45] attempted to answer this question by adapting the SES-CD and MaRIA scoring systems to evaluate deeper small intestinal lesions with the use of MR enterocolonography and single-balloon enteroscopy. They demonstrated good correlation between the adapted SES-CD and MaRIA scores to assess terminal ileal and proximal ileal segments ($r = 0.808$, $P < 0.001$).

Owing to its cumbersome nature, the widespread use of the MaRIA score in clinical practice has been limited. An additional concern is the fact that it does not take into account total extent of disease.

6.3.2.2 Crohn's Disease MRI Index (CDMI) Score

The CDMI score was created to provide a simple MRI index of disease activity for luminal small bowel CD [42]. The derivation study included 16 patients who underwent MRE within 2 weeks of an elective small bowel resection. Transmural histopathological sampling and scoring was performed for comparison with MRE findings at 44 different locations. Mural thickness and T2 signal had the best predictive value for acute inflammation and are utilized in calculating the CDMI score.

Though the use of the CDMI is more simplistic than the MaRIA score, it too is limited in its ability to assess disease extent. Furthermore, additional validation and assessment of utility in clinical practice are required.

6.3.2.3 Magnetic Resonance Enterography Global Score (MEGS)

MEGS was developed as an attempt to expand upon the CDMI score by including disease length, colonic haustral loss, and extraenteric complications [44]. The initial study included a cohort of 71 CD patients who underwent MRE, fCal, and CRP.

MRI features of wall thickness, T2 signal, peri-mural mesenteric edema, and post-contrast T1 enhancement level were scored within nine predefined segments of the bowel, utilizing the same system as the CDMI. Measured length of

disease provided a multiplication factor for segmental scores, and points were added for the presence of extraenteric findings.

Wall thickness, mural T2 signal, and disease length were significant predictors of active disease, defined as fCal >100 μg/g. Additional validation was performed in a more recent study of CD patients receiving anti-TNF therapy, in which the median MEGS score decreased significantly in clinical responders compared to non-responders [48].

Strengths of this score include its ability to account for disease length and extramural complications. However, it is cumbersome for radiologists and due to its complexity in reporting would likely require advanced training, limiting widespread clinical use.

6.3.2.4 Clermont Score

The Clermont score resulted from a derivation study aiming to determine if diffusion-weighted imaging MRE (DWI-MRE) could accurately assess small bowel inflammation compared to standard MRE and the previously validated MaRIA score [49]. DWI provides benefit over conventional MRE in its avoidance of gadolinium.

DWI-MRE was found to have high correlation with standard MRE technique. Furthermore, there was good inter-observer agreement for qualitative measures of activity including wall thickening ($r = 0.83$, $P < 0.001$) and RCE ($r = 0.65$, $P < 0.001$), as well as the quantitative measure of apparent diffusion coefficient (ADC) ($r = 0.74$, $P < 0.001$). The score had a high sensitivity and specificity (82.4% and 100%) to differentiate active from inactive CD [49, 50].

Advantages of this score include its use of DWI imaging, which allows for avoidance of gadolinium administration. However, like other scoring systems, clinical use is limited due to its time-consuming nature.

6.3.3 Computed Tomography Enterography

CTE has been validated against endoscopic and histologic standards of reference in the accurate identification of active small bowel disease [37, 38]. Typical findings with active inflammation include mucosal hyperenhancement, wall thickening, mesenteric fat stranding, and dilated vasa recta [51, 52]. Importantly, these parameters improve with CD-directed therapies, making them useful in monitoring response to therapy [53]. With its high sensitivity (>90%) for the detection of small bowel inflammation, it has become an important adjunct in the assessment of CD patients.

Faubion et al. demonstrated that combining information from CTE and ileocolonoscopy provided an assessment of disease activity that better correlated with inflammatory biomarkers compared to endoscopic assessment alone [54]. Furthermore, supplementary information obtained during CTE may influence management plans, as Bruining and colleagues reported that treatment strategies were altered based on CTE results in 51% of patients [55].

Like MRE, CTE is advantageous in its ability to detect extraluminal or transmural disease and can potentially differentiate inflammatory from fibrostenotic strictures. CT may be preferred in patients who suffer from claustrophobia or are unable to lie still for prolonged periods due to shorter acquisition time. Despite these benefits, exposure to ionizing radiation remains a major drawback. Though validated radiation dose-reduction techniques have demonstrated adequate sensitivity in detecting active small bowel CD [56], further exploration is required and availability of such techniques among centers may vary.

Currently, no validated scoring systems exist for CTE. Given the use of ionizing radiation, CTE is unlikely to be of major use in clinical trials and is not advocated for repeated use in young patients. However, it may hold merit in the situations described above or when MRE is unavailable. Due to its wider availability compared to MRE in nonacademic centers, further studies to develop and validate CTE-based scoring systems are needed.

6.3.3.1 Abdominal Ultrasonography

The use of abdominal ultrasonography (US) is of growing interest in the evaluation of luminal

CD. Several advantages exist over the use of CTE and/or MRE, including low cost, portability, wide accessibility, avoidance of ionizing radiation, and (in some protocols) avoidance of oral or IV contrast administration. In a recent review by Calabrese and colleagues [57], US was reported to have good correlation with both endoscopy and cross-sectional imaging at detecting CD lesions, assessing disease extent, determining level of disease activity, and identifying postoperative recurrence. Performance characteristics were best for terminal ileal followed by colonic lesions, with lower accuracy for identifying more proximal small bowel lesions.

In the same review, the ability of US to detect complications such as strictures, fistulas, or intra-abdominal abscesses was felt to be comparable to CTE and MRE [57]. Furthermore, a newer US modality utilizing shear wave elastography (SWE) may be able to better differentiate inflammatory from fibrotic strictures with the use of acoustic radiation force impulse technology to assess the elastic properties of tissue [58, 59]. This affords SWE a unique advantage in determining the composition of a stricture, a factor which has significant impact on the selection of either medical or surgical management.

Contrast-enhanced ultrasound (CEUS) has also been proposed as another modification to current US protocols to assess disease activity. Examination requires IV administration of contrast microbubbles, which are reportedly quite safe and without an increased risk of nephrotoxicity, likely due to elimination via the lungs within 10–15 minutes of IV administration [60]. However, use may be limited in patients with significant cardiac comorbidities. A recent systematic review and meta-analysis on the use of CEUS by Serafin et al. reported a pooled sensitivity of 94% and specificity of 79% for detecting active CD [61]. However, due to methodologic heterogeneity, lack of objective diagnostic thresholds, and small sample sizes of available studies, the quality of available evidence on this topic is limited. Larger prospective studies are needed prior to implementing its widespread use in clinical practice.

The use of US in the evaluation and serial monitoring of CD patients is promising, though its widespread adoption has been curtailed for a number of reasons. First of all, its utility is highly reliant on the experience of the operator and the location of bowel attempting to be visualized, the latter of which can become a significant issue particularly in obese patients or in deep-lying bowel segments. Furthermore, there exists no widely accepted consensus or objective diagnostic threshold by which to classify CD activity. Further study is required before defining the specific roles that varying US modalities will play in clinical practice.

6.3.4 Role of Imaging Modalities in Diagnosis and Monitoring

MRE and CTE have become indispensable tools in the evaluation of small bowel CD, with US and its varying modalities gaining wider acceptance. In current practice, MRE is often preferred due to its lack of ionizing radiation. Emerging data has demonstrated promise for the use of both CTE and MRE in monitoring treatment response, and the potential utility of radiographic response as a treatment endpoint continues to evolve. Studies are currently lacking in this regard for US techniques. Though further studies are needed, these radiographic tools hold a promising role in the ongoing assessment, risk stratification, and guidance of therapeutic decisions for CD patients.

6.4 Endoscopy

Ileocolonoscopy (IC) has long been utilized for diagnosis, assessment of disease extent and severity, prognostication, and surveillance of dysplasia in IBD. In recent years, IC has found an expanding role in assessing therapeutic response, paralleling the recognition of mucosal healing as a preferred treatment target over clinical remission. With increased use of endoscopy in monitoring response to therapy, attempts have been made to standardize the process by creating a number of endoscopic scoring systems.

The two validated endoscopic scoring systems for CD activity include the Crohn's Disease Endoscopic Index of Severity (CDEIS) [67] and

Simple Endoscopic Score for CD (SES-CD) [63]. Additionally, the Rutgeerts score has been deemed the gold standard in prognosticating postoperative disease recurrence [64].

6.4.1 Crohn's Disease Endoscopic Index of Severity

CDEIS has historically been deemed the gold standard for endoscopically classifying disease activity in CD. The system measures multiple endoscopic parameters within five different segments of the bowel, for a total score of 0–44 (Table 6.1) [62]. A score >12 typically constitutes severe disease, while a score of 0–3 suggests remission [65]. It has demonstrated accuracy and reliability with good inter- and intra-observer correlation, making it popular for use in clinical trials [66].

Despite its widespread use in clinical trials, no well-established values to define endoscopic response or remission exist. In several trials, clinical response has been described as a decrease in the baseline CDEIS by 3–5 points. However, a more recent post hoc analysis of data from the SONIC trial suggested that a decrease in CDEIS >50% results in higher rates of corticosteroid-free remission [67–69]. Not only has there been disagreement regarding cutoff values defining response, but the definition of endoscopic remission varies among studies as well, with cutoffs ranging from 0 to 4 [70, 71].

Due to its complexity and requirement for specialized training, it lacks practicality in clinical settings. Furthermore, concerns exist regarding the potential underestimation of disease severity when the disease is localized to only one segment. For these reasons, along with the lack of well-defined cutoff values, its use is largely reserved for clinical trials.

6.4.2 Simple Endoscopic Score for Crohn's Disease

The SES-CD was developed as an attempt to provide a simpler scoring system for use in clinical practice. It has since demonstrated reproducibility and good correlation with CDEIS ($r = 0.920$) [67]. Scoring involves assessment of four endoscopic variables within the same five segments of bowel to produce a score between 0 and 56 (Table 6.2).

Table 6.1 Crohn's Disease Endoscopic Index of Severity (CDEIS) [62]

	Values for scoring					Totals
Variable	Ileum	Right colon	Transverse	Left colon	Rectum	
Deep ulcerations (12 if present, 0 if absent)	0–12	0–12	0–12	0–12	0–12	Total 1
Superficial ulcerations (6 if present, 0 if absent)	0–6	0–6	0–6	0–6	0–6	Total 2
Surface involved by disease (cm VAS)	0–10	0–10	0–10	0–10	0–10	Total 3
Surface involved by ulcerations (cm VAS)	0–10	0–10	0–10	0–10	0–10	Total 4
	Sum of totals 1–4 =					Total A
	Number of segments visualized in part or entirely (from 1 to 5)					n
	Total A/n =					Total B
	If *ulcerated stenosis* in any segment, add 3					Total C
	If *non-ulcerated stenosis* in any segment, add 3					Total D
	Total B + C + D =					CDEIS score

VAS visual analogue scale (range 0–10, as the VAS is 10 cm long)
CDEIS scores (range 0–44)
0–3 = remission
3–9 = mild disease
9–12 = moderate disease
>12 = severe disease

Table 6.2 Simple Endoscopic Score for Crohn's Disease (SES-CD) [63]

	Values for scoring			
Variable	0	1	2	3
Size of ulcers	None	Aphthous ulcers (ø = 0.1–0.5 cm)	Large ulcers (ø = 0.5–2.0 cm)	Very large ulcers (ø > 2.0 cm)
Proportion of ulcerated surface	None	<10%	10–30%	>30%
Proportion of affected surface by any lesion	Unaffected	<50%	50–75%	>75%
Presence and severity of stenosis	None	Single, can be passed	Multiple, can be passed	Cannot be passed

Ø = diameter
SES-CD score (range 0–56)
0–2 = remission
3–6 = mild disease
7–15 = moderate disease
>16 = severe disease

Although initially intended to offer a more simplified scoring system, it remains complex. As with CDEIS, no standardized cutoffs exist to define endoscopic response or remission. It has been suggested that endoscopic response be defined as a >50% decrease in SES-CD, while a score of 0–2 should indicate remission [68, 69, 72].

SES-CD has higher utilization than CDEIS in clinical practice, with many software programs for endoscopic reporting simplifying its calculation. Though like CDEIS, caution should be taken in that an incomplete examination may result in underestimation of disease severity, given the presumption that any unexamined segments are "lesion-free."

6.4.3 Rutgeerts Score

The cumulative incidence of intestinal resection in CD patients over 10 years is >50% [73]. Moreover, endoscopic recurrence within 1 year of ileocolonic resection is high, even in the absence of symptoms [64, 73]. For this reason, Rutgeerts et al. developed a scoring system to predict postoperative recurrence in CD patients following ileocolonic resection [59].

The Rutgeerts score ranges from i0 to i4 based on extent and severity of lesions involving the ileocolonic anastomosis and neoterminal ileum (Table 6.3). Scores of i0 and i1 are low risk and considered to indicate remission, while scores of i2 or higher suggest endoscopic recurrence.

Table 6.3 Rutgeerts' score for prediction of postoperative disease recurrence [64]

Score	Endoscopic findings
i0	No lesions
i1	≤5 aphthous ulcers
i2	>5 aphthous ulcers with normal intervening mucosa, skip areas of larger lesions, or lesions confined to the ileocolonic anastomosis
i3	Diffuse aphthous ileitis with diffusely inflamed mucosa
i4	Diffuse inflammation with larger ulcers, nodules, and/or narrowing

This scoring system has been shown to have good correlation with future risk of endoscopic recurrence postoperatively. In the initial study, only 20% of those with low-risk scores had further evolution of disease at 3 years, while 92% of those with severe lesions experienced disease progression [64, 69].

Despite a lack of formal validation, this scoring system has been widely accepted and used in clinical practice. It provides a tool that allows for risk stratification and escalation of therapy when appropriate, in hopes of curtailing clinical relapse and further complications which have the potential to result in additional surgeries and morbidity.

An optimal monitoring strategy for assessing postoperative CD recurrence has yet to be established, but typically a timeframe of 6–12 months post-resection is advocated to avoid performing assessment too early when inflammation may not have yet developed or alternatively too late so that disease activity becomes excessive and difficult to control.

In the randomized postoperative Crohn's endoscopic recurrence (POCER) trial [74], De Cruz and colleagues evaluated whether the addition of early postoperative endoscopic assessment with subsequent escalation in therapy was superior to providing optimum drug therapy alone. Using the Rutgeerts scoring system, they found that patients who underwent early postoperative endoscopic assessment at 6 months had significantly lower rates of recurrence at 18 months (49%) compared to those receiving appropriate medical therapy without early endoscopic assessment (67%). Furthermore, it was noted that 41% of patients with early endoscopic remission at 6 months still developed endoscopic recurrence at 18 months despite ongoing therapy. These results highlight the need for assessment of subclinical endoscopically identifiable lesions, as it allows for appropriate escalation of therapy prior to the development of clinical symptoms – the presence of which often suggests that a complication may have developed.

6.5 Global Disease Activity Scores

6.5.1 Lémann Index

The Lémann index was created to provide a more comprehensive assessment of cumulative bowel damage in CD patients, as opposed to many other scoring systems which only describe disease activity at a specific time point [75]. Resulting from a collaboration between 24 different centers in 15 different countries, this scoring system utilizes a combination of clinical, radiographic, and endoscopic data within 4 segments of the digestive system, including the upper digestive tract, small bowel, colon/rectum, and anus – each of which are further subdivided. Segmental scores are generated based upon prior surgical interventions as well as the presence of stricturing and/or penetrating lesions. The combination of these scores results in the final Lémann index, a quantitative tool that provides a more global assessment of the long-term impact of CD. Unfortunately, its complexity and requirement for specialized training as well as multidisciplinary collaboration renders it impractical in routine clinical practice. However, it has the potential to hold great merit in clinical trials, considering that prevention of structural damage is a major target of our therapeutic endeavors.

6.5.2 Role of Endoscopy, Scoring Indices and Mucosal Healing in Diagnosis and Monitoring

In the current landscape of IBD management, operating under the tenet that achieving mucosal healing results in more favorable outcomes, routine use of ileocolonoscopy is increasingly recognized as a standard of care. However, to what degree mucosal healing is required to achieve such improved outcomes is not well-defined. A few reports have also demonstrated that patients with partial mucosal healing experienced similarly improved outcomes compared to those with complete mucosal healing, further underscoring our lack of clarity regarding a specific threshold [5].

The development of endoscopic scoring systems has attempted to provide quantitative tools to measure disease activity, though they lack validated thresholds by which we can predict response to therapy, endoscopic healing, or prognosis. Implementation and routine use of these systems in clinical practice is limited due to their complexity, though software programs for endoscopic reporting may provide support for use of SES-CD. Even if endoscopic scoring systems are not utilized in clinical practice, it remains paramount to provide adequate description of disease activity.

6.6 Limitations and Future Needs

Recognizing that up to half of patients in clinical remission may have endoscopically active disease and that a significant proportion of patients with mucosal healing will continue to experience

distressing symptoms highlights the fact that focusing solely on clinical remission will inevitably lead to either undertreating those with clinically silent disease or overtreating others with symptoms unrelated to CD. Therefore, a significant need exists for simple, widely available, reproducible, and prospectively validated tools to objectively monitor CD patients.

Serologic and fecal biomarkers are a useful adjunct in the assessment of treatment response and perhaps prediction of future complications; however, they lack validated cutoff values, may not be sufficiently specific for active CD, and have potential heterogeneity in their response among individuals and varying disease locations. Though they perform better in the prediction of active disease than clinical symptoms alone, their purpose may lie in risk-stratifying patients that require further evaluation with radiographic or endoscopic studies.

With the substantial heterogeneity in disease expression and clinical outcomes, there is an increased need for biomarkers that help to risk stratify and personalize therapy for CD patients. Beyond the use of CRP and fCal in simply predicting the presence of disease activity, increasing attention has been directed toward the identification of markers that may provide prediction regarding disease phenotype and natural history. For instance, a number of molecular studies have suggested that ileal and colonic CD may exist as distinct phenotypes, owing to the existence of site-specific susceptibility genes [76, 77]. Moreover, Weiser et al. were able to demonstrate two distinct molecular phenotypes (ileum-like and colon-like CD) within samples of colonic tissue from adult CD patients, each of which appeared to be associated with different clinical phenotypes as well as disease-related outcomes [78]. Currently these markers are solely used on an experimental basis, as additional studies are required to understand their utility and implications in clinical practice.

In addition to laboratory-based biomarkers, cross-sectional imaging with CTE and MRE has become the mainstay in the management of CD, providing essential data regarding terminal ileal skipping, isolated transmural disease, and extraintestinal complications that would otherwise be missed with conventional ileocolonoscopy. Despite the existence of multiple scoring systems, data is lacking to suggest that improvement in such parameters carries any prognostic significance and further study is needed.

The use of ileocolonoscopy in the evaluation of disease activity and assessment of response to therapy is well-established. Although a close monitoring strategy during induction and maintenance therapy is advocated to document mucosal healing, the frequency and timing with this should be accomplished remain unclear. Further complicating this issue is the lack of a widely accepted definition for mucosal healing, which would be an important definition not only for clinical practice but for clinical trials which are increasingly utilizing this as an endpoint. Although a few available endoscopic scoring systems are capable of documenting improvement in disease activity, further validation studies are required to establish thresholds for these systems and to delineate what magnitude of change results in improved clinical outcomes.

6.7 Summary

As awareness regarding the disconnect between patient-reported symptoms and underlying disease activity rises, the importance of having objective measurement tools to guide the management of CD patients has become increasingly important. However, due to a lack of standardized definitions and prospectively validated measurement systems for the currently available tools, the use of a combination of factors is required. It is also important to recognize that the management of CD patients is a complex task – one that requires the amalgamation of multiple factors, including patient symptoms and provider assessment, in addition to the use of objective measurement tools to formulate an overall assessment and direct therapeutic decision-making.

Summary Points

- Clinical symptoms have a poor correlation with disease activity.
- C-reactive protein and fecal calprotectin provide a more objective assessment of disease activity than clinical symptoms.
- Several radiographic and endoscopic scoring systems are available to objectively measure disease activity in Crohn's disease.
- Radiographic and endoscopic responses to therapy may result in improved clinical outcomes.

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Fibrosis and Stricturing Disease in Crohn's Disease

7

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Abstract

Crohn's disease (CD) has a protean presentation including inflammatory, stricturing, fistulizing, and perianal disease morphologies. The incidence of fibrostenosing CD and the need for surgery have largely remained unchanged despite the use of anti-inflammatory drugs including biologics. Fibrosis is a common occurrence in ulcerative colitis. Clinical, serologic, and imaging markers lack accuracy to predict, diagnose, and prognosticate fibrostenosing CD. There are no established clinical trial end points to measure efficacy of anti-fibrotic drugs. Management of fibrostenosing CD needs a multidisciplinary approach involving medical, endoscopic, and surgical management. Targeted anti-fibrotic therapies are necessary to treat fibrostenosing CD in the future.

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7.1 Introduction

CD has a protean presentation. The variability in disease phenotype over time leads to the need for classifying the disease into inflammatory, stricturing, fistulizing, and perianal disease per the Montreal Classification [1]. It is common for stricturing and fistulizing CD to coexist. One study on surgical specimens suggested that 64% of patients had fistulizing CD of which 41% of specimens had fistulae within a stricture and about 56% had fistulae proximal to the stricture speculating that mechanical factors may contribute to their formation [2]. The anatomic distribution of strictures follows the sites affected by inflammation and includes the ileum followed by ileocolic region, albeit strictures can occur in other locations including the upper gastrointestinal tract, colon, and rectum [3, 4]. Long-term studies continue to show a 20–40% prevalence of fibrostenosing Crohn's disease (CD) in populations across Asia, Europe, and North America with a follow-up over a timeframe of 4–10 years [5–9]. Pathophysiologically, intestinal fibrosis is associated with increased extracellular matrix (ECM) generation and mesenchymal cell proliferation leading to progressive narrowing of the intestinal lumen, which ultimately progresses to mechanical obstructive symptoms. Animal models of inflammation-induced fibrosis indicate the continuation of the fibrotic process despite abatement of the inflammatory process [10].

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Population-based studies demonstrate that 20% of patients develop fibrostenotic complications within 20 years of a CD diagnosis while >30% develop this complication within 10 years of diagnosis at tertiary referral centers [6, 11, 12].

Fibrostenosing CD continues to be a significant risk factor for surgery, and there has been no significant change in the need for surgery despite introduction of immunosuppressant therapy [6, 8]. A recent population-based study from Europe indicates that while the use of immune modulators and biologics has increased over the past decade, there has been no change in the progression of disease from an inflammatory to a complicated course [13]. Other studies show that the incidence of fibrostenosing CD and the need for surgery have either remained unchanged or have decreased in some parts of the world while it has increased in other parts and the exact etiological factors remain elusive [8, 9, 14]. Attempts to develop predictive models to determine occurrence or speed of disease progression and attempts to "personalize" IBD care continue to be made. This is critical to define patient populations at risk amendable to tailored anti-fibrotic therapies or to learn about the pathophysiology of fibrosis [15, 16]. Despite numerous projects to develop genetic, epigenetic, serologic, radiologic, or clinical predictors to direct physicians at personalizing IBD treatment, none have achieved clinical applicability due to variable penetrance of genetic and epigenetic factors and low accuracy of serologic, radiologic, and clinical predictors [15, 16].

7.2 Overview on the Mechanisms of Fibrogenesis

Common etiopathogenic mechanisms of fibrosis include an expansion of the mesenchymal cell pool, consisting of fibroblasts, myofibroblasts, and smooth muscle cells. Multiple sources of mesenchymal cells have been described, namely, endothelial to mesenchymal transformation, epithelial cell to mesenchymal transformation, stellate cells, or fibrocytes derived from the bone marrow [17]. Activation of myofibroblasts causes increased ECM production [18].

7.3 Risk Factors for the Development of Fibrostenotic CD

Animal models show that the predisposition for and degree of fibrosis in general may be variable in differing genotypes in fibrotic conditions including PSC and lung fibrosis [19, 20]. Also a phenomenon of rapid progression of fibrosis has been observed clinically in patients undergoing liver transplantation [21]. These concepts may be extrapolated to fibrostenosing CD.

Clinicians continue to rely on certain clinical factors to predict and prognosticate fibrostenosing CD. These factors include need for corticosteroids, early onset of disease, frequency of flares, smoking, perianal disease, and small bowel involvement [22–24]. However, using these factors for prediction may often be too late as they already represent a more complicated CD course and fibrosis may already be present at the time of prognostication. Also these clinical predictors lack validation in large prospective or ad hoc studies. Hence, there is an increased interest in the discovery of accurate and noninvasive biomarkers.

7.4 Biomarkers as a Diagnostic and Predictive Tool for Fibrostenosing CD

Several biomarkers have been purported for the prediction of complications, association, or diagnosis of fibrostenosing CD. Prognostic biomarkers that have been tested for the prediction of fibrostenosis include genetic markers and antimicrobial antibodies.

7.4.1 Predictive Biomarkers

In general, genetic markers include those that are predominantly associated with autophagy (e.g.,

ATG16L1), recognition of muramyl dipeptides (MDP) bacterial components (e.g., NOD2), interleukin and interleukin receptor-associated genes (e.g., IL-23 receptor), epithelial cell adhesion (e.g., discs large homologue 5 (DLG5)), or matrix regulation (e.g., metalloproteinase-3 (MMP-3)).

A meta-analysis of predictive genes included assessing the occurrence of high-risk NOD2 single nucleotide polymorphism (SNPs) among CD patients which predicted an increased risk of stenosing CD (OR = 1.94, (95% CI, 1.61–2.34)) with an OR for small bowel involvement at 2.53 (95% CI, 2.01–3.16) [25].

It is speculated that gut injury followed by exposure of microbial components to the intestinal immune system initiates an immune response and may be responsible for eliciting the formation of antibodies which are detectable in the serum. The first such antibody studied included anti-*Saccharomyces cerevisiae* (ASCA) [26]. This was followed by other antibodies, including the anti-glycan antibodies; anti-mannobioside carbohydrate antibody (AMCA), anti-laminaribioside carbohydrate antibody (ALCA), or anti-chitobioside carbohydrate antibody (ACCA) and non-glycan antibodies to bacterial components including anti-outer membrane protein C (OmpC), anti-I2, or anti-CBir1.

The odds ratio of predicting combined penetrating and stricturing CD showed an incremental rise as the degree of immune responses to a combination of glycan and non-glycan antibodies increased, i.e., by the number of positive antibodies (OR = 1.1, 2.3, 5.5, and 11 for 1, 2, 3, and 4 positive immune responses, respectively) using OmpC, anti-I2, anti-CBir1, and ASCA [27]. In another study, anti-I2, anti-CBIR1, or ASCA antibodies aided in predicting complicated CD but were unable to differentiate stricturing and nonstricturing CD [28]. Findings similar to this combination were seen when anti-glycan antibodies were used exclusively [26]. Overall, these predictors are not accurate enough to be used in clinical practice and show variable sensitivity and specificity based on the number of antibodies used to predict complications including strictures with sensitivities and specificities varying from 42% to 71% and 48% to 75%, respectively, and are elaborated in Table 7.1 [26, 29–31].

Table 7.1 Diagnostic and predictive biomarkers in fibrostenosing Crohn's disease

Biomarker (type)	Country of origin	Study characteristics	Sensitivity	Specificity	Accuracy (ROC)	Additional comments
ATG16L1 (genetic) [51]	Australia	Cohort study	–	–	–	Frequency of ATG16L1 T300A GG genotype associated FSCD with a frequency of 0.39, $p < 0.001$
NOD2 mutations (genetic) [52]	USA	Meta-analysis	36% (pooled)	73% (pooled)	0.56 (pooled)	RR for NOD2 mutant allele for complicated CD (stricturing or fistulizing) = 1.17 (95% CI) 1.10–1.24; $p < 0.001$). RR for P.G908R mutation for complicated CD (stricturing or fistulizing) = 1.33 (95% CI 1.11–1.60; $p = 0.002$)
CX3CR1 (genetic) [53]	Germany	Retrospective cohort	–	–	–	Prevalence in FSCD vs non-FSCD for V249i (55% vs 41%, $p = 0.035$) 3020insC (23% vs 6.7% $p = 0.001$)
MMP-3 (genetic) [54]	Denmark	Prospective	–	–	–	Significant differences were seen between MMP-1, MMP-2, MMP-3, and MMP-9 relative to TIMP-1, TIMP-2 which were increased in inflamed and non-inflamed IBD on surgical resection. No significant difference was noted between inflamed and fibrotic specimens

(continued)

Table 7.1 (continued)

Biomarker (type)	Country of origin	Study characteristics	Sensitivity	Specificity	Accuracy (ROC)	Additional comments
IL12B (genetic) [33]	Western Europe	Cohort	–	–	–	OR for homozygosity for the rs1363670 G-allele (IL12B gene) = 5.48 (95% CI 1.60–18.83; $p = 0.007$)
JAK2 (genetic) [34]	Europe	Cohort	–	–	–	HR for stenosis for combination of NOD2, JAK2, ATG16L1; =1.29, $p = 3.01 \times 10^{-02}$
MAGI1 (genetic) [55]	Europe	Cohort	–	–	–	Variants in *MAGI1*, *CLCA2*, *2q24.1*, and *LY75* loci were associated with FSCD ($p_{\text{combined}} = 2.01 \times 10^{-8}$)
miRNA-200a and miRNA-200b (epigenetic) [56]	China	Prospective	–	–	–	Mean serum miRNA-200b FSCD vs control, $P < 0.05$, FSCD vs non-FSCD $p > 0.05$
miRNA-29b (epigenetic) [39]	Italy	Cohort	–	–	–	Notes a reduction in the mean levels of *miR-29a* in FSCD relative to inflammatory CD ($p = 0.049$)
miRNA-19a/b (epigenetic) [40]	Italy	Cohort	–	–	0.67	Mean serum miR-19-3p (miR-19a-3p and miR-19b-3p) was reduced in FSCD vs non-FSCD subjects by >2-fold, $p = 0.007$ and 0.008
ASCA (AMA) [29]	Germany	Cohort	–	–	–	OR for complicated CD (stricturing/fistulizing) ASCA: 3.5 (95% CI, 1.9–6.4)
Anti-CBir1 (AMA) [27]	USA	Cohort	–	–	–	Prevalence of anti-CBir1+ vs anti-CBir1- in complicated CD (stricture/fistula) was 19% vs 12%, $p = 0.36$
Anti-I2 (AMA) [27]	USA	Cohort	–	–	–	Prevalence of anti-I2+ vs anti-I2 - in complicated CD (stricture/fistula) was 31% vs 12%, $p = 0.003$
Anti-OmpC (AMA) [27]	USA	Cohort	–	–	–	Prevalence of anti-OmpC+ vs anti-OmpC- in complicated CD was 36% vs 12%, $p = 0.006$
Anti-glycan antibodies (AMA) [29]	Germany	Cohort	–	–	–	OR for complicated CD (stricturing/fistulizing) were ASCA: 3.5 (95% CI, 1.9–6.4) AMCA: 2.4 (95% CI, 1.2–4.8) ALCA: 2.3 (95% CI, 1.1–4.8)
AMA [57]	Ireland	Cohort	–	–	–	% age of involvement distinguishing inflammatory CD vs complicated CD (stricturing and fistulizing CD) differed using anti-OmpC, ASCA IgA, and anti-CBir on univariate analysis ($p < 0.05$)
Anti-glycan antibodies [37]	Canada	Cohort	–	–	–	ASCA IgG positivity was predictive of complicated CD (stricturing/penetrating) (OR = 3.01; 95% CI, 1.28–7.09; $P = 0.01$)

Table 7.1 (continued)

Biomarker (type)	Country of origin	Study characteristics	Sensitivity	Specificity	Accuracy (ROC)	Additional comments
Anti-glycan antibodies (AMA) [26]	USA	Meta-analysis	–	–	–	Pooled diagnostic OR to detect complications (fistulizing and stricturing CD) for ASCA and ACCA were 2.8 (95% CI: 2.1, 3.8) and 2.5 (1.9, 3.2), respectively, when analyzed individually or a combination of >2 anti-glycan antibodies pooled diagnostic OR was 2.8 (2.2, 3.7)
YKL-40 (AMA) [48]	Turkey	Cohort	–	–	–	Mean YKL-40 levels in FSCD vs non-FSCD (167.50 ± 119.30 ng/mL vs 80.12 ± 56.38 ng/mL ($P = 0.003$))
N-terminal propeptide of type III collagen (ECM) [42]	Italy	Case series	–	–	–	Serum N-terminal propeptide of type III collagen before surgical resection vs after surgical resection, (5.0 ± 1.8 vs 2.7 ± 0.7 microg/l ($p = 0.0001$))
Basic-FGF (growth factor-cytokine) [46]	USA	Cohort	–	–	–	Mean FGF levels between non-FSCD, FSCD, and fistulizing CD were 13.18 ± 1.85, 11.94 ± 2.93, and 11.96 pg/ml ± 2.64, $p = 0.91$
Basic-FGF [47]	Italy	Cohort	–	–	–	Significant difference between serum b-FGF and bowel wall thickness FS-CD when compared to other phenotypes
Fibronectin (ECM) [43]	Denmark	Cohort	16%[a]	83%[a]	–	Significant drop of mean fibronectin after surgery
Peripheral fibrocytes [50]	Japan	Prospective	–	–	–	Surgical specimens showed increased fibrocyte/total leukocyte % age in inflammatory lesions (22.2%) vs non-affected areas of the intestine (2.5%) and fibrotic areas ($p < 0.001$). Percentage of circulating $CD45^+$ collagen I^+ fibrocytes/total leukocytes were higher in patients with Crohn's disease (3.5%) than in healthy controls (1.5%)

Abbreviations: *ALCA* anti-laminaribioside, *AMA* antimicrobial antibodies, *AMCA* anti-mannobioside, *ATG* autophagy protein, *ASCA* anti-*Saccharomyces cerevisiae* IgG, *CD* cluster of differentiation, *CLCA* calcium-activated chloride channel, *ECM* extracellular matrix, *FGF* fibroblast growth factor, *FSCD* fibrostenosing CD, *HR* hazard ratio, *JAK* Janus kinase, *MAGI* membrane-associated guanylate kinase, *MMP* matrix metalloproteinases, *NOD* nitric oxide dismutase, *miRNA* micro-RNA, *TIMP* tissue inhibitor of metalloproteinases, *RR* risk ratio, *USA* United States of America
[a]To detect FSCD prior to or after surgery (normal plasma fibronectin being 206–379 mg/L)

7.4.2 Association Biomarkers

Biomarkers used for association include genetic markers and antimicrobial antibodies. Genetic biomarkers associated with fibrostenosing CD include variations in matrix metalloproteinase-3 (MMP-3) genes, i.e., 5T5T genotype at MMP-3 SNP-1613 5 T/6 T, which significantly increases the risk of stenotic complications in CD during follow-up (91.2% vs 71.8%) [32]. In another study, the presence of at least one of three NOD 2 SNPs was significantly associated with development of stricturing disease (40% vs 33%) [33]. Other SNPs significantly associated with development of stricturing disease include rs1363670 G-allele (69% vs 35%) [33]. Genetic markers

such as NOD2 mutations, JAK2, or ATG16L1 polymorphisms were associated with stenotic CD as derived from a large European GWAS study called the IBD chip study showing a hazard ratio of 1.42 [34]. In this study, the association between stenosis and genetic scores created using significant single nucleotide polymorphisms (SNPs) in a univariate analysis demonstrated a HR for stenosis of 1.29 using a combination of NOD2, JAK2, and ATG16L1 among patients with low and high score [34].

The IBD-5, disks large homologue 5 (DLG5), autophagy-related protein (ATG16L1), and IL-23 receptor (IL23R) were associated with CD and UC and their complications in a large Dutch-Belgian cohort; however, no clear association with fibrostenosing CD was identified [35]. In a meta-analysis of two studies, the pooled diagnostic odds ratio (DOR) to assess association with complications (fistulizing and stricturing CD) was calculated to be highest with ACCA and ASCA at 2.8 (95% CI: 2.1, 3.8) and 2.5 (1.9, 3.2), respectively, when analyzed for individual antibodies or 2.8 (2.2, 3.7) when using a combination of >2 anti-glycan antibodies [26]. More recent studies since the publication of this meta-analysis continue to suggest association with complicated disease phenotype with the highest association using AMCA and ASCA antibodies [36, 37].

7.4.3 Diagnostic Biomarkers

Biomarkers include a combination of genetic biomarkers, micro-RNAs, ECM molecules, fibroblast growth factors, and circulating fibroblasts.

miRNAs are RNA molecules that inhibit posttranscriptional expression of genes. Serum miR-200b was noted to be elevated, and serum miR-29a was noted to be decreased in ten fibrostenotic CD subjects compared to inflammatory CD [38]. Mucosal biopsy samples also demonstrate decreased expression of miR-29 in strictured versus nonstrictured segments of the bowel which mirrored decreased serum expression of miR-29 [39]. Similarly serum miRNA-19 was diminished in stricturing CD yielding a AUC = 0.67 to detect stricturing CD phenotype [40].

ECM biomarkers include inhibitors of metalloproteinases (TIMPs) in intestinal resection samples, propeptides of collagen in serum, and serum fibronectin. These markers are noted to be elevated in the serum of patients in stricturing CD; however, these studies failed to establish any measure of sensitivity, specificity, or accuracy due to lack of reliable cutoff values [41–43]. Contradictory findings were noted for the marker serum propeptide of collagen III which was noted to be elevated in fibrostenosing CD prior to intestinal resection followed by a drop after surgery when compared to controls, while another study showed no correlation to disease phenotype or activity [42, 44]. Other ECM markers that were analyzed include MMP-induced changes in vimentin (VICM), MMP-induced changes of biglycan molecules (BGM) that cross-link collagen, neutrophil elastase (ELNE), MMP-mediated type V collagen degradation (C5M), and type V collagen propeptide (Pro-C5) [45]. Although this study showed high AUC (>0.8) using such biomarkers to differentiate IBD from IBS and UC from CD, this study failed to diagnose fibrostenosing CD as a disease phenotype [45].

Growth factors promoting fibroblast expansion and activation, including basic fibroblast growth factor (b-FGF), have been demonstrated to be elevated in serum and biopsy specimens of surgically resected bowels of subjects with stricturing CD when compared to healthy controls. The Pearson correlation between b-FGF and disease activity was statistically significant at 0.53 [46]. These findings were also correlated in vivo showing an association between serum b-FGF levels and bowel wall thickness as measured on doppler studies [47]. Another growth factor of endothelial cells and fibroblasts called YKL-40 has been demonstrated to be elevated in the serum of fibrostenosing CD subjects compared to controls in one study which was contradicted in another study [48, 49]. Although sensitivity and specificity could not be established, there was significant correlation between YKL-40 levels and clinical disease activity

($r = 0.681$) and the presence of intestinal strictures ($r = 0.457$) [48].

Circulating fibrocytes have been tested as biomarkers in other fibrotic diseases, and examination of this phenomenon in CD yielded significantly higher ratios of the percentage of $CD45^+Col^+$/leukocytes, $ICAM^+$ fibrocyte/leukocyte, and $CXCR^+$ fibrocyte/leukocyte ratios in CD, and these fibrocytes were shown to produce higher amounts of collagen I after stimulation by lipopolysaccharides in vitro [50].

The lack of predictive and diagnostic accuracy and consistency between studies precludes the use of these biomarkers for predicting or diagnosing fibrostenotic CD [28]. This has also limited the use of these biomarkers as end points to assess outcomes in fibrostenosing CD in clinical trials [28].

These biomarkers are elaborated further in Table 7.1.

7.5 Diagnostic Evaluation

Histopathologic analysis of surgical resection specimens indicates stenotic CD is associated with inflammation and fibrosis and rarely are they exclusive of each other [58]. Identifying and distinguishing inflammation and fibrosis has implications for management [35]. Endoscopy with biopsies is limited in detecting deeper intramural inflammation or fibrosis and cannot assist in their differentiation. Cross-sectional imaging allows for the detection and characterization of mural and extramural complications and is encouraged at first presentation of intestinal stenosis [35, 59].

7.5.1 Imaging

Common imaging techniques used include CT enterography (CTe), MR enterography (MRe), and ultrasound (US). Stenosis is usually defined as a thickening of the bowel wall with a narrowing of the small bowel lumen with pre-stenotic dilation [60]. The pooled sensitivity and specificity of US for detecting small and large bowel stenosis ranged from 75% to 100% and 90% to 93%, respectively [61–63]. The sensitivity for ileal stenosis was 86% and was 58% for colonic stenosis in one study, indicating that certain locations of strictures may represent a limitation of this method [62]. High-resolution US showed a sensitivity and specificity for detecting stenotic CD of 86% and 90% when correlated to clinical symptoms and surgical findings [64]. US imaging is radiation-free and is useful to visualize the terminal ileum and colon as well as for therapeutic interventions including abscess drainage [59]. Some limitations include inadequate visualization due to gas-filled bowel loops and large body habitus. High-frequency (5–17 MHz) linear array probes help with better visualization of wall thickness and wall layer discrimination [65]. Delineation between fibrosis and inflammation in stenotic lesions remains a challenge although contrast-enhanced US (CE-US) may offer some advantages wherein microbubble contrast agents are injected intravenously prior to US imaging [66]. However, these techniques have not achieved clinical applicability in the delineation of fibrosis and inflammation in IBD strictures.

CTe enables the detection of stenotic lesions, pre-stenotic dilation, fistulae, and abscesses with high accuracy. CTe is able to detect small bowel stenosis with a sensitivity and specificity of 85% and 100%, respectively [67]. Data on colonic stenosis is limited. However, CTe may lead to significant cumulative exposure to radiation more so in young patients with childbearing potential and the risk of occurrence of radiation-induced cancers [68]. Enteroclysis which entails fluoroscopic or endoscopic placement of an enteroclysis tube into the duodenum distal to the ligament of Treitz with infusion of contrast media may be used to image jejunal anatomy better due to limited distension proximal to mid-ileum on CTe [69].

Sensitivity and specificity of MRI in detecting stenotic lesions ranged from 82% to 100%, respectively [63]. The inherent advantages of MRe include lack of radiation exposure and multi-planar and cine imaging with high contrast enhancement, while increased costs are a major limitation. In addition, MRI may help grade inflammation based on certain characteristics

including hyperintensity on T2, mucosal enhancement, ulcerations, and blurring of margins [70]. In this same study, fibrosis correlated with the percentage of enhancement gain (enabling the discrimination between mild-moderate and severe fibrosis), the pattern of enhancement at 7 minutes, and the presence of stenosis [70]. Although not available yet for clinical use in humans, magnetic transfer (MT) may be used for distinguishing stiffer tissue including muscle and fibrotic tissue from inflammation as shown in some animal studies [71].

Taken together the accuracy of cross-sectional imaging for the detection of stenosis is high, but none of the currently available techniques has been validated to distinguish inflammation from fibrosis.

7.6 Management: Monitoring and Therapeutics

We describe the clinical management of fibrostenosing CD as recommended by the European Crohn's and Colitis Organisation [ECCO] [72]. A multidisciplinary approach is suggested in the management of fibrostenotic CD including a collaborative effort from gastroenterologists, colorectal surgeons, radiologists, and pathologists. A brief overview is illustrated in Fig. 7.1. Management of fibrostenosing CD can be broadly categorized into medical management (anti-inflammatories), endoscopic management, surgical management, and often an overlap between medical and surgical co-management.

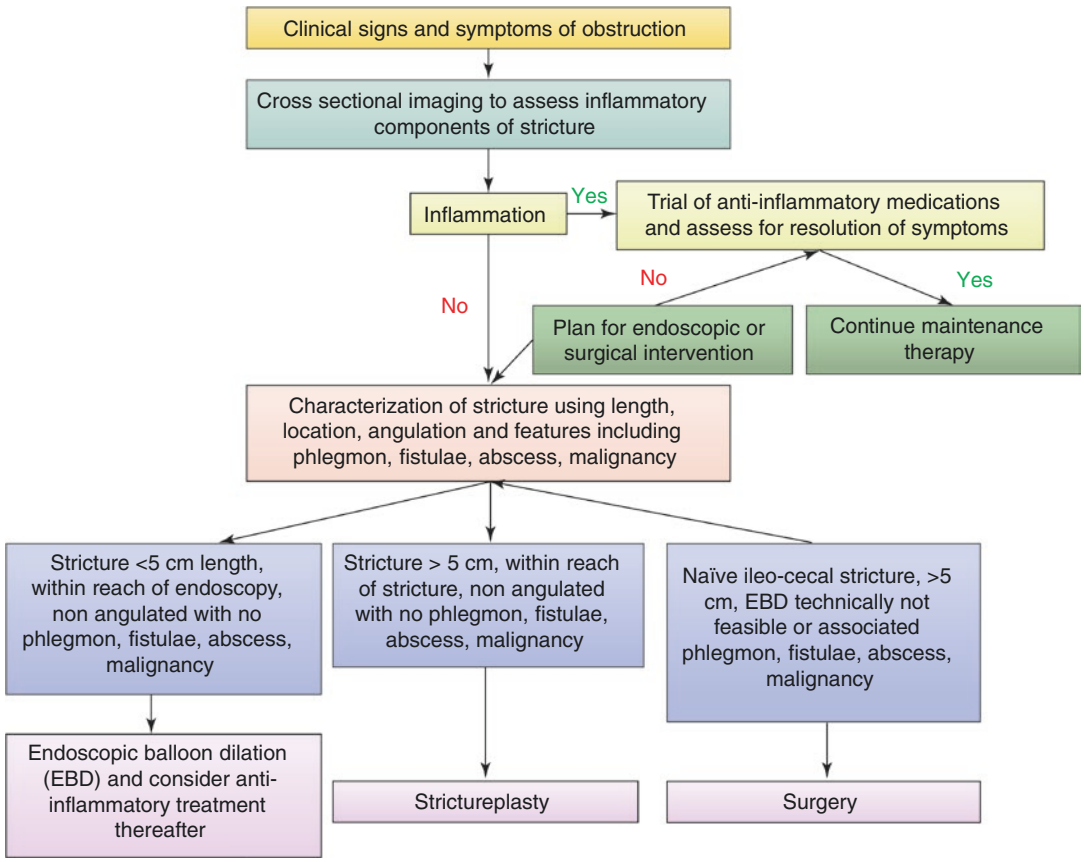

Fig. 7.1 Management of fibrostenosing Crohn's disease

7.7 Medical Management

An acute intestinal obstruction should be managed with appropriate cross-sectional imaging, bowel rest, nasogastric decompression, intravenous hydration, and electrolyte replenishment guided by laboratory data. Signs of peritonitis should warrant a surgical evaluation [35].

Since most strictures have fibrotic and inflammatory components, steroids and biologics help relieve obstruction by decreasing inflammation in an acute and subacute setting after bowel rest [73]. However, current therapies lack efficacy in reversing fibrosis, making endoscopic balloon dilation (EBD) with through the scope (TTS) balloons, and surgical management necessary.

7.8 Endoscopic Balloon Dilation (EBD)

EBD may be feasible for short segment strictures (<5 cm) within reach of a traditional colonoscopy or upper GI endoscopy, including terminal ileal and anastomotic strictures, upper GI strictures, and small intestinal strictures, respectively. Successful dilation with a technical efficacy of 89% and clinical efficacy of 81% has been quoted in a pooled analysis with a complication rate of 2.8% [74]. EBD and strictureplasty are contraindicated in the presence of an abscess, phlegmon, fistula, high-grade dysplasia, or malignancy [72]. Serial EBD of recurrent strictures is an efficacious approach, and a decision about surgical approach versus serial dilation may be made on technical feasibility, symptom-free interval, and patient preferences [35]. Other approaches including intralesional steroids, anti-TNFs, and stents are not currently recommended by the ECCO consensus [35].

7.9 Surgery

Surgical intervention including surgical resection and/or strictureplasty should be the preferred option for longer strictures (>5 cm). Fibrostenosing jejunal and ileal disease can be managed by "conventional" or side-to-side [Heineke-Mikulicz and Finney] and "nonconventional" strictureplasties. Nonconventional methods are used in patients who have multiple strictures in close proximity with the short gut from prior surgeries [75].

Short strictures defined as <10 cm are best treated with the Heineke-Mikulicz technique, and longer strictures (10–25 cm) are treated with Finney's strictureplasty. A meta-analysis comparing conventional and nonconventional strictureplasties showed no difference in the rates of complications between the two techniques [76]. Laparoscopic surgery for fibrostenotic CD is increasingly common in experienced centers to enable superior recovery, better cosmesis, less adhesions and incisional hernias, and similar surgical recurrence rates [77].

7.10 Future Therapies

While no specific anti-fibrotic therapy is available for fibrostenosing CD, this approach would be highly desirable given the potential to prevent or treat strictures without the need for endoscopic or surgical intervention. Potential targets include the blockade or administration of cytokines including TGF-β, TNF-α, IL-13, and IFN-ϒ and/or effector pathways to these cytokines as derived from other fibrotic diseases [78–81].

TGF-β plays a central role in fibrogenesis through its TGF-β effector pathways which are often classified as canonical pathways (SMAD pathways) and noncanonical pathways (phosphoinositide 3-kinase (PI3K), PAK22-abl, the mechanistic target of rapamycin (Akt-mTOR), cellular Abelson non-receptor kinase (c-Abl-protein kinase C-δ/c-Abl-PKC-δ), and c-Jun N-terminal kinase (JNK)) [81]. In addition, TGF-β increases inhibitors of MMPs and decreases matric metalloproteinases.

Targets used for anti-TGF-β therapy include using neutralizing antibodies to TGF-β receptors (metelimumab, CAT-152, LY238770), peptide inhibitors to TGF-β1, 3 (P144), ligand traps to TGF-β (sTβRII), or blocking the production of TGF-β (pirfenidone). These therapies have been

used predominantly in systemic sclerosis, prevention of fibrosis after trabeculectomy, diabetic nephropathy, and idiopathic pulmonary fibrosis and are in phase 2 and 3 clinical trials as described in Table 7.2. Other therapeutic interventions include blocking canonical pathways that are expressed downstream of TGF-β receptors (Wnt pathway, ALK5/SMAD pathway, SMAD3) and noncanonical pathways (c-Abl, sarcoma tyrosine kinase inhibitor, rho-associated kinases (ROCK), protein kinase (PKC-δ)) [80, 81]. Of these, c-Abl inhibitors (imatinib) or ROCK inhibitors (fasudil) are in clinical trials for treatment of bone marrow fibrosis in CML and diabetic retinopathy (Table 7.2).

Table 7.2 Anti-fibrotic therapies in phase 2 and phase 3 clinical trials for fibrotic conditions

Potential drug	Mechanism of drug action	Clinical trial	Disease applicability Ref
Drugs targeting TGF-β and its effector pathways			
CAT-192 (metelimumab)	Monoclonal antibody to TGF-β1	Phase 1, 2	Systemic sclerosis [85]
CAT-152	Antibody to TGF-β2	Phase 3	Fibrosis after trabeculectomy [86]
LY238770	Antibody to TGF-β1	Phase 2	Diabetic nephropathy, CKD (NCT01113801: trail terminated due to lack of efficacy)
Avotermin	Recombinant hTGF-β3	Phase 2	Surgical scars (NCT00432211/NCT00656227: trials unfinished)
P144	Peptide inhibitor to TGF-β1, 3	Phase 2	Systemic sclerosis (NCT00574613: results pending)
Pirfenidone	Blocks production of TGF-β	Phase 3	IPF [87]
IFN-gamma	Inhibition of TGF-β through SMAD3 pathway	Phase 3	SSc-IPF [88]
Imatinib	c-Abl (noncanonical pathway)	Phase 2, 3 Phase 3	Bone marrow fibrosis in CML [89] Sclerotic skin GVH reaction
Losartan	Anti-inflammatory (anti TGF-β activity?)	Phase 2, 4	IPF (NCT00879879: results available, publication pending) Liver fibrosis (NCT01051219: results pending) [90] HIV fibrosis (NCT01529749: results pending)
Fasudil	Inhibition of ROCK (noncanonical TGF-β pathway)	Phase 3	CAD [91], vascular modulation in diabetic macular degeneration (NCT01823081: results available, publication pending), Raynaud's (NCT00498615: results available, publication pending)
CC-930	JNK inhibitor (noncanonical TGF-β pathway)		IPF, DLE (NCT01203943, NCT01466725: both studies terminated due to risk profile)
Other cytokine targets			
Tocilizumab	(anti-IL-6)	Phase 2	Systemic sclerosis [92]
Humanized IL-13 antibody (lebrikizumab)	Monoclonal antibody to IL-13 which inhibits activation of fibroblasts	Phase 2	IPF (NCT01629667: terminated due to lack of efficacy) Asthma [84]
Miscellaneous mechanisms			
D-penicillamine	Prevention of collagen cross-linking by copper containing lysyl oxidase enzyme leading to decreased ECM stiffness	Phase 3	Scleroderma [93]

Abbreviations: *Abl* Abelsen, *IL* interleukin, *JNK* c-Jun N-terminal kinase, *JAK* Janus kinase, *PKC* protein kinase C, *ROCK* rho-associated kinases, *TGF* transforming growth factor, *TNF* tumor necrosis factor
NCT numbers are obtained from clinicaltrials.gov

IL-13 promotes TGF-β activity and decreases MMPs [82, 83]. However, monoclonal antibody trials were terminated due to lack of efficacy in lung fibrosis, but show good efficacy in moderate to chronic asthma [84].

TNF-α has pleiotropic effect and is considered both anti- and profibrotic [78]. Other relevant mechanisms of drugs in phase 2 and 3 clinical trials are explained in Table 7.2.

7.11 Future Directions

Significant advances have been made in the management of fibrostenosing IBD, but key questions remain. At the pathogenesis stage, these include the discovery of mechanisms that cause a switch from inflammatory to fibrosing disease and determination of factors that auto-propagate fibrosis despite reduction in mucosal inflammation. Major challenges include the need for more representative experimental animal models for fibrostenosing CD as current approaches may not be ideal [94]. Mouse models of intestinal fibrosis have been reviewed recently [94–96]. At the clinical level, major challenges that have been identified include the lack of diagnostic and prognostic biomarkers that enable the definition of suitable patient populations or depict their response to therapy. Future directions should include a concerted effort to develop standardized imaging scores. These biomarkers and imaging scores may lead to measurable outcomes in clinical trials with new drug therapies. In the therapeutic realm, limiting systemic toxicity from anti-fibrotic treatment in CD will remain an essential milestone, considering the localized and patchy distribution of fibrostenotic CD and its association with internal penetrating disease. Several initiatives are currently ongoing making the testing of specific anti-fibrotics in IBD in the near future a realistic prediction.

Summary Points

- The incidence of fibrostenotic Crohn's disease and need for surgery have not changed significantly despite increased use and availability of anti-inflammatory agents.
- Accurate and reproducible biomarkers to diagnose, predict, and prognosticate fibrostenotic CD are lacking, which is true for clinical practice and clinical trials.
- Management of fibrostenotic CD involves a multidisciplinary approach and includes medical, surgical, and endoscopic management.
- There are multiple candidate pathways to block the fibrogenic process.
- Anti-fibrotic treatments for other organs are currently in preclinical or early clinical trial phases bringing an anti-fibrotic therapy in IBD within reach.

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Postoperative Crohn's Disease

8

Kurvi Patwala and Peter De Cruz

Abstract

This chapter will cover prognostic and predictive biomarkers that have been identified in association with postoperative recurrence of Crohn's disease. It will discuss the optimal management of Crohn's disease following resectional surgery with reference to the natural history of Crohn's disease after surgery, risk factors for earlier postoperative recurrence, diagnosis and monitoring of recurrence and therapeutic strategies to address prevention of recurrence. It will provide a clinical algorithm encompassing biomarkers to help guide clinical management as well as identify future directions for ongoing research.

8.1 Introduction and Background

Despite the advent of biologic therapies, up to a third of patients with Crohn's disease (CD) require intestinal resection within 5 years of their diagnosis [1]. Intestinal resection for CD is indicated for patients who develop obstructive symptoms, internal penetrating disease or inflammatory disease that is refractory to medical therapy [2]. Although surgical intervention is effective at inducing remission and improving quality of life, it is not curative. Within 1 year of surgery, 90% of CD patients will develop endoscopic recurrence, 30% will present with clinical recurrence, and 5% of patients will require further surgical intervention [3–5]. Thereafter, clinical recurrence occurs at a rate of approximately 10% per year. Overall, up to 70% of patients who have had previous intestinal resection will require reoperation for the management of their CD [6].

8.2 Natural History of Crohn's Disease Recurrence

Following surgical resection of macroscopically affected CD and the creation of an ileocolic anastomosis, recurrent disease typically occurs at, and above, the surgical anastomosis. Subclinical lesions characterised by ulceration and inflammation are seen on endoscopy and precede the development of clinical symptoms. The severity of subclinical endoscopic lesions predicts the likelihood of developing subsequent symptomatic clinical disease and the requirement for further surgical intervention [5].

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Both animal and human studies have indicated that flow of the faecal stream through the surgical anastomosis and neo-terminal ileum precipitates the development of disease, highlighting the importance of the role of gut microbiota in disease pathogenesis [7]. Diversion of the faecal stream with a loop ileostomy is protective against CD recurrence; however, restoration of bowel continuity is associated with disease recurrence within weeks to months [8].

8.3 Risk Factors for Earlier Recurrence

Meta-analyses suggest that smoking, perforating disease and previous resection are independent risk factors for the development of postoperative CD recurrence [9]. There is also a cumulatively increased risk when more than one risk factor is present [10].

8.3.1 Smoking

Smoking is known to double the risk of postoperative recurrence despite best available medical therapy [10]. There is insufficient evidence to adequately assess the length of smoking cessation required to improve outcomes postoperatively; however, given that smoking is a modifiable risk factor, all patients should be counselled on the risks and encouraged to quit.

8.3.2 Perforating Disease

Perforating disease in CD refers to acute free perforation, subacute perforation with subsequent abscess or chronic perforation with fistula formation and is a risk factor for postoperative recurrence. Moreover, reoperation rates are also more common in patients with perforating disease. A meta-analysis examining the role that perforating disease plays in postoperative CD recurrence suggests that perforating and non-perforating disease are two different entities and perforating disease is a factor that needs to be considered when assessing postoperative relapse risk [11].

8.3.3 Previous Resection

Previous resection has been shown to be a risk factor for recurrence postoperatively. However, the extent of disease and length of previous resection appear to play a less clear role in the risk of recurrence [9].

8.4 Diagnosis and Monitoring of Post-op Recurrence

8.4.1 Endoscopy

Ileocolonoscopy is currently the gold standard investigation for the diagnosis of postoperative CD recurrence. Postoperative inflammation and ulceration may be seen at the surgical anastomosis and within the neo-terminal ileum endoscopically within weeks of resection. These findings include aphthous ulcerations which are mostly found in the pre-anastomotic ileum, anastomotic stricturing and myenteric plexitis [12]. Endoscopic disease precedes the development of clinical disease. Furthermore, the extent and severity of early lesions are predictive of the duration of time between surgical intervention and recurrence of symptoms and need for reoperation [12].

Most studies to date use the Rutgeerts' score for diagnosis which comprises of the following [5]:

- i0 – no lesions
- i1 – <5 aphthous ulcers
- i2 – ≥5 aphthous lesions confined to the ileocolic anastomosis
- i3 – diffuse aphthous ileitis
- i4 – diffuse inflammation with large ulcers or anastomotic narrowing

Stages i0 and i1 represent postoperative remission, while stages i2 and above are indicative of endoscopic recurrence. Patients with evidence

of stage i3 or i4 disease are classed as having severe endoscopic recurrence. The severity of endoscopic findings at 1 year postoperatively based on this scoring system has been shown to predict symptomatic relapse [5]. However, Rutgeerts' score is yet to be formally validated and may lead to subjective scoring given the minimal difference between i1 and i2 [9].

The correlation between scoring systems such as the Crohn's Disease Activity Index (CDAI) or the Harvey Bradshaw Index (HBI) and endoscopic findings is poor and is likely related to the subjective nature of the clinical indices of disease activity in contrast to the objective findings at colonoscopy [9].

Routine practice currently suggests endoscopy should be performed at 6 months postoperatively [10]. Ileocolonoscopy done too soon after surgery might not detect patients likely to have recurrent disease, and disease relapse might be well established and resistant to treatment if ileocolonoscopy is performed too long after surgery. Ileocolonoscopy at 6 months with subsequent step up of prophylactic therapy is more advantageous than symptom-based monitoring alone and aids in preventing endoscopic and clinical recurrence [9].

8.4.2 Faecal Inflammatory Markers

Faecal inflammatory markers are derived from neutrophil cytosol which is released in the setting of inflammation [13]. They are simple, cost-effective investigations which are stable at room temperature for up to a week [14, 15]. While ileocolonoscopy with subsequent histological examination remains the gold standard for the detection of postoperative recurrence, faecal inflammatory markers can be used to non-invasively monitor for postoperative recurrence [16].

Faecal calprotectin (FC) is a member of the S100 family of calcium-binding proteins and is currently used in the diagnosis and monitoring of IBD [17]. Elevated levels are found in body fluids and are proportional to the degree of inflammation present [18]. In the postoperative CD population, patients who have FC concentrations ≥100 μg/g are significantly more likely to have endoscopic recurrence, and levels decrease significantly in response to escalation of therapy [19]. Analysis of results from the Postoperative Crohn's Endoscopic Recurrence (POCER) study suggested that if the normal range in postoperative CD patients is raised to 100 μg/g, FC has a sensitivity of 90% and negative predictive value of 91% compared to a specificity of 57% and a positive predictive value of 52% [19]. However, FC may also be raised in a number of other conditions including colorectal carcinoma, adenomatous polyps, obesity, non-steroidal anti-inflammatory use and physical inactivity [16].

Other faecal markers include lactoferrin which is derived from an iron-binding glycoprotein and S100A12 derived from calgranulin [16]. Studies evaluating lactoferrin have been small, and few have made routine comparisons to endoscopy. Studies with small cohorts have shown that lactoferrin falls to normal concentrations within 2 months of resection and can correlate with symptomatic recurrence (measured by the HBI) but not endoscopic recurrence [16]. In one prospective study, 20 patients in clinical remission defined as CDAI <150 between 6 and 12 months following surgical resection underwent lactoferrin testing and ileocolonoscopy. Lactoferrin correlated with endoscopic recurrence and levels were significantly higher in patients with clinical recurrence than those in remission [16]. S100A12 is currently emerging as a useful marker of gut inflammation but has not yet been evaluated in the post resection setting.

Routine FC testing can play a role in the postoperative setting as part of a management algorithm in asymptomatic patients and is superior to both CDAI and CRP as a screening tool. Colonoscopy should be reserved for asymptomatic patients with an increased calprotectin concentration ≥ 100 μg/g, those with symptom recurrence or to monitor treatment response to a step up in therapy.

8.4.3 Serological Markers

Serological markers directed against specific antigens have been identified in IBD and can be used to aid the diagnosis and differentiation of CD from ulcerative colitis (UC). These antibodies are directed against enteric microbial antigens such as oligomannan, cell wall porin proteins and flagellin subunits [20]. Higher titres of these serological markers are present pre-resection, and many do not decrease following surgery suggesting a permanent immune change rather than disease burden [20].

Anti-saccharomyces cerevisiae antibodies (ASCA) are antibodies to mannan cell wall proteins and are highly prevalent in CD. ASCA has the highest sensitivity and specificity for CD and when combined with perinuclear anti-neutrophil cytoplasmic antibody (pANCA) can aid in distinguishing CD from UC [20]. Stricturing and penetrating disease is associated with a positive ASCA, and the severity of disease may be predicted by the magnitude of the ASCA result. A number of studies suggest an association between a positive ASCA and the need for abdominal surgery and the requirement in some cases of earlier intervention [20]. However, ASCA alone is insufficient to predict relapse. In one study, endoscopic recurrence at 18 months did not differ between patients who were positive prior to resection compared to those with a negative result [21]. In a separate early paediatric study which has not been replicated since, levels of ASCA decreased postoperatively [22].

Anti-Fla-X, A4-Fla2 and CBir are antibodies against flagellin subunit proteins linked to clostridium cluster XIVa [21]. Patients with recurrence at 18 months are more likely than those without relapse to be positive for anti-Fla-X. This result is particularly significant at baseline and 12 months but not at 6 months or 18 months [21]. Anti-Omp-C, an antibody to a bacterial outer membrane protein derived from *Escherichia coli*, is more likely to be positive when patients have previously undergone ≥2 resections [21]. Omp-C antibody positivity is not associated with an increased risk of postoperative recurrence. However, when all four of the aforementioned antibodies are elevated at baseline, patients are more likely to have endoscopic recurrence at 18 months compared to negative antibody testing [21]. Adjustment for clinically relevant risk factors improves the predictive ability of these serological investigations.

While a number of serological markers exist, their role in the diagnosis of postoperative CD is uncertain and thus clinically unreliable. As further markers evolve, further research is required, and in the future, highly sensitive and specific serological tests may become available.

8.5 Clinical Trials of Therapeutics

A number of trials evaluating the benefit of different drug therapies in the prevention of postoperative CD exist.

Metronidazole and ornidazole are antimicrobials of the nitroimidazole class which lead to DNA breakdown. Use of metronidazole provides a modest benefit in the reduction of postoperative relapse [23], whereas metronidazole combined with a thiopurine is moderately effective [24]. In studies to date, a dose of 400 mg BD for 3 months is suggested with step down to 200 mg BD, 200 mg daily or cessation of the drug if the initial regime is not tolerated. Such a strategy is tolerated by 80% of patients. Another medication of the same class, ornidazole is moderately effective in preventing endoscopic and clinical recurrence of postoperative CD when given for 1 year [25]. Both therapies reduce the risk of endoscopic and clinical relapse relative to placebo [26]. However, their long-term use is limited due to the potential risk of toxicity.

Thiopurines form the initial mainstay of therapy for patients diagnosed with CD. They are immunosuppressive drugs and supress the action of T cells which lead to inflammation. Azathioprine in particular targets Rac1, a GTPase that is involved in the activation of intestinal T lymphocytes [27]. Azathioprine used in combi-

nation with metronidazole has been found to significantly reduce endoscopic recurrence at 12 months compared to patients receiving placebo (44% vs 69%) [24]. In the TOPPIC trial which compared mercaptopurine to a placebo in the prevention of CD in patients undergoing resection, clinical recurrence requiring rescue anti-inflammatory therapy occurred at a lower rate in those taking mercaptopurine (13%) versus placebo (23%). Complete endoscopic remission was maintained in proportionately more patients on mercaptopurine than placebo [28]. Furthermore, mercaptopurine was more effective in patients with preoperative thiopurine exposure than in thiopurine naïve patients [28]. In a subgroup analysis assessing the effectiveness of thiopurine therapy in smokers, postoperative recurrence was significantly reduced in patients on mercaptopurine therapy compared to those taking a placebo [28].

Antitumour necrosis factor (anti-TNF) therapy inhibits tumour necrosis factor in bowel mucosa resulting in significant clinical improvements in patients with IBD. Infliximab is highly effective in the reduction of endoscopic and clinical recurrence at 12 months post resection [29]. Furthermore, commencement of therapy 1 year postoperatively also induces and maintains endoscopic remission in patients who demonstrate endoscopic recurrence [29]. However, studies to date are small, and larger studies are required to establish its optimal use postoperatively. Adalimumab also prevents postoperative recurrence in the short term [30]. A proportion of patients treated with anti-TNF therapy will be primary nonresponders, some will lose response, and factors such as smoking may diminish efficacy.

8.5.1 Top-Down Versus Tailored Approach

Two approaches exist in the management of postoperative CD: firstly a "top-down" approach in which anti-TNF prophylaxis is administered for all high-risk patients immediately after surgery and secondly a "tailored" approach whereby initial drug therapy is based on clinical risk and ongoing therapy is tailored to endoscopic recurrence.

The "top-down" approach was demonstrated by the PREVENT study which compared infliximab and placebo in the prevention of postoperative clinical and endoscopic recurrence. In this study, all high-risk patients defined as any patient that smoked, had perforating disease or had previously undergone resection, received either infliximab (5 mg/kg) or placebo therapy immediately following surgery. The primary end point was clinical recurrence at or before week 76 defined as an increase in CDAI score of 70 points or a total score $\geq$ 200 and endoscopic recurrence comprising of Rutgeerts' score $\geq$ 2. Secondary end points included clinical recurrence at or before week 104 or endoscopic recurrence at or before week 76. Analysis of results revealed that infliximab is not superior to placebo in preventing clinical recurrence after CD-related resection [31]. However, infliximab does significantly reduce the rate of endoscopic recurrence. Furthermore, the PREVENT study identified that preoperative anti-TNF exposure and a history of previous resection are independent risk factors for postoperative clinical recurrence [31].

Comparatively, the POCER study examined the role of a tailored approach. This study was devised to address best drug therapies and strategy by administering initial drug therapy based on clinical risk and adjusting ongoing therapy based on endoscopic findings. Patients were risk stratified into high-risk ($\geq$1 risk factors) or low-risk (no risk factors) based on whether they were smokers, had previously undergone an intestinal resection or had perforating disease. The low-risk patients received no therapy, while the high-risk patients received mercaptopurine or adalimumab if they were thiopurine intolerant. Thereafter, one third of patients were randomised to standard care comprising of endoscopy at 18 months. The remaining two thirds of patients received early endoscopy at 6 months with subsequent step up

of therapy if endoscopy revealed recurrent disease. If step up therapy was required, low-risk patients were prescribed mercaptopurine or adalimumab if they were thiopurine intolerant, adalimumab if already on a thiopurine or an increase in adalimumab dose for patients already on anti-TNF therapy. All patients received metronidazole for up to 3 months. The primary end point was defined as endoscopic recurrence based on a Rutgeerts' score of ≥2 at 18 months. At 18 months, there was a significantly lower rate of endoscopic recurrence in the active care arm with 49% of patients in the active care arm with endoscopic recurrence versus 67% in the standard care arm. Furthermore, 22% of patients in the active care arm had complete mucosal healing, defined as Rutgeerts' score of zero, versus 8% of patients in the standard care arm. Stepping up treatment at 6 months brought 39% of patients with recurrence into remission 1 year later [10]. However, remission at 6 months with no change in therapy was associated with recurrence in 39% of patients 1 year later suggesting that intensifying treatment at 6 months is helpful in bringing patients with recurrence into remission. However, remission at 6 months does not guarantee that remission is maintained 1 year later. Overall, treating according to risk of recurrence with 6-month colonoscopy and step up for recurrence is significantly superior to optimal drug therapy alone in preventing CD recurrence [10]. Additionally, selective potent immunosuppression, adjusted if needed on the basis of colonoscopy rather than its use in all high-risk patients, leads to effective disease control and avoidance of overtreatment in a majority of patients.

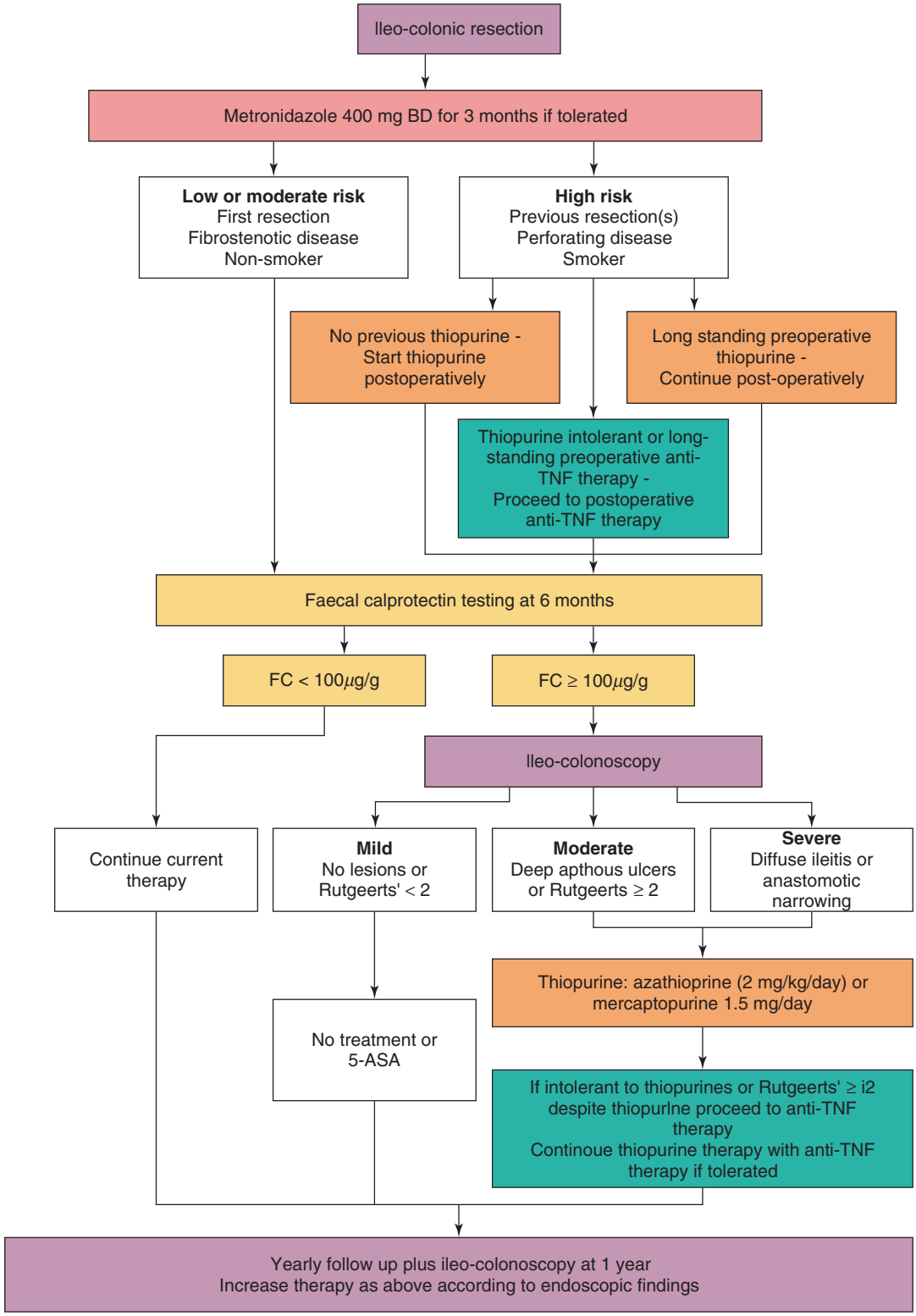
In a subgroup analysis of the POCER study comparing thiopurine therapy and adalimumab, adalimumab was superior compared to thiopurines in preventing early disease recurrence in high-risk patients and more likely to maintain complete mucosal macroscopic normality [32]. However, thiopurine metabolites were not routinely utilised during this study due to standardised dosing [10]. In clinical practice, drug optimisation might result in an improved benefit from thiopurines. CDAI scores, with recurrence defined as ≥150, did not differ between treatment groups and did not correlate consistently with endoscopic recurrence [32]. However, the study duration for the latter sub-analysis was out to 6 months; hence, a significant difference in clinical recurrence between treatment groups would not have been expected over such a short period. Moreover numerous studies in CD have demonstrated a discordance between clinical symptoms defined by CDAI and endoscopic disease activity.

Vedolizumab, a selective adhesion molecule, has become available for patients intolerant to thiopurines or TNF antagonists. In a recent analysis of postoperative CD patients, vedolizumab proved to be effective in preventing recurrence of ileal disease in six out of seven participants who were nonresponsive to anti-TNF therapy [33, 34]. The one patient who did not respond required further surgical resection. However, the sample size was small, and thus it is difficult to extrapolate data. A further randomised, double blind, placebo controlled study of vedolizumab for the prevention of postoperative CD is currently underway [35]. Ustekinumab, another monoclonal antibody, has proven efficacy in the treatment of luminal CD. However, no data examining its use in the postoperative setting exists currently, and further studies are required to test its efficacy in preventing postoperative recurrence.

8.6 Algorithm

Postoperative drug therapy, according to clinical risk of recurrence, with colonoscopy at 6 months and treatment step up for recurrence, is significantly better than standard care alone for prevention of CD recurrence. Immunosuppression restricted to patients at high risk is likely to be more cost-effective than its use in all patients. Although all high-risk patients could be treated with anti-TNF therapy, this would carry increased cost, an increased rate of side effects and may constitute overtreatment in some patients.

A proposed clinical algorithm for the post-operative management of Crohn's disease is shown below:



8.6.1 Current Limitations and Future Directions

Although the gut microbiota are known to play a key role in postoperative CD pathogenesis, there are limited data on therapeutic manipulation of the gut microbiota in the postoperative setting beyond the use of antibiotics. Probiotics have not been found to be effective, and the efficacy of faecal microbiota transplantation for the prevention and treatment of postoperative recurrence is unknown. Future studies should seek to characterise the changes that occur in the gut microbiota overtime in an effort to find bacteria-specific targeted therapies. A systems biology approach to carefully characterise an individual's risk of recurrence based on clinical, genetic, microbiologic and immunologic factors should ultimately be undertaken to tailor the most appropriate postoperative prophylaxis to the individual patient.

Summary Points

- Stratifying patients into high risk or low risk based on smoking status, previous resection or perforating disease allows patients to receive tailored therapy based on their risk of postoperative recurrence.
- Postoperatively, faecal calprotectin with an increased upper limit of >100ug/g can be used as a screening tool, with colonoscopies reserved for asymptomatic patients with an increased calprotectin concentration or those with symptom recurrence.
- Ileocolonoscopy should be performed within 6 months post resection and severity scored based on Rutgeerts' score with patient's scoring ≥i2 receiving step up of therapy as appropriate.
- Selective potent immune suppression, adjusted if needed on the basis of colonoscopy rather than its use in all patients at high risk of recurrence, leads to effective disease control in most patients.
- Although serological markers have been identified, the evidence is limited, and application in clinical monitoring postoperatively is not recommended.

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Perianal Crohn's Disease

9

Wing Yan Mak and Siew Chien Ng

Abstract

Perianal disease affects one-third of Crohn's disease patients. It mainly affects the young and is associated with significant morbidity and multiple surgical interventions. Perianal Crohn's disease involves a spectrum of disorders, from fistulising to non-fistulising disease. The aetiology of perianal Crohn's disease is not well understood. Several biomarkers including the presence of anti-*Saccharomyces cerevisiae* antibodies (ASCA) and OmpC antibody and persistently high CRP level have been shown to be associated with the development of perianal Crohn's disease. The presence of proctitis is associated with poorer prognosis. Diagnosis of perianal Crohn's disease requires careful history, physical examination, imaging with MRI or endoanal ultrasound and examination under anaesthesia.

Management of perianal Crohn's disease is challenging. Antibiotics and immunomodulators, although can improve symptoms, do not heal perianal fistulas. About one-third of patients with perianal Crohn's fistula respond to antitumour necrosis factor (TNF) agents, but prolonged treatment is usually necessary, and the risk of recurrence is high. Only 34% of patients remained free of relapse after 1 year of treatment. Combination of optimal medical therapy with surgical therapy (drainage of sepsis and insertion of seton) leads to a higher complete remission rate compared with single therapy. Overall, long-term infliximab therapy with combined medical and surgical management produces clinical remission in 36–58%. Importantly, deeper radiological healing on MRI has been shown to lag behind clinical remission by a median of 12 months. Therefore, multidisciplinary management including medical and surgical therapy, with radiological guidance, should be the gold standard in perianal Crohn's disease management.

Recently, studies have shown that higher infliximab levels are associated with fistula healing. Therapeutic drug monitoring might have a role in the management of perianal Crohn's disease. New treatment modality including mesenchymal stem cell therapy and newer generation of biological therapy includ-

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ing vedolizumab and ustekinumab also show promising results in perianal Crohn's disease.

Management of perianal Crohn's disease should have a multidisciplinary approach. Future studies focusing on identification of biomarkers associated with a complicated course of perianal Crohn's disease are required to improve patients' quality of life and remission rates.

9.1 Introduction

Perianal Crohn's disease affects one-third of patients with Crohn's disease. Its presence indicates a more aggressive disease course, with a higher rate of recurrence following treatment cessation and a shorter median time to recurrence [1, 2]. Beaugerie et al. reported that the presence of perianal Crohn's disease at presentation was associated with an increased likelihood of repeated courses of corticosteroids (and risk of dependence), increased number of hospital admissions and increased surgical resections in the subsequent 5 years and predisposes patients to chronic disabling symptoms. [3]

Perianal Crohn's disease is defined as inflammation surrounding the anal region. While Crohn's disease is typically described as progressing from inflammatory to penetrating and/or stricturing phenotypes, patients with perianal Crohn's disease do not always follow this pathway. Perianal disease can precede intestinal symptoms of Crohn's disease with a varying frequency from 5% to 46% in different series [4–9]. However, less than 5% have perianal involvement as the sole presentation of Crohn's disease [10]. When perianal disease is the first presentation, it may be difficult to differentiate from simple anal fissures, haemorrhoids or fistulas to those without Crohn's disease. Diagnosis of underlying Crohn's disease should be suspected in patients: (1) whose perianal disease does not improve with conventional treatment, (2) who have unusual presentation (e.g. anal fissures are not located in the midline) and (3) who report symptoms suggestive of underlying Crohn's disease, e.g. diarrhoea, joint pain, rash and eye involvement.

The epidemiology of perianal Crohn's disease is poorly understood. The reported prevalence was highly varied, ranging from 10% to 80% [11–13]. The disease spectrum of perianal Crohn's disease is also wide, from fistulising to non-fistulising diseases. Various classification systems have been proposed to stratify the spectrum, but none have been generally accepted [14–16]. Details of description of various perianal Crohn's lesions are shown in Table 9.1.

In this chapter, we have approached perianal Crohn's disease with reference to the AGA technical review. We will focus on diagnostic evaluation, risk factors for development, management and prognostic factors/biomarkers.

9.2 Anal Skin Lesions and Anal Canal Lesions

Table 9.2 summarises the features, management and prognosis of anal skin and anal canal lesions.

9.3 Perianal Abscesses

Perianal abscesses occur in 23–62% of patients with underlying Crohn's disease [26, 48]. They can be divided into four types according to their anatomical relationship to the internal sphincter and levator muscles (see Fig. 9.1). Ischiorectal abscesses are the most common and account for 39–43% of all perianal abscesses [49, 50].

Patients with perianal abscesses usually present with acute pain (82%) and occasionally incontinence (7%) [50]. An active search for the internal fistula opening is recommended. In a prospective study of perianal Crohn's disease patients, 73% of all perianal abscesses were associated with an ischiorectal fistula, and 50% were associated with a transsphincteric fistula [50].

Table 9.1 AGA definition of various types of perianal lesions in patients with Crohn's disease [17]

Type of lesion	Description
Skin tag	Two types: 1. Large, oedematous, hard, cyanotic skin tags. Typically arising from a healed anal fissure or ulcer. Excision contraindicated due to problems with wound healing 2. "Elephant ear" tags that are flat and broad or narrow, soft painless skin tags. May cause perianal hygiene problems and can be safely excised
Haemorrhoids	Prolapsing internal haemorrhoids. Uncommon in Crohn's disease. Often present as large external skin tags
Fissure	Anal fissures are broad based and deep with undermining of edges. There may be associated large skin tags and a cyanotic hue to the surrounding skin. They tend to be multiple and may be placed either eccentrically around the anal canal or in the midline in contrast to idiopathic fissure-in-ano, which tend to lie in the midline. Typically painless (pain should raise suspicion for perianal abscess or acute/chronic conventional anal fissure). Conventional anal fissures occasionally are treated by conventional fissure treatment including lateral sphincterotomy
Anal ulcer	Anal ulcers are usually associated with rectal inflammation and may lead to destruction of the anorectum, anorectal strictures, complex anorectal fistulas and perianal abscess
Low fistula	Superficial, low intersphincteric or low transsphincteric fistulas. May arise from either the anal glands (cryptogenic) or from penetrating ulceration of the anal canal or rectum
High fistula	High intersphincteric, high transsphincteric, suprasphincteric, extrasphincteric fistulas. May arise from penetrating ulceration of the anal canal or rectum
Rectovaginal fistula	Superficial, intersphincteric, transsphincteric, suprasphincteric and extrasphincteric fistulas. May arise from penetrating ulceration of the anal canal or rectum into the vagina
Perianal abscess	Potential anorectal spaces may become infected with an abscess, including perianal, ischiorectal, deep postnatal, intersphincteric and supralevator
Anorectal stricture	May be short annular diaphragm-like strictures <2 cm in length or longer tubular strictures arising from rectal inflammation. May arise from either the anal glands (cryptogenic) or from penetrating ulceration of the anal canal or rectum
Cancer	Squamous cell carcinoma, basal cell carcinoma or adenocarcinoma arising from malignant degeneration of non-healing perianal fistulas or sinus tracts

The management approach to perianal abscesses involves drainage of sepsis without damaging the sphincter complex. Adequate drainage should be achieved to prevent reaccumulation of sepsis (see Fig. 9.2).

9.4 Perianal Crohn's Fistulas

Fistulising perianal Crohn's disease (pCD) represents a distinct, aggressive and disabling phenotype of Crohn's disease. It occurs in up to 40% of CD patients [9, 51–53]. Perianal fistulas can be the initial manifestation of Crohn's disease in 10% of patients [54].

9.4.1 Factors Associated with Development of Perianal Crohn's Fistulas

The aetiology of fistulising perianal Crohn's disease is unknown. The influence of gender on development of pCD is conflicting [8, 55]. Onset of Crohn's disease less than 40 years old was found to be significantly associated with development of penetrating complications of CD, including perianal fistulas [56]. pCD was found to be more common in non-Caucasian and Sephardic Jews (as compared to Ashkenazi Jews) [8, 56]. Of note, perianal complications are more commonly reported in

Table 9.2 Characteristics of anal skin lesions and anal canal lesions

	Incidence	Clinical features	Risk factors	Diagnostic methods	Management	Prognosis
Anal skin lesions						
Anal skin tags	37% of patients with CD; 68% of CD patients with perianal disease [18]	Divided into 2 types: [17] 1. Typical CD skin tags: large, oedematous, hard and cyanotic; may arise from lymphedema secondary to lymphatic obstruction 2. "Elephant ear" tags: soft, flat and board and painless; may interfere with personal hygiene	First type (typical CD skin tags) often co-exist with intestinal inflammation [19]	By typical appearance on physical examination Excisional biopsy not recommended in view of poor wound healing, post-operative complications and rare requisites for proctectomy	Usually expectant management *Typical CD skin tags*: Excision is contraindicated due to poor wound healing [17] Management should be targeted on treating underlying intestinal inflammation *"Elephant ear" tags:* Excision can be considered if extremely bothersome symptoms and active proctitis have been excluded	86% asymptomatic; 40% resolved spontaneously [20] May increase in size and thickness and become more firm during active flare of CD [21] Very rare malignant transformation (only one case reported so far) [22]
Haemorrhoids	Uncommon in CD Reported incidence: 0.04–0.4% in patients with CD [11, 23]	Often present as large skin tags Usually asymptomatic	May become problematic if severe diarrhoea	By careful digital rectal examination and proctoscopy Prolapsed internal haemorrhoids appear as dark pink, glistening, and occasionally tender masses at the anal verge	Conservative management advocated as haemorrhoidectomy is associated with serious complications including poor wound healing, anorectal stenosis and high rate of proctectomy Elastic band ligation/selective haemorrhoidectomy may be used in patients with severe prolapsing and bleeding haemorrhoids in the absence of proctitis [17, 24]	Conservative management was reported as having >60% in one series [25] Consider diagnosis of CD in patients with non-healing haemorrhoidectomy wounds 2–3 months after operation
Anal fissures	Common in CD; reported in 35–59% of CD patients [26, 27]	Usually painless; however, anal discomfort reported in 44–70% of cases [27, 28] Other symptoms include discharge, pruritus and bleeding Most located in the posterior midline; lateral fissures present in up to 20% [27, 28] Multiple fissures in 1/3 of cases [27, 28]	Likely to be secondary to direct ulceration from underlying CD Raised anal resting pressures causing compromised rectal blood flow may predispose to fissures formation in CD [29]	Fissures can usually be identified when buttocks are parted Digital or proctoscopic examination is often impossible due to pain	80% heal spontaneously Surgical treatment in unselected cases should be avoided Fissurectomy should only be considered in cases where the edges of fissures are densely fibrotic and unlikely to heal aftersphincterotomy alone [25] Lateral sphincterotomy in selected symptomatic cases without proctitis may achieve healing without incontinence in most cases Topical treatment (glyceryl trinitrate/diltiazem ointments, tacrolimus) can be effective [25]	Chronic fissures are uncommon; only 19% had persistent anal fissures after 10-year follow up [30] Unhealed fissures can progress to fistula or abscess in up to 26% [27]

Anal ulcer	Reported incidence 1.9–5.1% [31, 32]	Characterised by perirectal pain; severe unremitting pain in 56%; associated constipation in 35% of cases [33] Large ulcer with craggy appearance and oedematous, irregular, undermined and detached edge Multiple ulcers in 14–33% [34] Rare for ulcer to extend from anal canal to perianal skin	Concomitant proctitis present in 75–96% of cases [34]	By careful history taking and inspection on physical examination Digital or proctoscopic examination is often impossible due to pain	Topical metronidazole and topical tacrolimus ointment are shown to improve symptoms and depth of ulcers respectively [35–37] Systemic therapy with cyclosporine and infliximab has been shown to be effective; but systemic side effects of cyclosporine probably limit its long-term use [38–40] Hyperbaric oxygen therapy and elemental diet may be beneficial [41–43]	Predict a more aggressive disease course in general High likelihood of future luminal disease activity and other perianal complications [10] Fifty percent will progress to anal stenosis with induration [21] Poor long-term outcome in those with cavitating ulcers; risk of proctectomy in up to 83% of patients reported [18]
Anal canal lesions						
Anorectal strictures	Reported incidence: 9–22% [18, 26]	Mostly asymptomatic due to underlying loose stool associated with CD Typical symptoms include urgency, incontinence, tenesmus, frequency, bloody diarrhoea and difficulty with defecation Can be short (<2 cm), annular and diaphragm-like or long and tubular in shape	Mostly occur as a complication and result of distal rectal inflammation and recurrent anal fissures	Multidisciplinary approach should be adopted Digital rectal examination may not be possible due to underlying severe degree of stenosis Examination under anaesthesia (EUA) with biopsy should be done to exclude malignancy MRI pelvis to look for anatomical location, length of stricture and associated perianal disease	Asymptomatic patients don't require treatment Mildly-moderately symptomatic cases can be treated by repeated gentle dilatations by finger, Hegar dilators or coaxial balloon dilatation Severe cases may require proctectomy	Most respond to simple dilatation Forty-seven percent had poor wound healing if there is concomitant perianal disease [44] Presence of fibrotic stricture is a strong predictor of future need of permanent stoma [45]

(continued)

Table 9.2 (continued)

	Incidence	Clinical features	Risk factors	Diagnostic methods	Management	Prognosis
Anal cancer	Reported rate of malignant transformation of perianal fistula in CD: 0.7% [46]	Squamous cell carcinoma (SCC), adenocarcinoma and basal cell carcinoma have been reported [19] Diagnosis often delayed due to underlying anorectal stricture and pain making thorough examination difficult	Increased risk with underlying chronic severe anorectal/ perianal disease	High index of suspicion is required Examination under anaesthesia with biopsy and curettage should be done if any new symptoms arise in patients with longstanding perianal fistula	Standard oncologic surgical principles and procedures should be followed	Poor in general with more aggressive disease and worse disease-free survival than general patients with anal SCC Higher rate of complications post-treatment expected due to poor wound healing High rate of complications after radiotherapy for IBD-related anal SCC reported [47]

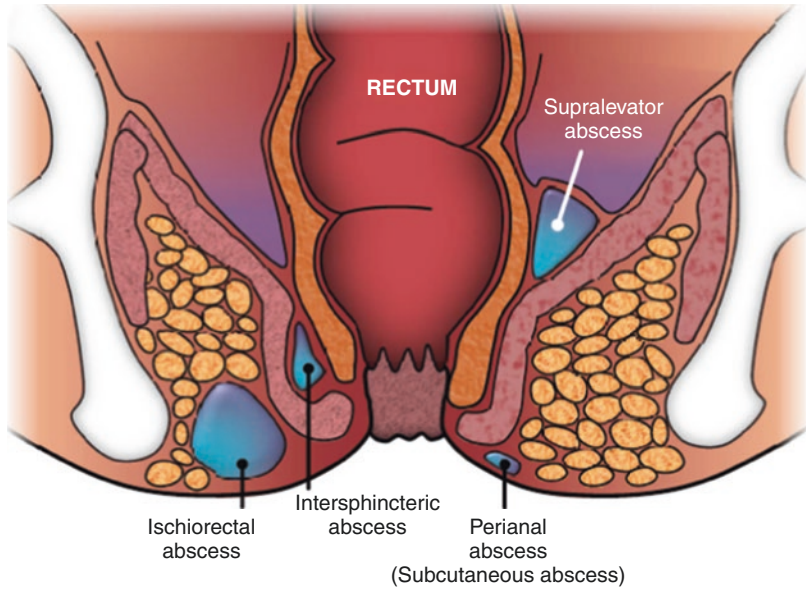

Fig. 9.1 Classification of perianal abscess. Intersphincteric abscess is located in the space between the internal and external anal sphincter; supralevator abscess lies above the anorectal ring in the supralevator space; ischiorectal abscess occurs in the ischiorectal space, while subcutaneous abscess occurs in the superficial soft tissues (https://www.fascrs.org/patients/disease-condition/abscess-and-fistula-expanded-information)

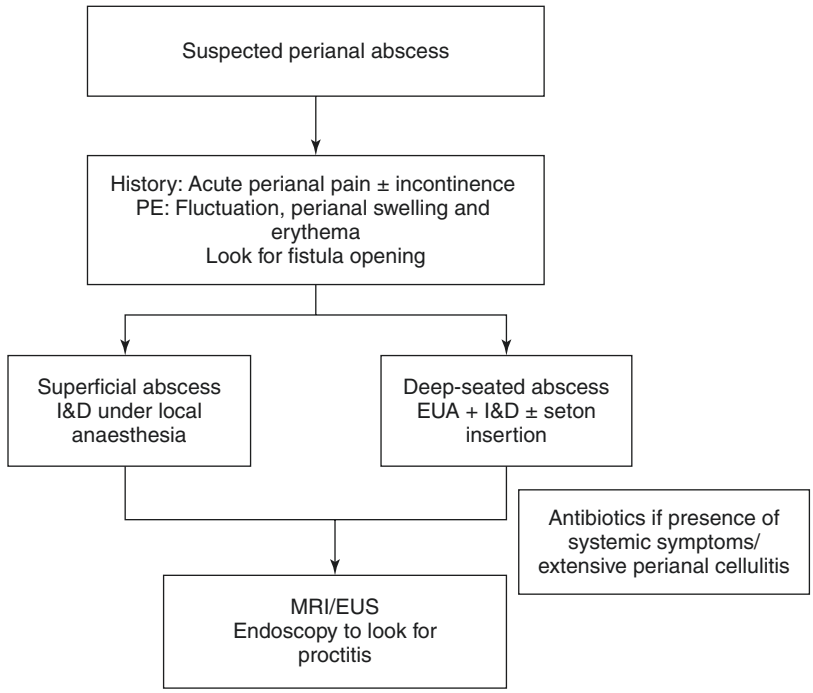

Fig. 9.2 Algorithm for management of perianal abscess. (PE physical examination, I&D incision and drainage, EUA examination under anaesthesia, EUS endoanal ultrasound)

the East Asian population than in Caucasian population [78]. pCD is more commonly found in patients with colonic involvement, especially those with active proctitis [OR: 4.32 (3.4–5.51), $p < 0.001$] [10, 57, 59]. A recent study by Kaur et al. showed that family history (4.98 [3.30–7.46], $p < 0.001$) and stricturing behaviour of CD were also associated with a higher chance of having pCD (OR 1.44 [1.14–1.81], $p < 0.002$) [7].

9.4.2 Biomarkers in Predicting the Development of Perianal Crohn's Fistulas

Besides the above clinical factors, several biomarkers are associated with the development of perianal Crohn's fistulas, as summarised in Table 9.3.

9.4.3 Diagnosis of Perianal Crohn's Fistulas

Diagnosis of perianal Crohn's fistulas includes a comprehensive history and careful physical examination. Typical symptoms of perianal Crohn's fistulas include anorectal pain, purulent discharge, per rectal bleeding, recurrent urinary tract infection and/or faecal incontinence. Presence of any associated perianal abscess, anorectal stricture and rectovaginal fistula should be actively searched for.

Endoscopy is essential to look for the presence of proctitis and to assess the extent and severity of luminal disease and the presence of internal openings and other complications. Presence of proctitis is an independent risk factor for persistent non-healing, high recurrence rates and higher proctectomy rates [69, 70].

Table 9.3 Biomarkers associated with development of perianal Crohn's disease

Biomarkers		Association with pCD
Serology	Anti-*Saccharomyces cerevisiae* antibodies (ASCA)	Presence of ASCA predicts a higher likelihood of pCD (OR 1.89 [1.04–3.44]) [58]
	OmpC antibody	pCD was associated with a higher level of OmpC antibody level ($p = 0.008$) [59]
Inflammatory marker	C-reactive protein (CRP)	Persistently high CRP > 31 was found to be an independent risk factor for the development of perianal Crohn's fistulas [60]
Genetic markers	IBD5/OCTN	Previous studies have described an association between pCD with the susceptibility locus on chromosome 5 (5q31). A particular OCTN (carnitine/organic cation transporter) was found to be associated with the development of pCD
	Immunity-related guanosine triphosphatase protein type M (IRGM)	IRGM rs4958847 predicts a higher likelihood of pCD (OR 1.21–1.61) [61, 62]
	NOD2/CARD15	No correlation was shown between this genotype and pCD. Presence of NOD2/CARD15 wildtype predicted greater response to antibiotic treatment in patients with perianal Crohn's fistulas [63, 64]
Microbiology – change in microbiota	Decreased *Bifidobacteria* in the rectum	Decreased *Bifidobacteria* was noted in rectal mucosa in patients with perianal Crohn's fistulas than patients with idiopathic anal fistulas and Crohn's luminal disease [65]
	Gram-positive organisms	Predominantly colonised by skin flora with Gram-positive bacteria (*Corynebacteria* spp., *Streptococci*, coagulase-negative staphylococci) in perianal Crohn's fistulas [66]
Immunology	Inflammatory cytokines	Increased serum (TNFα, IL-6) and rectal mucosal inflammatory cytokines (IL-1β, IL-6, TNFα) in patients with pCD [67, 68]
	JAK-STAT pathway	Genetic variation in the JAK-STAT pathway was associated with pCD ($p_c = 3.72 \times 10^{-5}$) [79]

MRI is the gold standard for the diagnosis of perianal fistulas. It is non-invasive and highly accurate. Its diagnostic superiority to endoanal ultrasound and examination under anaesthesia has been demonstrated. [71, 72] The specificity of MRI in diagnosing perianal fistulas ranges from 76% to 100% [73].

Endoanal ultrasound (EUS) can be a reasonable alternative to MRI in diagnosing perianal fistulas. Its accuracy is around 86–95% in correct classification of the fistulas and around 62–94% in identifying the internal openings [74–76].

Examination under anaesthesia (EUA) stands as both diagnostic and therapeutic as immediate drainage and seton insertion for associated perianal abscess can be done after the diagnosis is made. EUA however should not be delayed if MRI cannot be performed immediately. Schwartz et al. have demonstrated in a prospective study that the diagnostic accuracy approached 100% when combining either two of the investigations (i.e. MRI, EUS or EUA) [77]. The priority of undertaking MRI or EUA first depends on availability of the diagnostic modality and the need to drain any underlying sepsis.

9.4.4 Management of Perianal Crohn's Fistulas

Management of perianal Crohn's fistulas is challenging. A study by Molendijk I et al. in 2014 revealed that only one-third of patients had durable remission of their complex perianal fistulas despite medical and surgical therapies [79]. The management principle is to surgically drain the underlying sepsis promptly and medically treat proctitis and fistulas aggressively [84]. Various studies have shown that the combination of surgical and medical therapy results in higher complete remission rates compared with either medical or surgical therapy alone [80–84]. Ultimately, the goal of treatment is to achieve and maintain disease remission while preserving continence.

9.4.5 Medical Treatment

Various medical treatments have been studied in the management of pCF. The limitations of the majority of studies have been in the definitions of clinical remission which have varied from reduction in drainage to fistula closure. There has also been heterogeneity in the treatments administered.

9.4.5.1 Antibiotics

Ciprofloxacin and metronidazole are the two most commonly used antibiotics in the management of pCF despite their association with slow and incomplete response, early recurrence and side effects with long-term use. In a case series by Bernstein et al., all patients had symptom improvement (reduction in drainage, decrease in erythema and induration). Nonetheless, only 10/18 (56%) achieved complete healing on maintained treatment [85]. A study in 1989 reported that all ($n = 8$) patients with pCF resistant to metronidazole improved on ciprofloxacin, but 50% still had persistent drainage [86]. Approximately two-thirds of patients responded to combination of ciprofloxacin and metronidazole in a series involving 14 pCF patients [86]. A randomised controlled trial comparing metronidazole and ciprofloxacin in 25 pCF patients failed to show any statistically significant difference in rates of response to treatment and remission [87].

9.4.5.2 Thiopurines

Although azathioprine and 6-mercaptopurine (6-MP) have been shown to be effective in the management of luminal Crohn's disease, thiopurine efficacy in pCF has only been assessed as a secondary end point in clinical trials. A meta-analysis of five studies reported a 54% clinical fistula response in the azathioprine/6-MP group compared with 21% in the placebo group [88]. Combining antibiotics (metronidazole or ciprofloxacin) to azathioprine has been found to be associated with a better response in the group receiving azathioprine at week 20 than those who did not (48% vs. 15%, $p = 0.003$) [89]. Patients aged 40 years or older and with a recent onset of

perianal involvement (less than 22 months) responded better to azathioprine or 6-mercaptopurine [90].

9.4.5.3 Calcineurin Inhibitors, Thalidomide and Others

A small placebo-controlled trial (n = 42) has demonstrated the efficacy of oral tacrolimus in closure of perianal fistulas (43% vs. 8%, $p < 0.05$) after 10 weeks of treatment (0.2 mg/kg/day) [91]. Topical tacrolimus failed to show any benefit in pCF [37]. The use of cyclosporine was studied in a small retrospective study with 20 patients being administered with intravenous cyclosporine (4 mg/kg/day) for 7 days, followed by oral cyclosporine. Although 80% had initial improvement in symptoms, the majority of patients relapsed after switching to oral cyclosporine or upon discontinuation [92].

Thalidomide has also been used in the management of pCF. A recent systemic review on its efficacy showed that one-quarter (10/40) of pCD patients in the four-case series achieved remission and 35% withdrew from treatment due to side effects [92].

There are only small case series studying the use of methotrexate monotherapy in the treatment of pCF. Mahadevan et al. reported a 31% partial closure rate and 25% complete closure rate with methotraxate [93].

Mycophenolate mofetil (MMF) has been used in pCF with several small studies demonstrating that cessation of fistula drainage was noted in 20–57%, while partial response rate was 28–50% [108–111].

Overall, there are only small studies showing moderate benefit from the use of calcineurin inhibitors, thalidomide and mycophenolate mofetil in pCF.

9.5 Biological Therapy

The development of biological therapy has revolutionised the management of pCF. Previous studies have demonstrated that anti-TNF therapy improved health-related quality of life in patients with Crohn's perianal fistula at 12 months which correlated most with patients with clinical and MRI healing [94]. It was also found that anti-TNF therapy may potentially heal the fistulas tracks and shorten the time to clinical improvement from several months to a few weeks.

9.5.1 Infliximab

The result of the seminal study on the use of infliximab in the management of perianal Crohn's disease was published by Present et al. in 1999. Among the 94 pCF patients who were given an induction course of infliximab at week 0, week 2 and week 6, 68% had clinical response, and approximately half of the patients had complete fistulas closure. The median time to achieve response was 2 weeks [95].

In the maintenance trial of infliximab in the setting of pCF (ACCENT II), complete absence of draining fistulas was noted in 36% of patients in the infliximab maintenance group, compared to 19% in the placebo group at week 54 (p = 0.009) [96]. There is also evidence that maintenance infliximab therapy could reduce hospitalisation, surgeries and procedures in fistulising Crohn's disease [97].

Several noncontrolled studies have also reported good treatment response to infliximab as both induction and maintenance therapy, with rates of complete cessation of fistula drainage ranging from 13% to 90% [98]. However, the risk of relapse is high after discontinuation of infliximab. Only 34% pCF patients were in remission after stopping infliximab [99]. The efficacy of concomitant therapy with immunomodulators and infliximab to achieve better outcome is questionable. Post hoc analysis of the ACCENT II data showed no major benefit of concomitant therapy. Another study revealed less perianal complications in patients on concomitant immunomodulators [100].

Combining infliximab with surgical management leads to better outcomes. An earlier study in 2003 revealed that the combination of seton placement and infliximab results in an earlier initial response (100% vs. 82.6%, p = 0.014), lower recurrence rates (44% vs. 79%, p = 0.001) and

longer time to relapse (13.5 months vs. 3.6 months, $p = 0.0001$) than infliximab alone [80]. Further studies in Japan and France evaluating the efficacy of combining seton insertion with infliximab also yielded positive results with a higher chance of fistulas closure [81, 82]. A recent systemic review and meta-analysis of 24 studies by Yassin et al. revealed that combination therapy led to higher complete remission rate compared with single therapy (52% vs. 43%) [83]. Overall, long-term infliximab therapy with combined medical and surgical management produced clinical remission rates of 36–58% [84].

9.5.2 Adalimumab

There are no randomised controlled trials assessing the efficacy of adalimumab versus placebo in pCF. The CLASSIC 1 trial including 32 patients with pCD showed no differences between placebo, 80 mg/40 mg induction group and 160 mg/80 mg induction group for fistula response or remission [101]. The efficacy of maintenance adalimumab has been demonstrated in the CHARM study. One hundred and thirteen patients with Crohn's fistulas were randomised to receive adalimumab at week 0 (80 mg) and week 2 (40 mg) followed by either maintenance adalimumab (weekly or every other week) or placebo. Thirty per cent of those in the adalimumab group had complete closure at 26 weeks and 33% at 56 weeks, compared to 13% in the placebo group ($p = 0.016$) [102]. In patients who previously lost response to infliximab or intolerant to infliximab, switching to adalimumab can still be efficacious. The GAIN study revealed no difference in treatment response between placebo and adalimumab as a second-line agent [103]. The CHOICE trial demonstrated 39% complete response rates with adalimumab after loss of response to infliximab [104]. Echarri et al. reported 50% (8/16) complete response rate after 4 weeks of multidisciplinary management with adalimumab, examination under anaesthesia and seton placement in patients with previous infliximab failure, of whom 87.5% remained in remission after 48 weeks [105].

9.5.3 Certolizumab Pegol

Similar to adalimumab, there are no randomised controlled trials examining the efficacy of certolizumab in the setting of pCF. The PRECISE 1 trial examined its efficacy as maintenance therapy for fistula healing as a secondary end point. There were no statistically significant differences in fistula healing (defined as ≥50% closure at two consecutive post-baseline visits ≥3 weeks apart) between certolizumab and placebo at 26 weeks in the subgroup of patients with baseline C-reactive protein ≥10 mg/L (30% in certolizumab group vs. 31% in placebo group) [106]. The PRECISE 2 trial, which assessed the efficacy and tolerability of certolizumab as a maintenance therapy after successful induction therapy in active Crohn's disease patients, included a small number of pCD patients ($n = 55$). Fistula closure rate was higher in the certolizumab group compared to placebo (36% vs. 17%, $p = 0.038$) [107].

9.5.4 Vedolizumab

Vedolizumab is a monoclonal antibody to α4β7 integrin which demonstrated efficacy and safety in the management of Crohn's disease. Exploratory data from the GEMINI 2 study supported its use in the management of pCD. Of the 57 patients with fistulising CD in the GEMINI 2 study (74% had pCF), 28% achieved fistula closure at week 14 with either 4-weekly or 8-weekly vedolizumab, compared to 18% in the placebo group. This difference was maintained up to week 52 [112]. Further studies are now underway to study its efficacy in fistulising Crohn's disease.

9.6 Surgical Treatment

The role of surgeons in the management of pCF has changed. Multidisciplinary management is now the gold standard for management of pCF. The goal of surgery in pCF is to control sepsis and relieve symptoms, followed by a stepwise

surgical treatment in order to achieve fistula closure without impairment of incontinence.

Examination under anaesthesia (EUA), incision and drainage of underlying abscesses and insertion of setons have become the cornerstone of combined medical and surgical management of pCF. Inadequate drainage of sepsis prior to biological therapy may lead to abscess formation and treatment failure. In fact, 21 patients (15%) developed at least one fistula-related abscess while on infliximab maintenance therapy in ACCENT II trial, which was not statically different from the placebo group (n = 27,19%; p = 0.526) [96].

The exact timing for seton placement and removal is controversial. The latest European Crohn's and Colitis (ECCO) guideline does not specify a time interval. A recent meta-analysis by de Groof EJ et al., which included 10 noncontrolled studies involving 305 patients treated with setons, reported 13.6–100% complete fistula closure rate and 0–83% recurrence [113]. The timing of seton removal ranged from 3 weeks to 40 months. Some recent evidence suggested that prolonged seton placement led to lower recurrence rates [81, 114]. Combining seton placement with biological therapy and/or immunomodulators is associated with better results than with seton placement alone. The ongoing PISA trial will hopefully provide us some insight into the optimal interval of seton removal. [115]

Fistulotomy is strongly discouraged in complex perianal Crohn's fistula because of the risks of incontinence, poor wound healing, high risk of recurrence and need for proctectomy [116].

Endorectal mucosal advancement flap (ERAF) has a success rate between 25% and 64% [117]. Proctitis is a contraindication for the procedure due to poor wound healing. A diverting stoma is sometimes created in order to improve the efficacy.

Infilling the fistula with glue/fibrin has been studied in various small trials with diminishing interest in this technique. The advantage of fistula glue/fibrin is a very low complication rate and risk of incontinence. A systemic review by O'Riordan JM et al. revealed a 55% fistula closure rate in a pooled analysis of 42 pCF patients [118]. There is limited data about its long-term efficacy in the setting of pCF.

Temporary faecal diversion may help alleviate the symptoms of around two-thirds of patients with pCD [119]. However, the rate of restoration of continuity of bowel remained low despite the use of biologics. Only around one-third of patients had attempted restoration of bowel continuity, and 16% had successful bowel restoration [119, 120]. Besides, recurrence is high even after restoration of bowel continuity. The use of anti-TNF does not seem to reduce the rate of proctectomy significantly. Only absence of rectal involvement is significantly associated with restoration of bowel continuity [119]. Eventually, 20–40% of patients required proctectomy [119–123]. Even following proctectomy, there is still a problem with poor wound healing. Yamamoto et al. reported that 28% of cases had a persistent perineal sinus after proctocolectomy for Crohn's disease [124].

9.7 Rectovaginal Fistula

Rectovaginal fistula (RVF) occurs in around 10% of women with Crohn's disease and is classified as a complex fistula [131, 132]. It typically arises from an anterior rectal ulcer and eventually erodes the vaginal wall [133]. Around one-third to one-half of patients with RVF are either asymptomatic or have minimal symptoms only and require no treatment [134]. Symptomatic fistulas will present with passage of stool or gas from vagina, dyspareunia, perianal pain and repeated genitourinary tract infections. Diagnosis of RVF is similar to that of perianal Crohn's fistula, and examination under anaesthesia is recommended for the definitive elucidation of RVF.

There are no randomised controlled trials on the optimal management of RVF. Management often depends on its anatomical location, complexity and presence of active colitis in the distal colon. Surgery is the mainstay of treatment in RVF. Medical therapy is often used as an adjunct in controlling underlying Crohn's disease.

Infliximab has been studied in a post hoc subset analysis of ACCENT II trial in evaluating its efficacy in Crohn's-related RVF. After induction with infliximab at weeks 0, 2 and 6, 60.7% (15/25) and 44.8% (11/25) of patients had their RVF closed at weeks 10 and 14, respectively. Patients receiving maintenance infliximab infusion (5 mg/kg) achieved a longer duration of closure of their RVF (46 weeks vs. 31 weeks) [134].

Surgical management of RVF typically involves repair of the fistula. It is important to have complete drainage of underlying perianal abscess before repair. A non-cutting seton may be inserted to facilitate drainage. Rectal advancement flap surgery is the most commonly used method. It is best performed in patients with low RVF and absence of proctitis and anal stricture. Success rate ranged from 40% to 92.3% with varying techniques, with or without diverting stoma [135–137]. Patients with refractory RVF or extensive colonic or anorectal inflammation may require proctectomy or panproctocolectomy.

9.8 Monitoring of Perianal Crohn's Disease

Monitoring of pCF patients includes both clinical and radiological assessment. Recent data has also identified several biomarkers in predicting response to treatment.

9.8.1 Clinical Monitoring

Two scoring systems are commonly used in the clinical setting in monitoring response to treatment – the Perianal Disease Activity Index (PDAI) (Table 9.4) and the Fistula Drainage Assessment. A PDAI score >4 resulted in 87% accuracy when using clinical assessment (active fistula drainage and/or signs of local inflammation) as reference. A fistula is considered closed if there is no drainage on gentle finger compression. Clinical response is defined as reduction of ≥50% in the number of draining fistulas, and remission is defined as absence of draining fistulas on two consecutive visits.

Table 9.4 Perianal Disease Activity Index [138]

Discharge	
0	No discharge
1	Minimal mucous discharge
2	Moderate mucous or purulent discharge
3	Substantial discharge
4	Gross faecal soiling
Pain/restriction of activities	
0	No activity restriction
1	Mild discomfort, no restriction
2	Moderate discomfort, some limitation activities
3	Marked discomfort, marked limitation
4	Severe pain, severe limitation
Restriction of sexual activity	
0	No restriction sexual activity
1	Slight restriction sexual activity
2	Moderate restriction sexual activity
3	Marked limitation sexual activity
4	Unable to engage in sexual activity
Type of perianal disease	
0	No perianal disease/skin tags
1	Anal fissure or mucosal tear
2	Less than three perianal fistulas
3	Greater than or equal to three perianal fistulas
4	Anal sphincter ulceration or fistulas with significant undermining of skin
Degree of induration	
0	No induration
1	Minimal induration
2	Moderate induration
3	Substantial induration
4	Gross fluctuance/abscess
Total score	

9.8.2 Radiological Monitoring

Clinical remission does not equate to deep remission as radiological healing lags behind clinical remission by a median of 12 months [124, 125]. Therefore pelvic MRIs are essential for monitoring of perianal Crohn's disease. Performing serial MRIs in patients on treatment can identify nonresponders, allowing for earlier management adjustments by switching to another biological agent or proceeding to further surgery. Several studies have studied response using the van Assche scoring system [16]. However, the results

are conflicting: one study found it insensitive to changes in some patients [132], while another study showed that T2 hyperintensity was the only factor most clearly associated with clinical benefit [126].

Previous studies have shown that higher numbers of fistula tracts were associated with lower rates of clinical remission, while fistula duration and complexity did not influence outcome [124, 125]. Another retrospective study of 36 patients showed that maximal fistula length <2.5 cm was a predictor of treatment response, while aggregate fistula length ≥ 2.5 cm was a predictor of disease progression [127].

9.9 Role of Biomarkers in Monitoring Response to Treatment

Therapeutic drug monitoring has been advocated in the management of luminal Crohn's disease in recent years. AJ Yarur et al. reported that patients with pCF who achieved remission had higher infliximab trough level compared to those with active fistulas [15.8 vs. 4.4 μg/mL, $p < 0.0001$], and those who developed anti-infliximab antibodies had a lower chance of achieving fistula healing (OR: 0.04 [95%CI: 0.005–0.3], $P < 0.001$). Infliximab trough level is significantly associated with fistula healing (AUC 0.82, $p < 0.0001$). An infliximab level of ≥10.1 μg/mL was associated with fistula healing [OR: 3.9 (95%CI: 1.34–11.8) $P = 0.012$] [128]. Another retrospective study by Davidov et al. showed that infliximab levels at weeks 2 and 6 were significantly associated with fistula response at weeks 14 and 30. Infliximab levels of 9.25 μg/mL at week 2 and 7.25 μg/mL at week 6 could predict response to treatment [129].

Genotypes may also influence the responsiveness to infliximab therapy. Polymorphisms in the Fas ligand 843 might be a genotypic predictor of response to infliximab in patients with fistulising pCD. Fas ligand 843 CC/CT genotype was the only predictor of response ($p = 0.002$; OR = 1.66; 95% CI: 1.21–2.29) [130].

9.10 Prognosis of Perianal Crohn's Fistula.

Medical therapy with anti-TNFs could only achieve remission in 30–40% of pCD cases. Surgical treatment alone only led to a favourable outcome in around 50% of patients [6] with a higher recurrence rate in patients with complex fistulas. At present, there are still no clear predictors for response to anti-TNF therapy except the presence of proctitis, but its performance is disappointing [124]. Further large-scale prospective studies incorporating radiologic parameters and the role of therapeutic drug monitoring are required.

9.11 Proposed Algorithm on Management of Perianal Crohn's Fistula

Based on the current evidence, a multidisciplinary approach with close collaboration between gastroenterologists, surgeons and radiologists is recommended. We suggest adopting a proactive therapeutic drug monitoring to assess primary nonresponders (see Fig. 9.3).

9.12 Latest Development and Future Direction

Despite the advancement in medical and surgical treatment for perianal Crohn's disease, durable remission rates of complex fistula are still as low as 37% [79]. New developments are desperately needed to respond to the unmet management needs.

Mesenchymal stem cell therapy appears to be a promising new treatment for perianal Crohn's fistula. Active inflammation with creation of epithelial defects is believed to be the pathological culprit of perianal Crohn's fistulas and may be addressed by the immunomodulatory potential of adipose tissue-derived mesenchymal stem cells [139–141]. A recent phase 3 randomised, double-blind, placebo-controlled study revealed higher combined remission rates, as defined by the

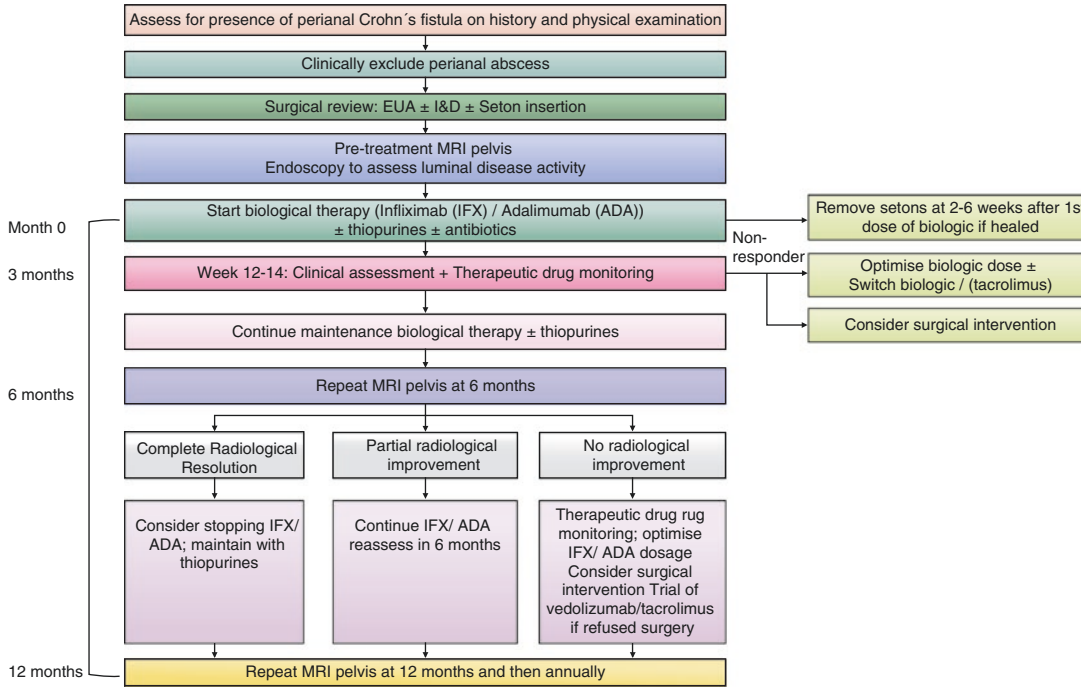

Fig. 9.3 Algorithm on the management of perianal Crohn's fistula

closure of the external opening and absence of collections >2 cm of the treated perianal fistulas, at week 52 (56.3% vs. 38.6%, $p = 0.010$). Of those who achieved combined remission at week 24, a greater proportion did not have a relapse at week 52 (75% vs. 55.9%, $p = 0.052$) [142].

Ustekinumab, an IL 12/23 inhibitor, is an effective treatment for Crohn's disease. Its efficacy in perianal Crohn's disease is now under study. In a small series of patients who had anti-TNF refractory Crohn's disease, ustekinumab was found to be effective in achieving reduction in the number of draining perianal fistulas. Two-thirds of pCD patients ($n = 6$) had more than 50% of their fistulas closed, while one-third had complete closure of all fistulas at ≥6 months [145]. Further large-scale prospective studies are required to study its efficacy in perianal Crohn's disease.

Other novel treatments include video-assisted anal fistula treatment (VAAFT) and fistula tract laser closure (FiLaC). Some promising results are available in the management of complex anal fistulas, but further prospective studies are needed to confirm these studies with small sample sizes [143, 144].

Therapeutic drug monitoring (TDM) of anti-TNF therapy is gaining increasing importance in the management of inflammatory bowel disease which may allow for a more personalised management approach [146]. There are only two retrospective reviews that have assessed the relationship between serum infliximab level and fistula treatment response [128, 129]. Further prospective studies are required in studying the role of TDM in the management of perianal Crohn's disease.

9.13 Conclusion

Management of perianal Crohn's disease remains a multidisciplinary challenge. Future studies focusing on identification of biomarkers associated with a complicated course of perianal Crohn's disease are required to improve patients' quality of life and remission rates.

Summary Points

- Perianal Crohn's disease involves a spectrum of diseases, from fistulising to non-fistulising disease.
- Perianal Crohn's fistulas (pCF) are common, occurring in around one-third of Crohn's patients and suggests a severe disease course.
- Diagnosis of perianal Crohn's disease requires careful history, physical examination, imaging with MRI or endoanal ultrasound and examination under anaesthesia.
- Management is multidisciplinary, involving gastroenterologists, surgeons and radiologists, in order to provide the best outcome.

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Part III

Clinical Algorithms Incorporating Predictive and Prognostic Biomarkers: Ulcerative Colitis

Biomarkers in Acute Severe Ulcerative Colitis

10

Matthew C. Choy, Dean Seah, and Peter De Cruz

Abstract

This chapter will cover prognostic and predictive biomarkers in acute severe ulcerative colitis (ASUC) that have been used to predict steroid response/failure and salvage therapy response/failure. It will provide a clinical algorithm encompassing biomarkers to help guide clinical management as well as identify future directions for ongoing research.

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10.1 Introduction

Acute severe ulcerative colitis (ASUC) is a potentially life-threatening condition that is associated with a significant risk of morbidity and mortality. Approximately 20% of patients diagnosed with ulcerative colitis (UC) will experience an acute severe flare during their lifetime [1], with one in five of these patients likely to undergo a colectomy during their first hospital admission [2]. Optimal management of ASUC is time-critical, and clinical, biochemical and endoscopic assessment is necessary in order to guide appropriate management for this challenging condition. In this chapter we explore how biomarkers can be utilised to predict the onset of ASUC, establish disease severity and monitor treatment response.

10.2 Diagnosis and Natural History of ASUC

ASUC is classically diagnosed according to the Truelove and Witt's (TLW) severity criteria [1] which consist of bloody stool frequency ≥6 per day and at least one of the following: pulse rate >90 bpm, temperature >37.8 °C, haemoglobin <10.5 g/dL and ESR >30 mm/h. Corticosteroids represent the first-line therapy; however, approximately one-third of patients do not respond [3]. Infliximab and cyclosporine

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Table 10.1 Predicting risk of ASUC at diagnosis

Number of risk factors present at diagnosis	Median (IQR) predicted risk of ASUC within 3 years
0/3	12% (10–16%)
1/3	25% (11–30%)
2/3	48% (36–56%)
3/3	69% (61–82%)

Adapted from Cesarini et al. [6]
One point for extensive disease, C-reactive protein [CRP] >10 mg/l or haemoglobin <12.0 g/dL for females or <14.0 g/dL for males at diagnosis

have demonstrated efficacy as medical salvage therapies for patients who fail to respond to corticosteroids [4]. However, despite these salvage therapies, a significant proportion fail to respond and there is a mortality rate of 1–3% [5].

Recently, it has been shown that disease features at diagnosis such as the presence of extensive disease (beyond the splenic flexure), C-reactive protein [CRP] >10 mg/l or haemoglobin <12.0 g/dL for females or <14.0 g/dL for males can be utilised to predict the individual risk of an ASUC episode within 3 years (Table 10.1) [6]. This Oxford risk tool has been validated on external cohorts with a high level of discrimination (c-index of 0.95 (Cambridge) and 0.97 (Uppsala). Given the high morbidity of ASUC episodes, early recognition of those at high risk of ASUC may prompt more rapid escalation of medical therapy in order to change the natural history of this disease.

10.3 Assessing Severity and Prognosis in ASUC

Clinical risk indices that incorporate clinical symptoms, signs and biomarkers have become widely used to determine prognosis and response to medical therapy. In addition to establishing the diagnosis of ASUC, Truelove and Witt's (TLW) criteria also enable clinicians to predict the likelihood of treatment failure and progression to colectomy, based on disease activity on admission. In a large retrospective study of UC patients in the UK, the number of TLW criterion present was positively correlated with the risk of colectomy (Table 10.2), with three or more criteria associated with a 48% risk of inpatient colectomy [2].

Table 10.2 Truelove and Witt's criteria to predict colectomy

Number of TLW criteria additional to ≥6 bloody bowel actions/day	Colectomy on admission
1	8.5% (11/129)
2	31% (29/94)
3 or more	48% (34/71)

Adapted from Dinesen et al. [2]

10.4 Medical Therapy in ASUC

10.4.1 Intravenous Steroids

Intravenous (IV) steroids remain first-line therapy in ASUC with steroid failure observed in up to 35% of patients. Doses higher than 60 mg of methylprednisolone are not supported by the literature [7], and prolonged courses greater than 7 days are not associated with improved outcomes [8]. As such, a thorough evaluation of treatment response is necessary to facilitate early recognition of steroid-refractory disease.

Steroid response is currently assessed on a case-by-case basis and aided by the application of clinical prognostic models. Traditional indices such as the Oxford criteria or Seo index [8, 9] involve evaluation of disease activity after 3 days of therapy via assessment of stool frequency and serum biomarkers of inflammation. The Oxford criteria were derived from findings of a landmark prospective cohort study, in which 51 patients hospitalised with ASUC were evaluated after 3 days of high-dose IV steroids. Stool frequency >8 times per day, or 3–8 times per day with a C-reactive protein (CRP) level >45 mg/L, was associated with an 85% risk of inpatient colectomy [8]. In a prospective Scottish study, investigators additionally correlated hypoalbuminemia <30 g/L and radiographic evidence of colonic dilatation with an increased risk of colectomy [10]. The clinical indices used in ASUC are outlined in Table 10.3.

More recently, research has turned to potentially more objective markers of steroid response,

Table 10.3 Clinical indices measuring disease activity in ASUC

Table of indices to predict steroid failure			
	Criteria		Interpretation and predicted risk
Travis risk score (Oxford index)	Assessed on day 3: Daily stool frequency ≥8 or Daily stool frequency ≥3 *plus* CRP >45 mg/L Assessed on day 7: ≥3 stools per day with visible blood		85% PPV for colectomy within same admission 40% PPV for colectomy within 3 months
Ho index	Assessed on day 3:		Total score: 0–1: Low risk of steroid failure (11%) 2–3: Intermediate risk of steroid failure (45%) ≥4: High risk of steroid failure (85%)
	Mean stool frequency		
	<4	0 points	
	4 ≤6	1 point	
	6 ≤9	2 points	
	>9	4 points	
	Colonic dilatation on plain XR		
	Present	4 points	
	Albumin		
	<30 g/L	1 point	
Seo index	Assessed on day 3: 60 × number of bloody stool + 13 × bowel movements + 0.5 ESR (mm/h) – 4 × haemoglobin (g/dl) – 15 × albumin (g/dl) + 200		Total score <150 = mild 150–220 = moderate >220 = severe *Total score >200 = 85% PPV of colectomy*

Adapted from Travis et al. Ho et al. and Seo et al.
Hb haemoglobin, *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate, *PPV* positive predictive value

as stool frequency can be confounded by disease chronicity and complications such as an intestinal ileus. A retrospective study in Ireland identified that a day 3 CRP/albumin ratio (CAR) of >0.85 combined with a stool frequency >3 was associated with a relative risk of steroid failure of 3.9 (95% CI 2.1–7.2, positive predictive value 74%, negative predictive value 81%) [11]. Results from a recent prospective cohort study in India determined that a faecal calprotectin (FC) level >1000 ug/g, on day 3 of steroid therapy, was predictive of steroid failure, with patients requiring escalation to medical salvage therapy and/or colectomy (PPV = 65%) [12]. More severe endoscopic disease was present in steroid non-responders, defined as a score of 6 or higher on the Ulcerative Colitis Endoscopic Index of Severity (UCEIS). Furthermore, a prospective pilot study of 46 patients assessing whether steroid failure can be determined at the time of admission identified that the combination of FC >1645/g and CAR >1.34 was strongly predictive of steroid failure (sensitivity 75%, specificity 92%, PPV 96% and NPV 58%) [13]. These indices require prospective validation, but may allow early risk stratification of patients for whom steroid therapy is likely to be futile, with currently available biomarkers. An algorithm outlining the approach to the initial management of ASUC can be found in Fig. 10.1.

10.4.2 Salvage Therapy

Once steroid failure is determined and a decision is made to proceed to medical salvage therapy, it is important to assess subsequent response to second-line therapy in a timely fashion (Fig. 10.2). Indeed, emergency colectomy has a higher risk of mortality over elective

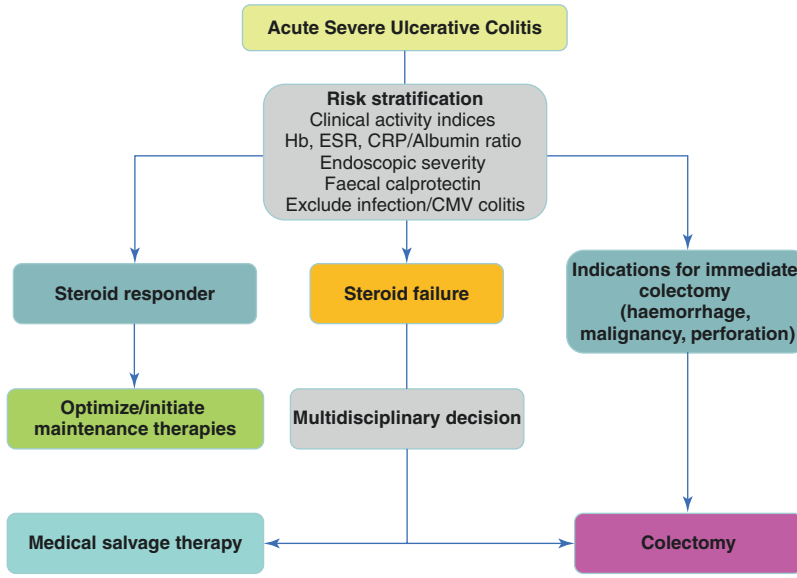

Fig. 10.1 Current approach to the initial management of ASUC

surgery [14], and delays in decision-making to perform colectomy increase the risk of complications [15].

10.4.3 Calcineurin Inhibitors

Cyclosporine (CyA) is effective as salvage therapy in ASUC. It has a rapid onset of action, and a dose of 2 mg/kg is recommended to reduce complications such as infection, renal injury and neurotoxicity [16]. CyA has been demonstrated to be equivalent to infliximab in terms of colectomy-free survival and quality of life outcomes [17]; however, a greater proportion of CyA-treated patients require retreatment, often with infliximab [18]. CyA is utilised for induction only and requires bridging to maintenance therapy, traditionally to a thiopurine. As prior exposure to azathioprine has been recognised as a predictive factor for CyA treatment failure [19], infliximab rescue or CyA rescue with vedolizumab maintenance [20] may be considered in this particular cohort.

Serum biomarkers may also have a role in predicting responsiveness to CyA rescue therapy. A CRP greater than 45 mg/L, at the time of CyA initiation, was predictive for colectomy within 6 months (hazard ratio, 1.70; 95% CI, 1.34–2.16) [21]. Another study examined the utility of the Ho clinical activity index which incorporates serum albumin as a biomarker to determine response to CyA; a Ho index ≥5 prior to salvage therapy predicted colectomy with an area under the receiver operating curve of 0.79 (95% confidence interval [CI], 0.59–0.99) [22].

10.4.4 Infliximab

Infliximab (IFX) is an antitumour necrosis factor (TNF) monoclonal antibody that has revolutionised therapy in inflammatory bowel disease. Although early studies in ASUC yielded conflicting results, its efficacy is now well established, and it has become the salvage therapy of choice in many centres, principally due to its ease of administration and the option for its use as both

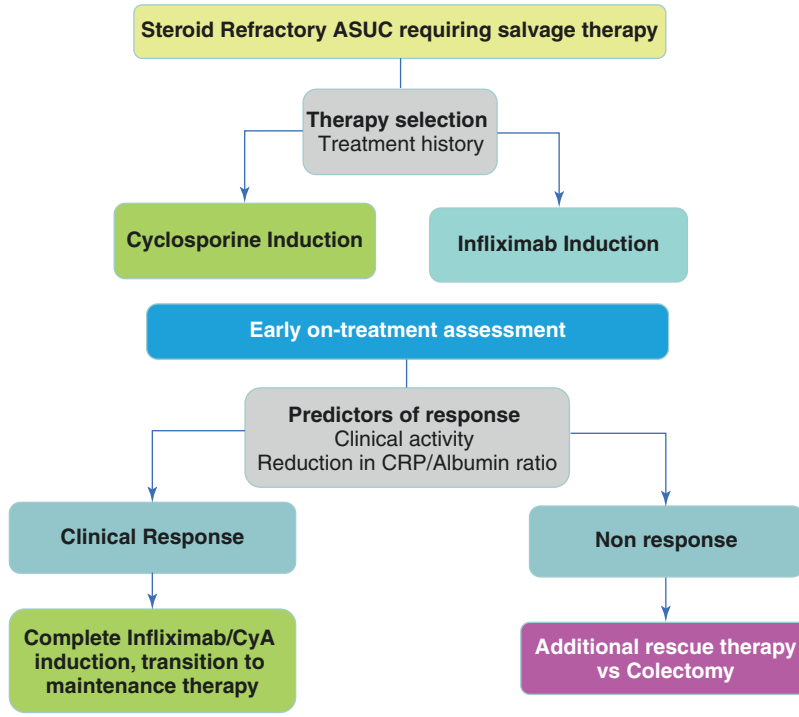

Fig. 10.2 Current approach to salvage therapy in ASUC

induction and ongoing maintenance therapy [23]. A recent meta-analysis has reported that the overall pooled colectomy-free survival following IFX therapy for ASUC from 40 studies including 1847 cases was 85.6% (95% CI 81.5–89.3%) at 1 month [24]. IFX and CyA have demonstrated equivalent efficacy as medical salvage therapies in ASUC in randomised controlled trials (RCT); however, non-randomised studies have suggested a better treatment response and reduced risk of colectomy at 12 months with IFX [4].

10.4.4.1 Dose Selection

IFX is traditionally dosed at 5 mg/kg given at weeks 0, 2 and 6. However, new insights into the pharmacokinetics of IFX in the setting of ASUC, which have shown increased drug clearance [8], low serum levels [9] and faecal drug loss [10], have led to an interest in dose intensification. However, the evidence to support such an approach is conflicting [24]. An elevated CRP >50 mg/L [25], low albumin and increased body mass index are factors that have been associated with increased IFX drug clearance and may be potentially utilised in pharmacokinetic models to determine initial dose selection.

10.4.4.2 Assessment of Response

Assessment of response to infliximab is not well defined. This is a crucial area of need as clinical response judgement determines further decisions for dose escalation or colectomy. Clinical trials in ASUC have typically utilised the Lichtiger score [26] to determine clinical response, and Canadian consensus statements recommend that response should be defined as improvement or resolution of abdominal pain and rectal bleeding [27]. However, clinically based indices can be subject to inaccuracy and there can be a disconnect between stool frequency, rectal bleeding scores

and mucosal healing. A retrospective analysis of 54 patients treated with infliximab induction identified that a failure to achieve a CRP/Alb ratio <0.37 prior to hospital discharge was a significant predictor of colectomy with 80% sensitivity, 62% specificity, a 42% PPV, a NPV 90% and an area under receiver operating curve (AUROC) 0.73 [28]. A cut-off of <0.32 had a 90% sensitivity, 55% specificity, 41% PPV and 94% NPV for avoiding colectomy in the first 12 months.

Faecal calprotectin (FC) levels may also have predictive value when assessing non-response to IFX. This was investigated in a large prospective observational study [29]. Analysis of 90 patients with ASUC was performed with outcomes defined as colectomy, corticosteroid response and IFX response. Elevated calprotectin levels on admission were seen in cases that exhibited steroid and IFX non-response as well as those that eventually progressed to colectomy within 1–8 days following IFX initiation. Of 21 patients, who received IFX, 11 (52%) did not respond and underwent emergency colectomy. IFX non-responders had higher levels of FC than infliximab responders (1795.0 and 920.5 ug/g; $p = 0.06$); however, this result was not statistically significant. Prospective evaluation of FC monitoring in ASUC is awaited.

10.4.4.3 Therapeutic Drug Monitoring

Therapeutic IFX drug monitoring of trough levels has an established role in maintenance therapy [30]; however, its utility in the ASUC setting has not been well characterised. In ASUC, a detectable trough serum IFX concentration at the completion of 3 doses of 5 mg/kg at week 0, 2 and 6 induction predicted clinical remission (OR 12.5; 95% CI, 4.6–33.9; $p < 0.001$) and endoscopic improvement (OR 7.3; 95% CI, 2.9–18.4; $p < 0.001$), whilst undetectable trough IFX levels were associated with higher rates of colectomy (OR 9.3; 95% CI, 2.9–29.9; $p < 0.001$) [31]. Similar data was reported in a multicentre prospective study, where serum IFX ≤ 7ug/mL at day 42, following week 0, 2 and 6 IFX dosing, was predictive of endoscopic non-response (OR 36; 95% CI, 1.9–719; $p = 0.03$) [25]. Paradoxically, a recent study by Beswick and colleagues, which prospectively examined IFX levels in 24 patients in the immediate post-induction phase, found that lower IFX levels as measured by area under the curve (day 1–3 <120 ng/mL; day 4–7 <216 ng/mL) were associated with an increased rate of clinical remission and CRP response at week 6, perhaps due to higher and more effective drug uptake in the tissue [32]. Faecal IFX loss may also be an important indicator of primary failure. This was first demonstrated in a moderate-severe UC cohort [33] and confirmed in an ASUC cohort, with day 1 faecal IFX >1ug/g predictive of colectomy within a year (OR 176 (2.1–14,452, $p = 0.01$)) [32].

CyA drug levels during the infusion have not shown to correlate with outcome. In a retrospective analysis of 81 patients of whom 47 underwent colectomy, CyA levels were not different between responders and non-responders. Responders, however, had a lower CRP (20 mg/L vs. 38 mg/L, $p = 0.01$), lower serum albumin concentrations (3.4 g/dL vs. 3.7 g/dL, $p = 0.03$) and higher rates of kidney injury (50% vs. 17%, $p = 0.002$) [34].

10.5 Post-induction Management

Following a successful induction of remission in ASUC, there remains a significant risk of relapse and surgery. In the aforementioned meta-analysis of 1847 patients treated with IFX for ASUC, colectomy-free survival was 80.4% (95% CI 76.0–84.6%) at 3 months and reduced to 69.8% (95% CI 65.0–74.3%) at 12 months, indicating a failure of maintenance therapy management in a subset of patients who proceed to surgery [24]. The optimal management strategy post salvage therapy induction has not been identified, with options including thiopurine monotherapy, IFX combination therapy or alternative biologics.

Strategic assessment of drug efficacy and disease activity following induction may assist therapeutic decision-making.

Following CyA induction, trough drug levels on oral CyA (150–300 ng/mL) need to be carefully monitored to prevent dose-related toxicity. Monitoring of thiopurine metabolites to achieve optimal 6-thioguanine levels may also improve remission rates [35]. As aforementioned, IFX monitoring is useful in moderate-severe UC, with levels on week 0, 2 and 6 dosing of >41 ug/mL at week 8 of therapy and >3.7 ug/ml at steady-state trough concentrations in the ACT1 and 2 studies associated with improved outcomes [36]. Proactive monitoring of drug levels may help to prevent the development of anti-drug antibodies leading to loss of response.

Objective disease assessment with the goal of mucosal healing is crucial as it does not always correlate with clinical symptoms. Endoscopy should be performed within 3–6 months of induction therapy, and both CRP and faecal calprotectin are useful surrogates to assess treatment response. It is unclear how quickly faecal calprotectin normalises in ASUC, and future research should shed light on the kinetics of this biomarker.

10.6 Experimental Biomarkers

Numerous experimental biomarkers have been evaluated to assess the likelihood of responding to therapy based on immunological, serological and genetic assessment. Cytokine profiles may have a role with higher levels of pretreatment TNF-alpha gene expression, within the colorectal mucosa predictive of infliximab non-response [37]. Higher serum and lower mucosal levels of anti-TNF also appear to predict for mucosal disease activity [38]. Gene array studies of UC mucosal biopsies have identified specific predictive panels of genes for response and non-response to IFX [39]. Patients who are ANCA seronegative and are homozygous for IL23R variants are more likely to respond to infliximab [40]. In steroid-refractory UC, the TT genotype of exon 21 MDR1 polymorphisms is associated with higher rates of CyA failure [41]. Although these markers have not yet entered clinical practice due to a lack of prospective validation, availability of testing and the present limited therapeutic choices in ASUC, they do provide important insights into disease mechanisms which may pave the way for future personalisation of drug therapy.

10.7 Current Limitations and Future Directions

Despite significant advances in our understanding of ASUC, an optimal approach to its management is yet to be established. The current treatment paradigm relies heavily on clinical predictors of disease activity; however, in the last 5 years, there has been a clear shift toward a treat-to-target approach based on more objective markers of treatment response. Traditional biomarkers used in this context, such as acute phase reactants, whilst helpful in indicating systemic inflammation, are relatively non-specific to the underlying disease process. It is also important to recognise that current predictive clinical indices such as the Oxford criteria were developed prior to the widespread implementation of CyA and IFX.

Evidence-based guidelines outlining standardised timing, interpretation and recommended thresholds for serum IFX, faecal calprotectin and cytokine levels are now needed. Therapeutic drug monitoring is currently utilised in the post-acute setting in order to guide IFX dose optimisation; however, its use remains largely reactive and an optimal monitoring schedule has not been clearly defined. Pharmacokinetic modelling is necessary to determine optimum IFX levels required to achieve primary response followed by on-treatment monitoring to ensure that levels remain therapeutic in order to prevent relapse and secondary loss of response. Further studies investigating the genetic and immunological mechanisms of treatment non-response are also required in order to inform selection of salvage

therapy. If flares of disease can be accurately predicted, potential cycling of active therapies to match disease activity patterns may be possible in the future. Methods to enable early identification of patients, in whom medical therapy is likely to be futile, are also necessary in order to expedite surgical intervention.

This landscape is likely to shift with current research ongoing into augmentation of steroid response via the application of hyperbaric oxygen (*ClinicalTrials.gov: NCT02144350*) or IL-1 receptor antagonists (IASO trial; ISRCTN43717130), as well as a randomised trial investigating the optimal dose of infliximab induction in ASUC (PREDICT UC; *ClinicalTrials.gov: NCT02770040)*. Newer, more specific biomarkers that stem from this research may facilitate earlier detection of treatment non-response and more timely escalation of medical therapy and personalised approaches to salvage therapy via immunological and pharmacokinetic profiling.

In summary, ASUC is a medical emergency in inflammatory bowel disease. Assessment should comprise of comprehensive clinical, biomarker and endoscopic evaluation in a multidisciplinary approach in order to risk stratify patients without delay. Steroid failure should be assessed systematically using currently available clinical indices and serum markers with swift implementation of second-line salvage therapy or consideration of colectomy.

Summary Points

- Acute severe ulcerative colitis is a life-threatening condition that requires prompt assessment and management.
- Clinical symptoms should be routinely combined with objective serum biomarkers and endoscopy to determine disease severity.
- Predictive indices such as the Oxford index are useful to guide assessment of steroid response.
- Biomarkers will have an increasingly important role in prospective algorithms for predicting steroid failure and assessing therapeutic outcomes.

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Chronic Active Ulcerative Colitis

11

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Abstract

Chronic moderately to severely active UC is a heterogeneous disease in which the incorporation of biomarkers into the baseline assessment plays an important role in understanding the individual disease phenotype. Disease activity should be proactively monitored with the non-invasive biomarkers CRP and faecal calprotectin, in addition to endoscopic assessment to establish mucosal and histological severity. Serial faecal calprotectins are particularly useful in UC and can be used to predict risk of disease flare and to titrate treatment. Cumulative inflammatory burden increases risk of dysplasia and should be considered over disease duration when assessing risk of colorectal cancer.

Intestinal ultrasound is emerging as an accurate tool to assess UC disease extent beyond the rectum and to monitor response to therapies.

The microbiome is an emerging biomarker as well as therapeutic agent and is awaiting further research before being incorporated into management algorithms. Engaging the patient in education and discussions about the ability of biomarkers to help predict response to treatment, risk of relapse or loss of response to therapy can facilitate shared decision-making, a vital part of the treat-to-target algorithm. These biomarkers may allow us to achieve frequent reassessment in a less invasive manner that is more acceptable to patients on the shared path towards UC disease modification.

11.1 Introduction

This chapter aims to contextualize the role of biomarkers with respect to the specific drug therapies that are currently used for moderately to severely active UC.

Biomarkers have come to the forefront of disease monitoring in ulcerative colitis due to strong evidence that clinical symptoms correlate poorly with endoscopic findings and lead to management errors. In clinical practice, a "treat-to-target" approach is emerging, with physicians aiming for control of objective markers of disease activity in addition to achieving the traditional targets of clinical (symptomatic) remission. This is in the hope that the natural history of ulcerative colitis can be altered and prognosis of an individual patient improved. This approach,

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however, requires a clear understanding of the utility of these new treatment targets. Endoscopic mucosal healing activity is the current gold standard objective target of disease control. In addition, the role of biomarkers such as CRP, faecal calprotectin, histological activity, cross-sectional imaging, the microbiome and serological antibodies used as composite targets will be discussed. A treatment algorithm is also proposed.

11.2 Serum Biomarkers

11.2.1 C-Reactive Protein (CRP)

C-reactive protein (CRP) is a protein found in the blood that is produced by the liver and is elevated in both acute and chronic inflammation in response to elevated levels of the cytokine IL-6. It has a short half-life ($t½$ = 19 hrs) [1], so it is a useful tool to monitor short-term changes in inflammatory burden. However, it is important to note that up to 15% of normal healthy individuals do not mount a CRP response in the setting of inflammation [2]. This may be related to a single nucleotide polymorphism, and this fact is important to recognize if considering the use of CRP as part of the assessment of patients with IBD [3].

CRP levels are lower in ulcerative colitis (UC) when compared to Crohn's disease (CD) [4] likely due the presence of transmural inflammation in Crohn's disease and associated higher IL-6 levels [5]. Despite this, CRP does not help in distinguishing CD from UC, due to the large spread in CRP concentrations in the individual patient. However, in patients who do mount a CRP response to inflammation, CRP levels are useful to monitor disease activity, with higher levels of CRP associated with more severe disease activity and improvements in CRP following medical therapy associated with treatment response [6]. Medical therapy has not been shown to reduce hepatocyte production of CRP, so a reduction in CRP is attributed to medically induced reduction of inflammation or in the relapsing and remitting natural history of IBD [4].

CRP has an established role in predicting treatment failure and need for colectomy in acute severe UC [7–9]. With regard to chronic moderately to severely active UC, the roles in predicting disease extent and mucosal healing are mixed [6]. Patients with more proximal disease are felt to have better correlation with CRP levels, compared to those patients with isolated distal disease such as proctitis [5]. In a retrospective study from the Mayo Clinic of 43 UC patients, CRP was shown to be significantly associated with clinical disease activity and endoscopically active disease but was not correlated with histological activity, possibly due to the small sample size [10]. In the Norwegian population-based inception cohort study (IBSEN), an increase in CRP levels was seen with greater disease extent [4]. However, CRP did not predict disease remission on endoscopic assessment at 5 years after diagnosis in 195 patients who underwent endoscopic assessment, with no difference between CRP levels seen in those in remission, compared to those with endoscopic inflammation (6 vs 7 mg/L, $p = 0.59$). In addition, all patients who underwent colectomy within the first month of diagnosis had a CRP above 23 mg/l ($p < 0.001$). CRP at diagnosis did not predict relapse risk in this cohort, but CRP levels above 10 mg/l at 1 year after diagnosis were associated with an increased risk of surgery during the subsequent 4 years (OR 3.0, 95% CI 1.1 to 7.8, $p = 0.02$) [4].

Overall data are mixed on the role of CRP as reflected in the consensus statement on treatment targets in IBD (STRIDE), in which CRP was only mentioned as an "adjunctive" target of treatment for UC [11]. We suggest that a fall in CRP may be best used as a marker of response to therapy. CRP may also be used as a cofactor in composite assessment of disease activity together with faecal calprotectin. Failure of CRP or faecal calprotectin normalization (below lab-specific cut-off) should prompt further endoscopic evaluation, irrespective of symptoms.

11.2.2 Anti-neutrophil Cytoplasmic Antibodies and Anti-Saccharomyces cerevisiae antibodies

Perinuclear anti-neutrophil cytoplasmic antibodies (pANCAs) are antibodies directed at neutrophil granules and were first reported to be

associated with UC in 1990 [12]. pANCA positivity is seen in 60–80% of UC patients. This marker is in an atypical pattern and differs from the ANCA pattern associated with vasculitis.

Anti-*Saccharomyces cerevisiae* antibodies (ASCA) are antibodies directed against cell wall mannan of the yeast *Saccharomyces* and are a marker of increased mucosal permeability, rather than a reflection of increased mucosal *S. cerevisiae* exposure [12].

ASCA and ANCA serologies have some utility for the diagnosis of undifferentiated IBD, whereas ASCA-negative/pANCA-positive serology is more associated with a phenotype consistent with typical UC [12]. These markers are stable over time and do not reflect disease activity. The serologies do not replace normal endoscopic or radiological criteria. In patients with UC, the presence of pANCA is felt to represent a more aggressive disease course and lower likelihood of response to infliximab, so there may be further utility in prognosis and treatment selection [13].

For patients who require a total colectomy and an ileo-anal pouch, the presence of serological markers such as ANCA and ASCA is also associated with a greater risk of pouchitis (ANCA or ASCA) or development of "Crohn's disease of the pouch" (ASCA). This is helpful in those with undifferentiated IBD who may be at risk of developing a Crohn's phenotype of their pouch with formation of perianal fistula and pouchitis, although these studies are conflicting [14].

11.3 Faecal Biomarkers

11.3.1 Faecal Calprotectin (FCal)

Calprotectin is a zinc-binding protein found in the cytosol of neutrophils, and its measurement in stool is used as a surrogate marker of intestinal inflammation [2]. It is, however, also increased by non-steroidal anti-inflammatory drug use, bleeding and malignancy. Higher levels are associated with the risk of colectomy in acute severe UC (ASUC) [15].

Outside of ASUC, the role of FCal in moderately to severely active UC is becoming clearer. In a meta-analysis including 744 patients with UC, a cut-off of 250 mcg/g produced an area under the curve for diagnostic accuracy of disease activity in UC of 0.93 (0.89–0.97) [16]. In addition, FCal has been demonstrated to predict clinical remission in those treated with infliximab. In a prospective study of refractory UC patients [17], patients who achieved clinical remission following infliximab induction, the median FCal value reduced from 507 mcg/g to 23 mcg/g ($p = 0.001$). In patients who failed to reach clinical remission, the fall from 312 mcg/g to 204 mcg/g was not significant.

Calprotectin can also be used to help predict which patients have achieved mucosal healing (MH, most often defined as a Mayo Endoscopic Subscore of 0 or 1) and histological healing (frequently defined as a Nancy score of 0 or 1). Patel and colleagues demonstrated that patients who achieve both MH and histological healing had significantly lower FCal levels compared to those who achieved MH but did not achieve histological healing (faecal calprotectin 31 mcg/g vs 231 mcg/g; $p = 0.001$) [18]. In this study, the outcomes of (1) deep remission (patient-reported outcome 2 (PRO2) remission and Mayo score 0) and (2) deeper remission (deep remission plus Nancy score 0 or 1) were defined. An optimal FCal cut-off of 60 mg/g accurately predicted remission with a specificity/sensitivity of 87/86% in deep remission and 90/83% for deeper remission [18].

Importantly, calprotectin has an established role in UC for predicting risk of relapse. De Vos and colleagues demonstrated in a prospective trial of UC patients that two consecutive calprotectin measurements of >300 mcg/kg obtained 1 month apart predicted a UC flare with a sensitivity of 61.5% and specificity of 100% in the following 12 months [19]. In addition, Costa and colleagues demonstrated that patients with higher median calprotectin values are more likely to have a clinical relapse. During their 12-month study, the median calprotectin was 220.6 mcg/g (95% CI 86–355.2) in those that relapsed versus 67 mcg/g (95% CI 15–119) in those that remained in remission ($p < 0.0001$) [20].

In practice, most importantly, consecutive measures of FCal offer utility in predicting

relapse in the following months [21]. More work to identify the most accurate cut-off level of calprotectin that correlates with deep remission is required.

11.3.2 Mucosal and Histological Activity

The concepts of mucosal healing and histological healing have been proposed as therapeutic goals in the treat-to-target paradigm, although due to currently insufficient evidence for histological remission, only mucosal healing has been recommended by multiple groups as a clinical treatment endpoint [11, 22].

Mucosal healing is an important prognostic factor for patients with UC with severe endoscopic disease associated with a worse prognosis. Patients who have severe endoscopic lesions on admission to hospital for acute severe colitis have a 41 times greater chance of requiring a colectomy compared to patients without severe lesions [23]. Furthermore, patients who achieve mucosal healing and are in clinical remission have a reduced chance of clinical relapse on follow-up compared to those who do not achieve mucosal healing [23–25].

The two main endoscopic scoring systems used in UC are the Mayo endoscopic subscore (MES) [26] and the UC endoscopic index of severity (UCEIS) [27]. The MES grades mucosal inflammation from 0 to 3 with 0 indicating inactive disease and 3 indicating severe disease. Historically, a score of ≤ 1 has been defined as "mucosal healing", with a score of 0 or 1 associated with better outcomes when compared to MES of 2 or 3 with a reduction in clinical relapse and colectomy [28]. However, more recently it has been demonstrated that a score of 0 is associated with improved outcomes when compared to a score of 1, with those achieving a score of 0 being less likely to require colectomy and more likely to remain in clinical remission [28, 29]. Due to these findings, for the MES an expert steering committee recommended a subscore of 0 as the optimal therapeutic target but that a subscore of 0 or 1 should be considered endoscopic remission [11, 22]. A decrease in Mayo endoscopic score ≥ 1 is defined as "endoscopic improvement" [22], with a similar definition also being used for regulatory labelling.

The UCEIS is a recently developed endoscopic score that has been partially validated with good inter- and intra-observer agreement and is suggested to be used alongside the Mayo endoscopic subscore in future clinical trials to further validate its role in defining remission. The UCEIS has good correlation with calprotectin [30] and patient-reported outcomes [11]. Furthermore, endoscopist knowledge of clinical activity minimally affects the UCEIS. Expert consensus has recommended a UCEIS score of 0 for the definition of endoscopic remission in clinical trials, with a decrease in the UCEIS ≥ 2 defined as endoscopic improvement, although only preliminary data exists to help validate cut-offs [11, 22].

As more stringent definitions of mucosal healing have been associated with improved outcomes in patients, there has been increasing interest in the role of histological activity on patient outcomes. Patients with endoscopic mucosal healing can have persistent histological activity, and this has been demonstrated to portend a risk of flare. In patients who achieve mucosal healing, persistence of histological activity has been found to increase the risk of relapse, hospitalization, colectomy and neoplasia [24, 31]. Furthermore, histological changes such as basal lymphoplasmacytosis, erosion and ulceration of the epithelium and moderate to marked architectural distortion predict clinical flares more accurately than endoscopic mucosal healing [24]. Histologic normalization has also been considered as the ultimate endoscopic target in ulcerative colitis and was found to be associated with a reduced risk of clinical flare when compared to both histological quiescence and endoscopically determined mucosal healing [25]. These studies demonstrate that incorporating a validated reporting system for histological disease severity into routine assessment in UC will become increasingly necessary. Validated histological scoring systems are currently being developed; however, no cut-offs for remission or healing have been defined, and therefore

histological remission so far has been recommended as an adjunctive target secondary to mucosal healing [11].

Adding to the importance of endoscopic outcomes as a prognostic factor, ongoing mucosal and histological inflammation has also been found to increase the risk of dysplasia and colorectal cancer in ulcerative colitis. In a long-term surveillance study of UC patients, the degree of endoscopic and histologic inflammation correlated with the risk of developing colorectal neoplasia, although on multivariate analysis, only histological inflammation was an independent predictor [32]. Histological activity has since been demonstrated in multiple studies to increase the risk of dysplasia and bowel cancer and has been suggested that histological activity scores should be used to stratify surveillance strategies [31]. When assessing neoplasia risk, considering the cumulative inflammatory burden which accounts for inflammatory burden documented in multiple prior surveillance procedures is much more accurate when compared to results of the most recent colonoscopy result [33]. Furthermore, in patients who have a macroscopically normal colon, colorectal cancer risk is similar to that of the general population on 5-year follow-up [34]. Chromoendoscopy increases rates of dysplasia detection and is postulated to contribute to the lower rates of advanced and interval cancers seen in the UC cohort [35]. Ideally we would perform randomized studies to clarify whether histologic healing should be a target that mandates medical therapy escalation [36]. Despite the absence of such trials and data, the US Food and Drug Administration has incorporated histologic assessments in their draft regulatory guidelines for UC (https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM515143.pdf).

11.4 Non-invasive Imaging: Intestinal Ultrasound

Intestinal ultrasound (IUS) is an appealing tool to assess for mucosal activity in IBD. The advantages over CT, MRI and endoscopy include lack of radiation exposure, no need for fasting and avoidance of endoscopy-related risk of perforation and discomfort. Its role in UC is less defined compared to Crohn's disease, although evidence is growing. Inflammation is demonstrated by bowel wall thickening >3 mm (Fig. 11.1). Increased Doppler signal and loss of stratification can also be present, although these findings are more prominent in Crohn's disease.

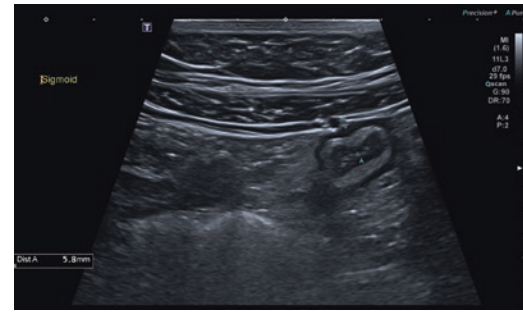

Fig. 11.1 Ulcerative colitis intestinal ultrasound findings: thickened sigmoid bowel wall to 5.8 mm

IUS is highly accurate at diagnosing UC and assessing the extent and severity of bowel inflammation. It has a sensitivity of 95% and specificity of 96% in diagnosing active UC when compared to endoscopy and performs better than MR colonography [37]. IUS has a high correlation with clinical, biochemical and endoscopic activity making it a useful tool for clinical assessment and further research [38]. A study of 51 patients demonstrated that endoscopic and IUS extent was concordant in 100% of patients [39]. Furthermore, IUS also correlated with CRP. Patients with proctitis were excluded due to anatomical technical difficulties with IUS access.

Most importantly, IUS has been demonstrated to be useful in predicting long-term outcomes in patients treated for UC. A prospective trial of 83 patients found that IUS scores had high concordance with endoscopic scores in ulcerative colitis following steroid therapy, proposing IUS as an accurate surrogate of colonoscopy, in those with disease extending beyond the rectum [40]. In this study, moderate to severe

activity on IUS at 3 months predicted ongoing endoscopic disease at 15 months following initiation of therapy.

IUS has high accuracy in detecting colonic inflammation more proximal to the rectum but cannot distinguish the exact cause of inflammation; thus, endoscopy is still required for diagnosis. Rather IUS is most useful in assessing response to therapy with resolution in inflammation. Further studies are required to completely outline the role of intestinal ultrasound and define clear cut-offs for disease activity, but its use in clinical practice is exponentially growing due to the advantages of real-time, point-of-care assessment, safety and lack of invasiveness.

Endoscopic US is another rarely used technique that can provide information about the spread of disease into different layers of the colonic wall and thus is helpful in distinguishing CD from UC [41]. However, endoscopic US is invasive and adds little information to standard endoscopy; hence its utility in clinical practice is limited.

11.5 Microbiome

The pathogenesis of IBD is felt to be related to the host's genetic makeup, the gastrointestinal microbiota and the interplay with a dysregulated or constitutively activated immune system. Dysbiosis has been identified in IBD with the advent of next-generation sequencing that allows a more accurate description of the microbial ecosystem.

Dysbiosis is greater in CD than in UC, with a lower microbial diversity, a more altered microbiome composition and a more unstable microbial community [42]. The utility of this knowledge, for example, shows that a lower relative abundance of *Faecalibacterium* seen in patients with CD, a genus that is not missing in patients with UC, can form a useful marker to discriminate patients with CD from patients with UC [42].

In a mixed IBD cohort of 128 patients and 9 healthy controls, 16S ribosomal RNA sequencing on faecal samples was obtained at 3-month intervals for up to 2 years. These results were combined with FCal, surgical resection status and host genetic markers. A "healthy plane" representing the normal microbial variation in the control group was established from microbiome samples over a time period of 2 years. Interestingly, although overall the FCal was higher in the IBD cohort compared to the healthy controls, this marker of inflammation did not correlate with deviation from the healthy plane. This study establishes the role of a microbiome signature and a predictive tool for IBD subtypes, particularly distinguishing ileal Crohn's disease from healthy controls, UC and colonic Crohn's disease [43].

Faecal microbiota transplantation has been used in the treatment of mildly to moderately severely active UC and resulted in a significant increase in α-diversity, which was durable 8 weeks after therapy completion in a randomized trial [44]. Particularly the role of *Fusobacterium varium*, isolated from colonic mucosa of patients with UC, may be a potential pathogenic factor that could be used as a biomarker for exacerbations [45].

The role of treatment-induced remission on the microbiome and the ability to prevent deviation from the "healthy plane" are yet to be studied. More research needs to emerge before the microbiome can be harnessed as a biomarker to its full potential.

11.6 Algorithm: Incorporating Biomarkers into a Treat-to-Target Model

Increasingly it is becoming evident that treating clinical symptoms alone does not provide optimal long-term outcomes. Incorporating biomark-

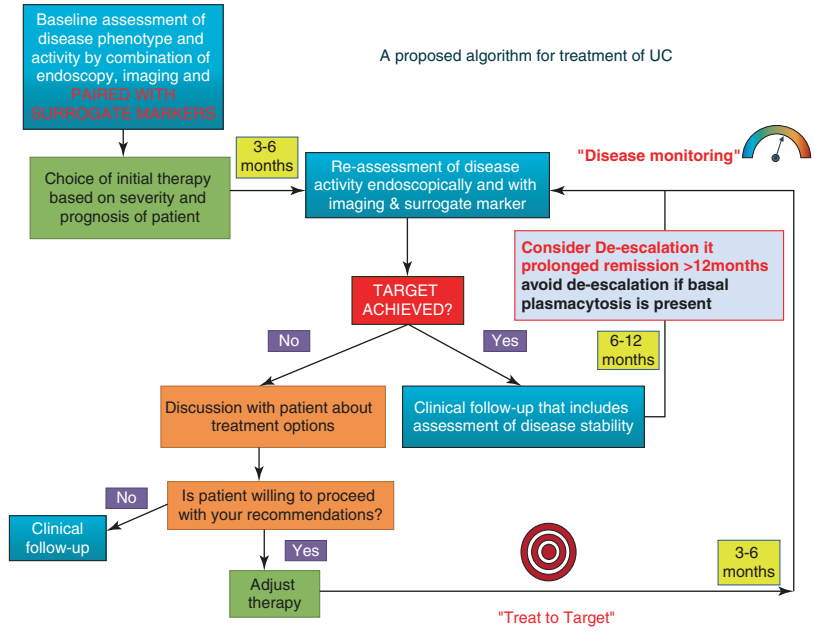

Fig 11.2 Proposed algorithm for treat-to-target incorporating biomarkers. (Adapted from Christensen and Rubin [46])

ers, particularly endoscopy, FCal, CRP and IUS, into routine clinical assessment and escalating treatment aiming for normalization of these biomarkers are associated with improved disease activity, reduced hospitalization and increased quality of life. We propose a treat-to-target algorithm that routinely incorporates these biomarkers into regular assessment of patients that should result in improved "tight control" and resultant better outcomes (Fig. 11.2).

De-escalation of therapy is also important to consider for a number of reasons including patient preference, cost, potential medication adverse events and, more recently, the appreciation of deeper levels of remission, such as the above-mentioned histological normalization.

However, it is important that de-escalation occurs only after confirmation of deep remission. This pathway is embarked upon in close consultation with the patient and must include discussions of the risk of relapse, need for subsequent disease monitoring and a rescue strategy to recapture response should relapse occur. The evidence surrounding this approach is evolving (Table 11.1).

11.7 Conclusion

Biomarkers of disease activity should be incorporated early in the care of UC patients from the time of suspected diagnosis and subsequently to characterize disease phenotype and then again in assessing response to therapy and risk of relapse. This approach is especially recommended for UC patients with moderately to severely active disease, in whom the prognosis for hospitalization or surgery is poorer and attention to their disease management is critically important [47]. As we move into a treat-to-target era, disease activity and therapeutic biomarkers to guide therapy decisions and optimize management are necessary. Ultimately we need models to predict individual disease prognosis to allow us to target those patients

Table 11.1 Biomarkers in chronic moderate to severe ulcerative colitis

	Advantages	Disadvantages
CRP	Widely available biomarker to monitor response to therapy	Does not correlate with extent of disease or risk of complications
pANCA	Can predict a more aggressive phenotype in UC	Does not replace need for endoscopy
Faecal calprotectin	Serial elevations predict disease flares	Patient reluctance to provide stool specimen
Mucosal and histological activity	Accurate predictor of risk of clinical relapse and complications such as surgery and malignancy	Invasive, costly
Intestinal USS	Non-invasive monitoring of disease activity and complications	Operator dependent and not widely available
Microbiome	Therapeutic target with FMT emerging	Further research required into risks and durability of effect

who are at the highest risk of morbidity. Biomarkers such as CRP (in those who make it), FCal and IUS may allow us to achieve frequent reassessment in a less invasive manner that is more acceptable to patients. High-quality research with standard definitions of key endpoints that include composite endpoints of PROs and objective measures are essential, including the development of a core outcome set to standardize the efficacy and safety data in UC trials [48].

Summary Points

- Chronic moderately to severely active UC is a heterogeneous disease in which the incorporation of biomarkers into the baseline assessment plays an important role in understanding the individual disease phenotype.
- Serial faecal calprotectins are particularly useful in UC and can be used to titrate treatment.
- Disease activity should be proactively monitored with the non-invasive biomarkers CRP and faecal calprotectin, in addition to endoscopic assessment to establish mucosal and histological severity.
- Engaging the patient in education and discussions about the ability of biomarkers to help predict response to treatment, risk of relapse or loss of response to therapy can facilitate shared decision-making, a vital part of the treat-to-target algorithm.

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Refractory Proctitis

12

Ian Craig Lawrance

Abstract

Ulcerative colitis (UC) is chronic and incurable and requires long-term management. Inflammation confined to the rectum occurs in 30% of UC patients with extension of disease in 50% at some stage. The use of faecal calprotectin can help in the assessment of disease activity and predict disease relapse, but C-reactive protein is frequently not of great use in ulcerative proctitis due to the rectum's dual blood supply. Genetic evaluation of UC has been of interest but has yet to provide any clinically useful insights into disease subtyping, management or patient-focused individualisation of therapy.

The topical 5-aminosalicylic acids are the first-line therapy for proctitis, and topical steroids should only be used for remission induction and not maintenance. When these fail to achieve remission, other topical agents could be considered like rectal tacrolimus cream, but the evidence for most of the other potential topical agents is limited to open-labelled studies alone and thus requires a great deal more investigation. The use of systemic, immunomodulatory and biological agents in proctitis is the same as for left-sided and extensive UC. Therapy has undoubtedly improved over recent years but with a goal of targeted therapies, treat to complete mucosal healing and therapy targeted to each individual, a great deal more has yet to be discovered.

12.1 Introduction

The external environment will impact an individual's intestinal microbiota due to the variety of foods, ambient microbiota and the individual's response to their natural external environment. This response to the intestinal microbiota is modified by the person's individual genetic characteristics and can result in tolerance to specific antigens or activation of the innate mucosal immune system. With the increased availability of antibiotics, refrigeration, hot water, higher standards of living, etc., there has been a corresponding increase in the immunologically based diseases such as asthma, systemic lupus erythematosus and also Crohn's disease (CD). Ulcerative colitis (UC), however, is also considered to result in an inappropriate activation of the innate intestinal mucosal immune system to normal colonic flora in genetically susceptible individuals.

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UC disease activity is characterised by a lifelong course of exacerbations and remission, with approximately 20–55% of UC patients suffering, at some stage, an episode of acute severe colitis (ASC), which is a severity of disease that requires management in hospital [1]. The Montreal classification categorises UC into ulcerative proctitis (E1) (disease limited to the rectum), left-sided colitis (E2) (disease extending to the splenic flexure) and extensive colitis (E3) (disease at some stage extending beyond splenic flexure) [2]. Inflammation confined to the rectum (E1) occurs in approximately 30% of UC patients, although, in almost 50% of patients, this may extend to involve more of the colon at some stage. Proctitis alone, however, can frequently result in distressing symptoms, including stool frequency, tenesmus (a feeling of incomplete evacuation), faecal urgency, faecal incontinence and rectal bleeding.

This can often be managed within the community with topical agents [3] including the 5-aminosalicylic acids (5-ASAs) or steroid-based suppositories and foams. There are also less universally used topical treatments also to be considered including rectal tacrolimus cream and epidermal growth factor (EGF) enemas, which have demonstrated clinical efficacy in randomised double-blind placebo-controlled clinical studies [4, 5]. Unfortunately, medication-resistant ulcerative proctitis can be extremely difficult to manage, and when the topical agents are not effective, the use of systemic agents is frequently required including oral prednisone and azathioprine/6-mercaptopurine (AZA/6MP), the antitumour necrotic factor (TNF)-α medications [6–8] and the anti-integrin therapy, vedolizumab [9, 10]. These biological therapies, however, are still not universally effective, are systemic and carry a considerable cost burden with a significant proportion of UC patients still not obtaining clinical improvement, let alone remission. It is for these patients that a better understanding of the disease and the investigation of new and novel therapies is still required.

12.2 Diagnostic Evaluation

The inflammatory bowel diseases (IBDs) have traditionally been divided into CD and UC. It has, however, been a long-held belief that these disease terms group together different disease subtypes with potentially different aetiologies, pathogenesis and genetics into a broad disease category. Recent genetic analysis has, to some extent, confirmed this by identifying three distinct genetic footprints, UC, colonic CD and ileo/ileocolonic CD, where UC and colonic CD are more similar than colonic CD and ileo/ileocolonic CD [11]. A second recent paper, using a custom designed immunochip for IBD containing 123,437 SNPs, identified that higher risk scores of the SNPs were associated with a more severe disease course [12]. Although these findings are not, at this stage, of clinical benefit, it does suggest that in the future there may be genetic determinants that could distinguish between disease subtypes and may help in predicting severity of flares, need for colectomy and responses to the various medications.

12.2.1 Disease Flare

Whenever a patient presents with a suspected exacerbation of their UC, whether it is extensive disease or proctitis, all need to be assessed for ASC defined, using a modification of the original Truelove and Witts criteria, as 6 or more bowel motions a day with large amounts of blood in each stool and with one of the following, a temperature greater than 37.8 °C, a resting heart rate >90 beats/min, a haemoglobin <10.5 g/dl or an ESR >30 mm/hour [13]. Both the American College of Gastroenterology [14] and the European Crohn's and Colitis Organisation [15] have accepted these criteria for ASC.

12.2.1.1 Disease Extension

Assessment of the disease also includes a limited sigmoidoscopy in an unprepared bowel, and this is of great use in determining if the disease is still

localised to the rectum or has extended proximally. Extension is not unusual and in paediatric patients, of the 30% suffering proctitis at diagnosis, disease extends in 50% [16]. In adults, again about 30% of UC patients initially present with proctitis [17]. Proximal extension then occurs, ranging from 14% at 4 years in a Turkish study [18] to 18% at 5 years and 37% at 10 years in a Greek cohort [19], with 34% at 10 years and 52% at 20 years in a Japanese cohort [20] and 53% of patients extending their disease after 25 years in the Danish population [21].

In the Greek cohort, progression occurred more commonly in non-smokers (hazard ratio [HR] 4.9, $p = 0.046$) [19]. While in the Danish population multivariate regression analysis undertaken in 467 patients observed no correlation with the number of clinical exacerbations, smoking, family history or parity [22]. A separate cohort of 145 patients with proctitis/proctosigmoiditis, of which 53 suffered disease extension, extension occurred in 16% at 5 years and 31% at 10 years, but this study identified that a younger age at diagnosis (HR 0.98, 95% confidence interval (CI) 0.96–0.99) and continuously active disease (HR 2.18, 95% CI 1.27–3.73) were independent risk factors for disease extension [17].

The prognosis for patients with ulcerative proctitis, however, is good. Despite the risk of disease extension, only 8–11% of patients require an operation over a mean of 11 years follow-up [22, 23], and the presence of proctitis (E1) at diagnosis was noted to carry a lower risk of colectomy than E2 or E3 disease (HR 0.43, 95% CI 0.22–0.86) [17].

12.2.1.2 Infection

The exclusion of infection is an absolute requirement when there is a suspected UC flare. Any time there is disease exacerbation, stool culture examination for *Salmonella*, *Shigella*, and *Campylobacter* infection is required. Patients with UC are also noted to be at an increased risk of *Clostridium difficile* infection, and its presence is associated with an increase in hospital stay [24], colectomy rate [25] and mortality [26] and thus must always be treated.

12.2.1.3 Appendiceal Orifice Inflammation (AOI)

There is an association between distal UC and the presence of appendiceal orifice inflammation (AOI), but although there is a protective association between appendicectomy and the development of UC (OR = 0.44; 95% CI [0.30, 0.64]), appendicectomy does not appear to influence the course of disease in proctitis either with (OR = 1.15, 95% CI [0.67, 1.98]) or without the presence of AOI (OR = 1.03, 95 CI [0.74, 1.42]) [27].

The presence of AOI in one study was associated with a mild disease course and lower probability of proximal disease progression, the need for immunosuppressive therapy and colectomy [28]. A second study comparing 48 patients with AOI to 46 patients without AOI, however, demonstrated no prognostic implication of AOI for disease remission, risk of relapse or the development of proximal disease extension [29]. In a Japanese cohort, however, all nine patients with AOI and proctitis had proximal disease extension suggesting that there could potentially be ethnic variability [30].

12.3 Biomarkers and Patient Monitoring

12.3.1 C-Reactive Protein (CRP)

The CRP is an acute phase reactant made by the liver that activates the complement system and promotes the clearing of necrotic tissue and bacteria by macrophages through phagocytosis. Interleukin-6 (IL-6) is a pro-inflammatory cytokine that is secreted by macrophages and lymphocytes [31], and when IL-6 is presented to the liver, CRP production is promoted, rises in the circulation usually within 2 hours and has a half-life of 18 hours. In the intestine, IL-6 arrives at the liver through the portal circulation, and a rise

in CRP is commonly associated with systemic inflammation. The rectum, however, has two blood supplies, the portal and systemic circulations. It is thus not uncommon that proctitis is not associated with a rise in CRP most likely due to IL-6 moving into the systemic circulation and thus CRP is frequently an unreliable marker of inflammation in the rectum. It is also estimated that around 15% of normal people will not mount a CRP response to IL-6.

12.3.2 Faecal Calprotectin (FC)

Calprotectin is a complex of the mammalian proteins and makes up to 60% of the soluble protein content of the neutrophil cytosol. It is secreted by an unknown mechanism during inflammation where it finds its way into the intestinal lumen through leucocyte shedding, active secretion and cell death. Calprotectin is resistant to enzymatic degradation, can be easily measured in the stool and is a highly sensitive marker for the presence of colonic inflammation. There are, however, numerous causes for a rise in FC so, although it is highly sensitive, it is not always specific for a flare of UC. Other gastrointestinal causes that result in a FC rise are listed in Table 12.1 and as expected include anything that is associated with colonic inflammation including untreated coeliac disease, colonic cancer, infection and microscopic colitis. The spondyloarthritides are also associated with intestinal inflammation including systemic lupus erythematosus and rheumatoid arthritis, with psoriasis having a FC rise in 16% of patient [32] and occurring in 5–10% of patients with ankylosing spondylitis [33]. Up to 50–60% of patients with a spondyloarthritis undergoing colonoscopy also have been noted to have microscopic lesions on histology [33, 34]. The use of a PPI is also associated with significantly elevated calprotectin values. In addition, the FC levels can vary on the patient's age and other comorbidities and can fluctuate on a day-to-day basis within the same individual.

Table 12.1 Gastrointestinal and non-gastrointestinal causes for an elevated faecal calprotectin

Gastrointestinal conditions	Non-gastrointestinal conditions
Ulcerative colitis	Rheumatoid arthritis
Crohn's disease	Psoriatic arthritis
Microscopic colitis	Systemic lupus erythematosus
Pouchitis	Gout
Radiation proctitis	Ankylosing spondylitis
Pouchitis	Juvenile arthritis
Diverticulitis	Protein pump inhibitor use
Intestinal infections	
Active coeliac disease	
Intestinal cancers and neoplasms	
Allergic gastroenteritis	
Allograft intestinal rejection	
Necrotising enteritis	

FC levels can be used to predict that a patient has IBD with a higher calprotectin level associated with a greater likelihood of IBD. The maximal predictive value occurs at 1000 μg/g with a predictive rate of 78.7%. Elevated FC may also be used to predict a relapse after ceasing infliximab in CD (STORI study) with FC concentration >300 μg/g at inclusion an independent factor of a disease relapse. An elevated FC also predicted relapse during maintenance of infliximab with a relapsing more likely with levels of 332 ± 168 μg/g and less likely at levels of 110 ± 163 μg/g ($P < 0.005$) [35]. It is similar for UC with a low FC <56 μg/g found to optimally predict an absence of relapse during follow-up [36], while an elevated FC >170 μg/g was noted to have a sensitivity of 76% and a specificity of 76% to predict a relapse (HR, 7.23; $P = 0.002$) [37], while a FC >75 μg/g had a HR 2.05 ($P = 0.0045$) of an UC patient flaring [38]. A review of studies predicting a flare rate in UC within 1 year have identified that a mean FC range between 190 μg/g and 615 μg/g is associated with a flare, whereas UC patients with a mean FC range between 47 μg/g and 282 μg/g are less likely to flare [39].

The use of FC is thus recommended as a marker of active colonic inflammation and the level of disease control and may also help in predicting a disease flare.

12.3.2.1 Blood Tests

Routine bloods assessing haemoglobin, liver function tests, renal function tests and iron studies should be undertaken. As detailed above the utility of requesting a CRP varies on the patient and can be used in individuals where an elevation has been observed and its reduction may correlate with improvement in the rectal inflammation.

12.3.2.2 Clinical Monitoring

Monitoring of patient symptoms using the partial Mayo score [40] or just scoring the stool frequency and faecal blood without including the physician score is an objective way to assess clinical improvement or worsening of disease and should be a routine practice at every clinical visit.

12.4 Topical Therapeutic Options

The use of systemic agents for the management of proctitis unresponsive to topical agents is the same as for left-sided and extensive UC and will not be discussed.

12.4.1 5-Aminosalicylic Acid (5-ASA) and Steroid Rectal Preparations

The rectal use of the 5-aminosalicylic acid (5-ASA) is efficacious for the induction and maintenance of remission in distal colitis [41, 42] with data suggesting no difference in effectiveness between a 5-ASA 500 mg suppository twice daily and 1 g at night for proctitis [43, 44], although oral agents may be frequently used as an alternative due to patient preference. Scintigraphic studies demonstrate that liquid enemas reach proximal to the splenic flexure, whereas foams provide medication to the proximal sigmoid colon and suppositories primarily treat the rectum [45, 46]. If a nightly suppository does not induce a clinical response, then increasing this to one suppository twice a day or adding in an oral 5-ASA can be considered (Fig. 12.1). Once remission is achieved, the remission can be usually be maintained with either an oral 5-ASA or a second daily suppository again depending on patient preference [47].

Steroid enemas/suppositories are superior to placebo in ulcerative proctitis; however, topical rectal 5-ASAs are at least twice as efficacious as rectal corticosteroids for patient symptoms and resolution of endoscopic and histological inflammation in distal colitis and proctitis. The 5-ASAs are thus considered first-line therapy [41, 48–50].

The 5-ASAs do not gain access to the colonic mucosa through the systemic circulation but primarily have effect at the mucosal level with rectal delivery providing high topical concentrations of 5-ASA to the area of inflammation. An inverse correlation between mucosal 5-ASA concentrations and level of disease activity in UC has been observed with greater drug levels associated with lower endoscopic scores and levels of s-interleukin (IL)-2 receptor, a marker of inflammation [51]. The topical nature of the medication results in less side effects, which is a consideration when steroid therapy can suppress the pituitary-adrenal axis (PA axis). There is also no evidence that topical steroid preparations will maintain clinical remission in UC [52]; thus with systemic absorption and a lack of long-term maintenance data, the use of topical steroids should be limited to induction therapy only, in patients who are unresponsive to the topical 5-ASA agents.

The second-generation corticosteroids (budesonide and beclomethasone dipropionate [BDP]) demonstrate efficacy in distal colitis and have limited systemic absorption with a high first-pass liver metabolism resulting in lower systemic steroid levels [50, 53, 54]. Despite the low systemic levels, however, PA-axis suppression has been observed with BDP, betamethasone and prednisolone enemas [55–57]. Again this suggests that these medications are more suited for induction and not maintenance therapy.

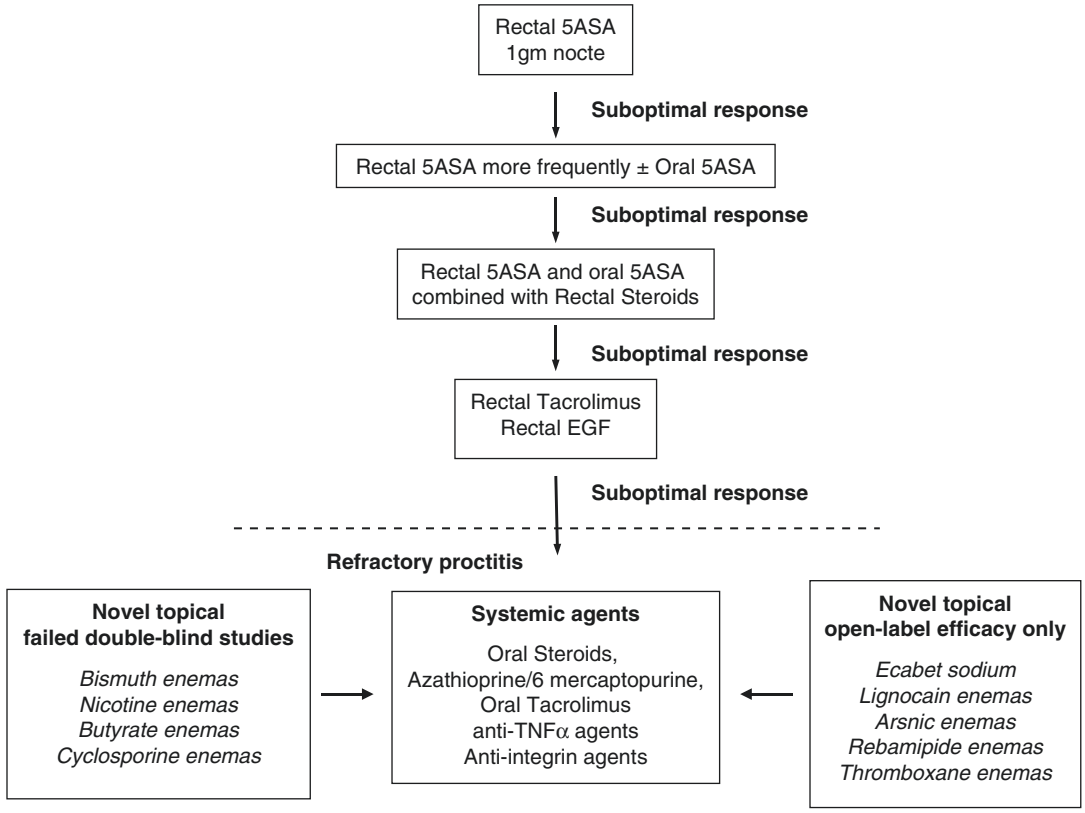

Fig. 12.1 Algorithm for patients with proctitis

12.5 Novel Topical Therapies with Double-Blind Studies

Open-labelled data may identify potentially effective therapeutic options, but randomised double-blind placebo-controlled trials are regarded as the gold standard for determining an agent's true efficacy. Tacrolimus rectal cream and epidermal growth factor (EGF) enemas are the only two agents with positive studies, while cyclosporine (CsA) butyrate and nicotine enemas failed to demonstrate efficacy in their randomised studies. This lack of efficacy, however, may potentially be due to the way the drug was administered, the mucosal contact time and mucosa concentrations and not that the agent itself does not have clinical potential.

12.5.1 Tacrolimus Rectal Preparations

Tacrolimus and cyclosporin are both calcineurin inhibitors that have demonstrated clinical efficacy in UC [47, 58]. Calcineurin, or protein phosphatase 2B (PP2B), is a cytosolic Ser/Thr protein phosphatase and dephosphorylates a variety of proteins and regulates the expression of IL-2, IL-4 and interferon (IFN) γ [59, 60] while modulating the transcription factor NF-κB's activity [61]. NF-kB activity is increased in UC, and this induces cytokine IL-1β, IL-6 and TNFα production, which are all pro-inflammatory. Reducing these cytokine levels is associated with clinical remission in IBD.

The use of topical tacrolimus has been investigated in UC patients with resistant distal colitis.

The first case series identified that 75% (6/8) of patients achieved clinical remission following 4–8 weeks of therapy with 0.3–0.5 mg/ml 3 ml twice a day of the tacrolimus rectal ointment [62]. Although tacrolimus is absorbed well transdermally [63], only low trough levels of tacrolimus were detected in the blood consistent with other studies of topical tacrolimus therapy [64]. In a second series that examined topical tacrolimus in patients with resistant distal colitis, the study demonstrated clinical and histological improvement in 10 of 12 patients with proctitis by 4 weeks. Again no major side effects were reported and the preparation was well tolerated [65]. The mechanism of action appeared to be local and not systemic with the concentration of the tacrolimus in the mucosa corresponding the clinical outcome [66]. A subsequent double-blind placebo-controlled 8-week induction study investigated the use of the tacrolimus rectal ointment [62] and identified a clinical response in 73% vs. 10% on placebo ($p = 0.004$), clinical remission in 45% vs. 0% on placebo ($p = 0.015$) and mucosal healing in 73% vs. 10% on placebo at 8 weeks ($p = 0.004$) without any significant side effects [5]. The findings suggest that this topic tacrolimus preparation can be effective for the management of resistant ulcerative proctitis.

12.5.2 Epidermal Growth Factor (EGF) Enemas

EGF, a 1207-amino-acid precursor, is found in gastric juices [67] and can stimulate healing topically [68, 69], and systemically it treats necrotising enterocolitis [70]. EGF is digested in the proximal gastrointestinal tract, and it is likely that very little luminal EGF reaches the colon.

Only one small randomised, double-blind placebo-controlled trial in 2003 investigated EGF enemas in the management of distal colitis in 24 patients. After 2 weeks of topical therapy, all patients receiving EGF improved with 83% (10/12) noted to be in remission compared to 8% (1/12) receiving placebo ($p < 0.001$). Endoscopic and histologic scores were also significantly better in the EGF group [4]. Despite these encouraging results, there are no further published studies, and pharmaceutical grade EGF is extremely difficult to source, and to date the author has been unable to find a supplier of the agent, thus making this purely a conceptual, but not practical, option for treatment.

12.5.3 Cyclosporine Enemas (CsA)

CsA administered intravenously is effective as rescue therapy in severe steroid-refractory ASC [71, 72]. Efficacy for CsA formulated as an enema for use in resistant distal UC has thus been suggested [73] with two open-labelled studies in treatment-resistant left-sided UC. In these small studies, 5 of 10 patients responded to a nightly 350 mg enema [74], and 7 of 12 responded to a daily 250 mg enema [75]. The only double-blind randomised placebo-controlled trial in 1994, however, did not demonstrate any superiority over placebo [76] with no further studies.

This is similar to the findings for tacrolimus enemas [65] and may be related to the concentration of the medication at the mucosal surface. To date the use of CsA suppositories has not been investigated, but the lack of efficacy may be due to the formulation as observed with the tacrolimus enemas compared to the tacrolimus suppository and cream [5, 62, 65].

12.5.4 Butyrate Enemas

Butyrate is a SCFA (short-chain fatty acid) that is actively metabolised by the colonic mucosa and if deficient in the lumen may promote a state of energy deficiency for the colonic mucosa resulting in tissue injury. Butyrate is also anti-inflammatory by decreasing NF-kB nuclear translocation in macrophages [77]. Initial open-labelled studies of butyrate enemas in distal UC were promising with a patient response following nightly butyrate enemas [78, 79] as well as endoscopic and histological improvement [80] observed.

These studies, however, were followed by three randomised, double-blind, placebo-controlled trials that were all negative with no significant difference to placebo treatment [80–82]. This promising concept has thus not demonstrated efficacy and is not of use in distal colitis.

12.5.5 Nicotine Enemas

As UC is more likely in non-smokers and not infrequently presents after the cessation of smoking, the use of nicotine is of interest as it may reduce intestinal inflammation through effecting both gut motility [83] and immune function [84]. Open-labelled use of a nightly 6 mg nicotine enema for 4 weeks in UC demonstrated clinical efficacy in 16 of 17 patients and endoscopic/histological improvement in 10 UC patients [85]. The only randomised placebo-controlled study, however, undertaken in 104 patients for 6 weeks demonstrated no clinical benefit for the nicotine enema (27% remission) over placebo (33% remission) [86].

12.6 Other Therapies with Only Open-Label Data

All other therapies that have been trialled for the management of resistant ulcerative proctitis have only limited open-labelled data. Further work is required for all the following agents, but there is potential that these may be effective in the difficult-to-treat patient.

12.6.1 Lignocaine Enemas

The proposal that distal colitis might be a result of autonomic nerve hyperactivity [87] leads to the investigation of lignocaine as a therapy following animal models of colitis demonstrating that lignocaine reduced the severity of acute inflammation [88, 89]. The first of 2 open-labelled studies of twice daily 2% lignocaine gel (400 mg) detected a response in all 28 patients after 2–12 weeks of therapy, while the second demonstrated a response in 41 of 49 patients following 6–34 weeks of therapy. Despite these impressive results, published over 25 years ago, there have been further publications and no placebo-controlled studies.

12.6.2 Ecabet Sodium (ES) Enemas

ES is an oral non-absorbable protectant derived from pine resin [90]. It has been used for gastritis and gastric ulceration due to its affinity for adherence to the mucosa and to fibrinogen found on the base of gastric ulcers [90]. Mucin, produced by intestinal goblet cells, is the major component of the intestinal mucus barrier. Loss of goblet cells reduces mucin production and thus the protective barrier that covers the colonic mucosa. This may result in the epithelial cell damage observed in actively inflamed UC. Rectally administered ES in animal models of colitis bound to damaged mucosa formed a protective barrier and was associated with reduced inflammation [91].

Two very small open-labelled studies in seven and six patients, respectively, demonstrated a clinical response after 2 weeks of twice-daily rectal administration for 2 up to 7 weeks [92, 93]. As ES has the ability to reinstate a barrier against the intestinal microflora, it is not unreasonable to predict a benefit in resistant proctitis. Further studies, however, are still required before any real therapeutic role is known.

12.6.3 Arsenic Enemas

Organic arsenic in the management of resistant proctitis was proposed more than 30 years ago [94]. Unfortunately, only one small open-labelled study of 10 patients has investigated its use. Acetarsol®, a suppository given twice a day, was associated with resolution of the symptoms and endoscopic signs of proctitis within 2 weeks in nine patients, but six of ten patients had inorganic arsenic blood levels in the hazardous range [95]. Anecdotal reports of efficacy have been made since then, but there have been no further publi-

cations and safety of this agent is still in question.

12.6.4 Thromboxane Enemas

Thromboxanes are associated with inflamed IBD mucosa [96]. Thromboxane synthesis inhibition reduces TNFα by human macrophages, and anti-TNFα therapy reduces thromboxane [97]. The open-labelled use of a Ridogrel® enema, a thromboxane synthase inhibitor and receptor antagonist, in 11 patients reduced mucosal thromboxane levels with 5 patients clinically responding, but this occurred without any endoscopic or histological improvement [98]. No further clinical studies have been undertaken.

12.6.5 Rebamipide Enemas

Rebamipide (2-(4-chlorobenzoylamino)-3-[2-(1H)-quinolinon-4-yl]-propionic acid) reduces inflammation in animal models of colitis [99, 100], stimulates endogenous prostaglandin production and accelerates healing [101]. Three open-labelled studies in distal UC have been undertaken: the first investigated 11 patients [102] with 9 achieving clinical remission and histological improvement by 12 weeks with twice daily 150 mg rebamipide; in the second study of 16 patients [103] 7 demonstrated clinical response after 4 weeks; in the third study of 20 patients [104] 16 responded endoscopically and 11 obtained clinical remission. Despite these studies being published more than 10 years ago, the medication being commercially available and other open-labelled studies suggesting efficacy in ischaemic distal colitis [105], pouchitis [106] and radiation proctitis [107], no other investigations have been undertaken in UC.

12.7 Future Directions

To date the tried and proved method of treatment in proctitis is to commence with topical agents. If these are unsuccessful, then the addition of oral lumenally active agents and then systemic agents is used. There is, however, a need for more options for topic agents. Rectal tacrolimus cream appears to be effective, but many of the other potential agents only have open-labelled studies, but blinded randomised, placebo-controlled studies are still required. Generally, biomarkers consist of assessing the FC, which identifies active and quiescent disease and may also be of benefit to predict a disease flare. CRP, however, is frequently not a good marker for rectal inflammation. Genetic analysis of the IBDs has identified at least three different IBD subsets, and there are probably more, while gene expression might correlate with worse disease progression, but this is not yet appropriate for clinical use. There is much work yet to be done before there is individual patient-directed therapy, but the process has commenced and will yet provide further information to the physician.

Summary Points

- Ulcerative colitis presents as proctitis in 30% of paediatric and adult patients with about 50% extending their disease over time.
- Infection must always be excluded if there is a suspected disease flare.
- Faecal calprotectin is highly sensitive for colonic inflammation, but it is not specific for a UC flare. It can differentiate between inflammation and irritability, and its level can be used as a predictive marker for the likelihood of a disease flare.
- C-reactive protein is not a reliable marker of inflammation in the rectum.
- There are no genetic or other biological markers of use in the management of proctitis.
- Topical 5ASAs are first-line therapy for proctitis, and steroids should only be used short term for induction of remission.
- Prior to the instigation of oral steroids, oral immunomodulation or biologic

therapies, consideration should be given to rectal tacrolimus cream.

- Other novel topical therapies like lignocaine, ecabet sodium, arsenic, thromboxane and rebamipide enemas could also be considered, but further work is still required to demonstrate efficacy of these therapies.

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UC Surveillance

13

Chang-Ho Ryan Choi and Ibrahim Al-Bakir

Abstract

Patients with inflammatory bowel disease (IBD) have a significant risk of developing colorectal cancer (CRC). Regular colonoscopic surveillance and dysplasia detection remains the mainstay of CRC risk management. The efficacy of colonoscopic surveillance in preventing CRC remains poor with a considerable number of surveillance patients presenting with interval or advanced CRC. This chapter will focus on clinical and molecular risk factors associated with CRC development. A particular consideration will be given to challenges in detecting and managing dysplasia, with reference to our latest understanding of how CRC evolves from a molecular perspective that could ultimately pave the way for biomarker discovery.

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13.1 Colorectal Cancer in IBD: Magnitude of the Risk and Recent Trends

The risk of CRC development in patients with IBD has been long recognised, with the first report of colitis-associated CRC described by Crohn and Rosenberg [1]. One of the earliest meta-analyses, published in 2001, reported a substantial risk of developing CRC, with cumulative incidence rates of 2% by 10 years, 8% by 20 years and 18% by 30 years [2].

These historically high CRC risks likely reflect a lack of efficacious IBD therapies, poor patient and clinician awareness of CRC risk and less advanced endoscopic technologies and techniques in early years. More recent studies report lower CRC risk in patients with IBD. The St Mark's Hospital group studied 1375 patients with extensive UC undergoing 1–2 yearly colonoscopic surveillance from 1971 to 2012 and found that the cumulative CRC incidence was considerably lower: 2.9% at 20 years, 6.7% at 30 years and 10.0% at 40 years after initial onset of colitis symptoms [3]. The authors found a substantial decrease in incidence rate of interval CRC (i.e. CRC occurring in between scheduled surveillance colonoscopies) from 2.5 to 0.4 per 1000 patient-years in the first and the last study decades, respectively.

Similarly, a Danish cohort study, published in 2012, that includes 47,374 colitis patients followed over a 30-year period showed that

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overall relative risk of CRC in patients with UC compared with the general population was only 1.07 (95% CI, 0.95–1.21). Furthermore, authors found that the overall relative risk has decreased from 1.34 (95% CI, 1.13–1.58) in 1979–1988 to 0.57 (95% CI, 0.41–0.80) in 1999–2008 for patients with UC [4]. These trends may be part explained by early detection of neoplasia: patients in the St Mark's cohort were found to have a significant increase in incidence rate of low-grade dysplasia (LGD) and early CRCs in the most recent decade (2003–2012) compared with the decade prior (1993–2002) [3]. This study and others [5] indicate that dysplasia is becoming a relatively frequent encounter during colonoscopic surveillance, highlighting the importance of optimal management strategies.

13.2 Clinical Biomarkers for Colorectal Neoplasia (CRN) Development

Numerous studies published to date have identified clinical risk factors for CRN development, which can be used in patient risk stratification. The most important risk factors are shown in Table 13.1.

Table 13.1 Main risk factors for colorectal neoplasia development in patients with IBD

Clinical risk factors	Degree of risk
Duration of colitis (cumulative incidence) [2, 3]	10 years = 1–2% 20 years = 2.9–8% 30 years = 6.7–18%
Extent of colitis (RR) [6, 7]	Proctitis = 1.7 Left-sided = 1.8–4.0 Extensive = 14.8–19.0
Severity of inflammation (OR or RR) [8–10]	2.6–4.7 per 1 unit increase in microscopic severity (4–5 point scales in order of increasing severity)
Colonic stricture (OR) [11–13]	4.6-fold increase
Primary sclerosing cholangitis (PSC) (OR or RR) [14, 15]	4–5-fold increase
Family history of CRC in FDR (OR or RR) [16, 17]	2.3–9.5-fold increase

OR odds ratio, *RR* relative risk, *PSC* primary sclerosing cholangitis, and *FDR* first-degree relative

13.2.1 Inflammation as the Most Important CRC Risk Factor

It is well established that chronic inflammatory conditions are associated with increased risk of cancer, such as oesophageal adenocarcinoma arising from Barrett's oesophagus, bladder cancer from chronic cystitis and lung cancer associated with cigarette smoking (reviewed in [18]).

Similarly, patients with long-standing IBD are predisposed to CRC due to recurrent courses of relapsing-remitting colonic inflammation. Indeed, observations from epidemiological studies demonstrate that the CRC risk increases with the increasing extent of colonic involvement [6], disease duration [2] and severity of inflammation [8]. Furthermore, patients with endoscopic features of disease chronicity (such as tubular, shortened or featureless colon) and severity (such as colonic stricturing [11, 13, 19], backwash ileitis and pseudopolyposis [11, 20]) are recognised risk factors for CRN.

Current international surveillance guidelines stratify patient risk based on their inflammatory profiles [21, 22]. For example, the British Society of Gastroenterology (BSG) recommends 5-yearly colonoscopies for left-sided colitis or extensive colitis with no active inflammation (either endoscopic or histological), 3-yearly colonoscopies for extensive disease with mild active inflammation or postinflammatory polyps and annual surveillance for those with moderate to severe inflammation in the most recent colonoscopy [21].

While the BSG guideline is pragmatic and comprehensive, there is an inherent drawback to this approach: determining the next surveillance interval is mostly based on inflammation profile seen on the most recent endoscopy only. For example, a patient with macro−/microscopically quiescent disease at colonoscopy may not undergo another surveillance procedure for 5 years, even though he/she has "accumulated" a significant aggregate of inflammatory burden in the preceding years [19]. This principle has been demonstrated in a recent large cohort study of extensive UC patients undergoing surveillance at St Mark's Hospital, with a surrogate quantitative

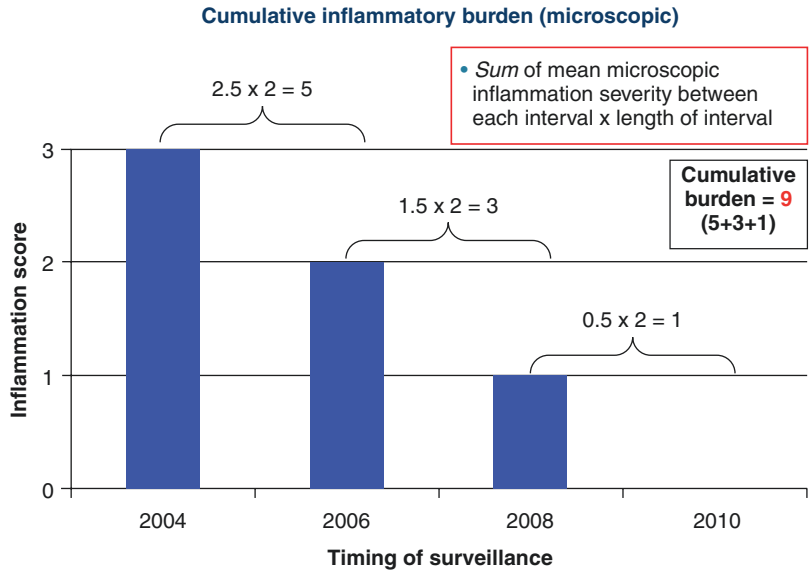

Fig. 13.1 An illustration of how cumulative inflammatory burden (CIB) for each patient can be calculated. This patient had severe (inflammation score = 3), moderate (inflammation score = 2), mild active (inflammation score = 1) and then quiescent microscopic disease (inflammation score = 0) in 2004, 2006, 2008 and 2010, respectively. The CIB for the first surveillance interval (i.e. from 2004 to 2006) would be an average microscopic severity between the two surveillance episodes (i.e. 3 + 2 / 2 = 2.5) multiplied by the length of surveillance interval (2 years), which is 5. The overall CIB is then obtained by summing the CIB scores from all surveillance intervals. (Reprinted from Choi et al [19])

score for cumulative inflammatory burden (example shown in Fig. 13.1) more accurately capturing future CRC risk [19].

Furthermore, the strength of this association is enhanced when the mean severity score is derived from additional colonoscopies performed over the preceding years [19]. The aforementioned study also showed that the risk of developing CRN was also independently associated with inflammation *persistency* regardless of its severity. Finally, multiple studies consistently show that inflammation scores calculated from histological findings better predicted the risk of CRN compared with scores based on the grading of endoscopically visible inflammation [8, 10, 19]. These data provide a strong argument for aggressively managing disease activity in order to achieve complete mucosal healing and prevent CRN development.

Post-inflammatory polyps (PIPs, or pseudopolyps) are a relatively common finding in chronic colitis; at least three studies performed to date showed that PIPs were associated with an approximately twofold risk of developing CRN [11, 20, 23]. It is yet unclear whether this indicates an increased risk of CRC arising from PIPs themselves or that PIPs simply reflect previous inflammatory burden. Evidence for the latter comes from a recent cohort study that showed PIPs had no statistically significant association with CRN development after adjusting for cumulative inflammation burden [19].

13.2.2 Other Clinical Risk Factors

Other important risk factors for CRC development in IBD include concomitant primary

sclerosing cholangitis (PSC) [7, 19, 24, 25] and a family history of CRC [16, 17, 26], which increases the risk of CRN by 4–5-fold and 2.3–9.5-fold, respectively. Intriguingly, patients with PSC and UC seem to have more frequent and severe inflammation in the proximal colon compared with patients with UC alone [27], which may explain higher-than expected frequency of proximal CRCs detected on observational studies [28, 29]. For this reason, an additional attempt should be made to actively search for lesions in the proximal colon when surveying these patients.

Data are contradictory on whether 5-aminosalicylates [30, 31] and thiopurines [32] provide any benefit in reducing the risk of CRN development, with all studies limited by immortal time bias [33]. Finally, there have been no studies to date that have comprehensively assessed the risk of CRN development in patients receiving anti-TNFα biologic therapy.

13.3 Predictors of Progression Once Dysplasia Has Developed

With the increasing incidence rate of dysplasia, current clinical practice should be optimised for:

(a) Accurate detection of dysplasia using suitable endoscopic techniques
(b) Appropriate management of dysplasia prior to the formation and progression to CRC

13.3.1 Detection of Dysplasia: Random Versus Targeted Biopsies

Historically, colonoscopic surveillance involved taking quadrantic biopsies every 10 cm of the colon, totaling approximately 33 biopsies [22, 34]. However, the overall dysplasia yield from such an approach has been very poor. The meta-analysis performed by SCENIC (consensus statement of surveillance and management of dysplasia in IBD) demonstrated that the dysplasia yield from random biopsy protocol was only 0.1–0.2% per 1000 biopsies [35]. Furthermore, they showed that the vast majority of dysplasia (90%) was detected from targeted biopsies with only a small minority (10%) detected via random biopsy protocol [35]. Indeed, studies consistently suggest that most dysplasia is endoscopically visible [36, 37] and the dysplasia detection is likely to improve further with modern endoscopic techniques and technologies, including high-definition endoscopy [38], chromoendoscopy [35, 39–41] and narrow-band imaging [42].

13.3.2 Detection of Dysplasia: Chromoendoscopy, Narrow-Band Imaging and High-Definition Endoscopy

Chromoendoscopy (CE) involves spraying a dye (e.g. indigo-carmine) directly onto the mucosa, highlighting subtle mucosal surface irregularities and abnormal pit patterns (Fig. 13.2). The majority of prospective studies included in the SCENIC meta-analysis showed favourable detection rates with CE use, improving the dysplasia yield by 1.8-fold (95% CI, 1.2–2.6) when compared with standard white light endoscopy (WLE) [35]. Similarly, a retrospective study using high-definition (HD) WLE with 1080 output improved detection rate by 2.2-fold compared with standard-definition (480 system) WLE [38].

On the other hand, addition of narrow-band imaging during IBD surveillance studies has proved non-superior to both standard and HD colonoscopy [42] and is therefore not a recommended technique of choice [42].

The added benefit of CE in a period where HD-WLE is becoming widely available remains open to debate. CE is associated with its own limitations, including longer procedure time, additional training and expertise. The most insightful study reflecting the utility of CE in routine clinical practice comes from a multicentre prospective trial involving 350 patients which showed that even when HD endoscopy is used, application of CE increased the dysplasia yield

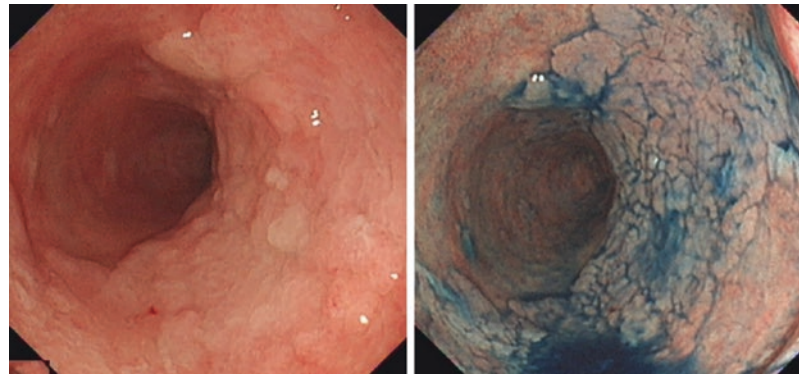

Fig. 13.2 Diffuse flat low-grade dysplastic lesion pre- and post-application of indigo-carmine dye spray. (chromoendoscopy; *Courtesy of Dr. Noriko Suzuki, St. Marks' Hospital, UK)*

by a further 52.3% compared with HD-WLE alone [43]. This study suggests that CE should be used with a HD scope wherever possible, an approach endorsed by the SCENIC consensus group [35]. Additional data from further prospective trials are eagerly awaited.

13.3.3 Dysplasia: What Are the Risk Factors for Progression to CRC?

The management of dysplasia depends on several factors including but not limited to endoscopic classification, histological grading and patient factors such as comorbidity and patient preference.

(a) *Histological Grade*

Confirmation of dysplasia grade should be made by at least two independent pathologists [21, 22]. There are three histological dysplasia grades: indefinite for dysplasia (IND), low-grade dysplasia (LGD) and high-grade dysplasia (HGD). Accepted management of HGD is relatively straightforward as it is an indication for colectomy: approximately 50% of these cases historically had concomitant CRC in colectomy specimens [3]. The clinical management challenge relates to LGD, which is by far the most commonly detected type of dysplasia during surveillance.

(b) *Location*

Lesions arising proximal to the maximal known extent of colitis are considered sporadic in nature and should be managed similarly to the conventional adenomas arising in colitis-free patients [21].

(c) *Endoscopic Features*

The recent SCENIC group consensus proposes dysplasia classification based on the morphological shape of the lesion and divides them into polypoid (pedunculated or sessile), non-polypoid (superficially elevated, flat or depressed) or invisible dysplasia (macroscopically invisible but detected via histological examination; Fig. 13.3) [35].

Polypoid LGDs have a favourable prognosis; multiple studies suggest that they can be managed safely with endoscopic resection [45–49]. A recent meta-analysis involving 376 patients who were followed up over 1704 years (mean number of colonoscopy performed per patient, 2.8) showed a pooled CRC incidence of only 5.3 per 1000 patient-year follow-up (95% CI, 2.7–10.1) [49]. However, these patients also had tenfold increased risk of developing further dysplasia, highlighting the need for ongoing close surveillance.

Compared with polypoid lesions, non-polypoid LGDs had a near ninefold increase in risk of developing CRC compared to polypoid

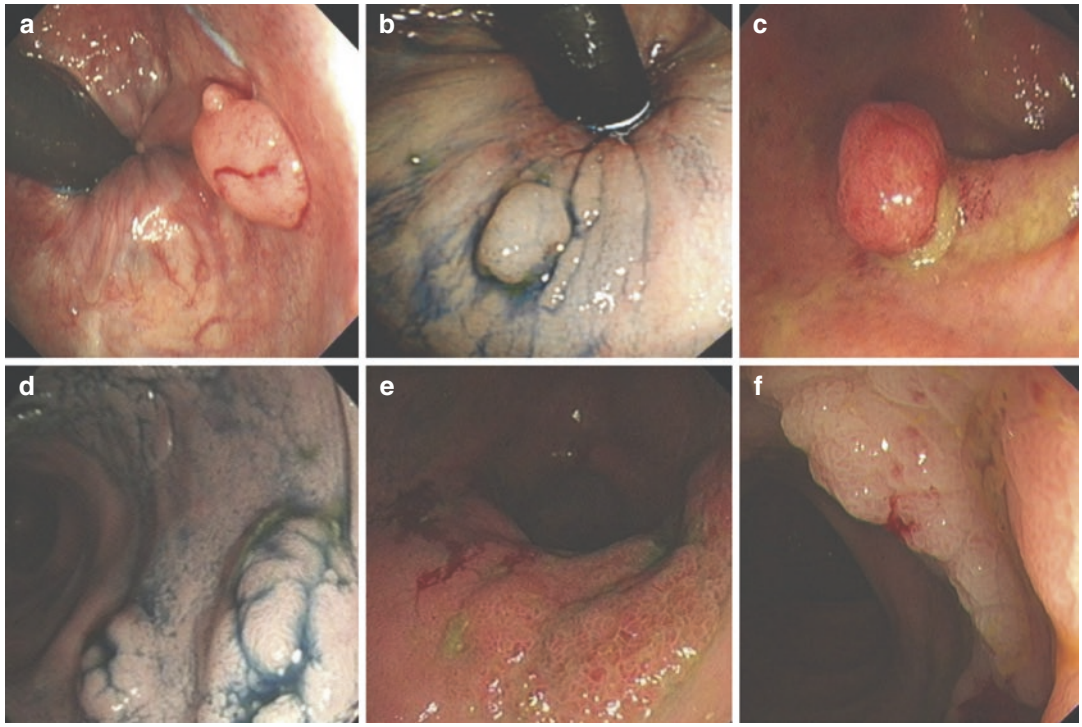

Fig. 13.3 Dysplastic lesions in IBD by endoscopic features. Polypoid dysplasia describes discrete sessile, pedunculated or sub-pedunculated lesions (**a**–**c**) that are usually well circumscribed from the surrounding mucosa. Non-polypoid dysplasia includes but not limited to superficially raised (**d** and **e**), visible flat (**f**), irregular or plaque-like lesions. (Reprinted from Choi et al. [44])

lesions [44]. They were often endoscopically unresectable (61.5%) and frequently associated with multi-synchronous CRC on progression [44]. The SCENIC consensus recommends endoscopic resection with close follow-up surveillance where possible and colectomy for unresectable lesions [35]. However, this remains highly controversial [50], and patients should be offered counselling so that informed decision can be made regarding the options of intensive colonoscopic surveillance or prophylactic colectomy.

Recent studies, including a meta-analysis, identified invisible dysplasia as an independent risk factor for CRC progression [44, 51], although most of these lesions were detected in an era prior to the advent of advanced endoscopic technologies and techniques. Where invisible dysplasia is detected, every effort should be made to visualise the dysplastic region under optimised endoscopic conditions. This includes pre-procedure control of mucosal inflammation, adequate bowel cleansing and use of high-definition chromoendoscopy by an expert endoscopist. Patients with truly invisible dysplasia should be counselled about CRC risk in a manner similar to those with non-polypoid dysplasia.

Finally, lesion size should be taken into consideration when managing dysplasia in IBD. Similarly to sporadic adenomas, low-grade dysplastic lesions in IBD that are 1 cm or larger confer an approximately fourfold risk of progressing to CRC [44].

13.4 Mechanism of Carcinogenesis in IBD

It is increasingly recognised that carcinogenesis occurs through a process of *clonal evolution*, whereby a cell population acquires genomic changes that provide them with a phenotypic advantage compared to other cells and which they can then pass on to daughter cells.

We hypothesise that the relapsing-remitting nature of IBD forms the key driver of mutant epithelial clonal evolution and expansion across the colonic mucosa: bouts of inflammation select for those clones that can survive this hostile environment and then more rapidly repopulate the healing mucosa during periods of remission. This unique environmental selection pressure is responsible for the major molecular differences between CRN and the sporadic counterparts.

This accelerated clonal generation and clonal expansion in IBD may in part explain the high incidence of synchronous neoplasia in IBD: longitudinal genetic analysis confirms the presence of multiple differing p53 mutant clones arising in the same patient, all with the potential to generate CRN, with one example demonstrating clonal spread over several years to involve the whole large intestine [52]. Corroborative findings of clonal expansion have been described using polyguanine microsatellite tracts as neutral lineage markers [53, 54].

(a) *Different Frequencies of Key Driver Genes Between Sporadic and IBD-Associated Neoplasia*

While the driver genes for cancer that are involved in both sporadic and colitis-associated CRC are mostly shared, the order and frequency of mutations in these genes differ significantly. One discriminant is the higher frequency of p53 mutations seen in IBD-CRC [55], which occurs early in carcinogenesis and is even observed in non-neoplastic IBD mucosa [56]. There is evidence that inflammation provides a selection advantage for p53 mutations: in vivo lineage tracing studies of intestinal crypt stem cells in recombinant mouse models demonstrate that p53 mutations provide a survival advantage over wild-type stem cells only in the setting of inflammation [57].

Another striking difference is the relative paucity of APC mutations in IBD-carcinogenesis compared to sporadic neoplasia. There is evidence to support that IBD-driven inflammation generates endogenous *Wnt* signalling without the need for constitutive *Wnt* pathway activation through an APC mutation: immunohistochemical analysis of colonic epithelium confirms increased nuclear to cytoplasmic β-catenin levels relative to a normal mucosa [58], and mouse models of intestinal injury show that epithelial healing requires the induction of *Wnt* signalling [59].

(b) *Accelerated Ageing with Field Cancerisation in IBD*

Evidence from molecular studies demonstrates that the increased cancer risk in IBD may be a consequence of accelerated ageing from rapid cell turnover. Analysis of point mutation signature of IBD cancers [55] showed typical signature associated with the ageing process [60], not with direct DNA damage from free radicals [61].

Epigenetic studies also confirm accelerated age-related methylation changes not only in neoplastic mucosa but also in adjacent non-neoplastic mucosa, at levels not seen in the normal mucosa distant from the neoplasia [62]. Finally, IBD-CRC seems to arise in a field of shortened telomeres and senescence [54, 63, 64].

13.5 Review of Current Biomarkers

Numerous single-molecule markers have been identified that allow CRN risk stratification (see Table 13.2). In practice, these potential biomarkers are limited by small sample size and a lack of validation through prospective studies. Moreover,

Table 13.2 Potential molecular biomarkers of CRC in IBD

Marker	Assay	Change	Reference
TP53 and chromogranin	Immunohistochemistry	Adjunct in accurate dysplasia severity grading	[66]
TRAP1	Immunohistochemistry	Increased expression in colonic mucosa of patients with concomitant dysplasia/cancer	[67]
AMACR (α-methylacyl-CoA racemase)	Immunohistochemistry	Combined with p53, can help predict low−/indefinite-grade dysplasia progression	[68]
8-ODhG (8-hydroxydeoxyguanosine)	High-pressure liquid chromatography	Increased in colonic mucosa of patients with concomitant dysplasia	[69]
Telomere length	Flow cytometry	Shorter in colonic mucosa of patients with concomitant dysplasia/cancer	[54, 64]
DNA – gene mutation panels *(CDKN2A, TP53 and KRAS)*	Targeted sequencing	Driver mutations detected 4 years prior to CRC formation	[52]
DNA – clonal expansion	Microsatellite genotyping	Larger clone sizes in colons with cancer	[53, 54]
DNA – aneuploidy	Flow cytometry, FISH, CGH	Increased in mucosa of patients with concomitant dysplasia/cancer and up to 2.5 years prior to CRC Predict indefinite- and low-grade dysplasia progression	[64, 70–74]
DNA – methylation panels *(RUNX3*, *MINT1*, *COX-2)* *(ER*, *MYOD*, *p16)*	Bisulphite sequencing	Significantly altered in normal mucosa adjacent to cancer	[62, 75]
miRNA – methylation *(MIR1*, *MIR9*, *MIR24*, *MIR137)*	Bisulphite sequencing	Increased in rectal mucosa of patients with concomitant dysplasia or cancer	[76]
RNA expression panels	Targeted sequencing	Altered expression profiles in non-neoplastic mucosa of patients bearing CRN	[77, 78]

single-pathway biomarkers may not capture the very diverse pathways a tumour clone can take to evolve into a fully malignant cancer.

The ideal biomarker is one that captures the "evolvability" of colonic mucosa as a surrogate marker of cancer while remaining "agnostic" to the dominant pathways driving carcinogenesis. Novel genomic sequencing and epigenomic analysis technique provide the greatest promise in this regard: the ENDCaP-C study [65] represents the first prospective, large-scale, multicentre attempt to using a five-marker methylation panel, with final results expected in 2019.

13.6 Microbiome and CRC Risk in IBD

To date, there have been no studies directly assessing the correlation between microbiomal composition and CRC risk in IBD. It is well known that IBD microbiomal dysbiosis results in alterations of bacterial species similar to those seen in association with sporadic colorectal adenomas and cancer; notable examples include an increase in *Fusobacterium* [79–81] and *Escherichia* [82] and a decrease in "protective" species such as *Roseburia* [83, 84]. The key research challenge is whether the altered microbiome in IBD and IBD-associated CRC risk represents causation or correlation.

Current IBD mouse models do not adequately replicate the multifactorial nature of both human IBD and colorectal cancer risk. However, they do indicate that the microbiome must play a critical role in modulating IBD carcinogenesis: germ-free azoxymethane-treated *IL10*$^{-/-}$ mice do not develop colitis-associated CRC compared to mice with a normal gut flora [85], while wild-type mice with a chemically induced colitis (using dex-

tran sulphate sodium and azoxymethane) are more likely to develop colitis-associated CRC in germ-free conditions [86].

Attempts have been made towards addressing microbiome-epigenome evolution in human IBD mucosa: UC mucosae with pro-tumorigenic methylation changes of key genes involved in CRC (e.g. *MINT2*, *MINT31*, *p16*, *NEUROG1*) are significantly correlated with heavy *Fusobacterium* spp. enrichment [87]. Further research into the microbiomal changes in IBD patients with CRN represents an unmet research need.

13.7 Conclusion and Future Areas of Research

In this chapter we reviewed the available evidence for recognised and potential clinical risk factors and molecular biomarkers for cancer development in patients with IBD. Rapidly developing endoscopic technologies and techniques, biological therapies and molecular biomarkers are likely to a have significant impact on neoplasia management in IBD and may change the landscape of IBD surveillance in the future. There are several important areas of future research, which include:

1. The prospective validation of inflammation-based risk scores to optimise surveillance intervals, in a clinical environment where "deep remission" and complete mucosal healing are recognised therapeutic endpoints [88].
2. Further evidence on the impact of chromoendoscopy at a time when high-definition colonoscopy becomes widely available.
3. Analysis of long-term clinical outcomes following endoscopic resection of larger or non-polypoid dysplasia, in order to prevent unnecessary colectomies.
4. The development and validation of dedicated training programmes for IBD surveillance, given the technical expertise required for these procedures that can significantly impact on outcomes.
5. Translational research towards the development of a molecule that captures the "evolvability" of colonic mucosa as a surrogate marker of cancer risk. Possible approaches include measurements of clonal expansion [53], alterations of genetic diversity over time [89] and epigenetic alterations [65].

Summary Points

- Patients with inflammatory bowel disease (IBD) continue to be at significant risk of developing colorectal cancer (CRC), but the risk of interval or late-stage CRC may be decreasing.
- In contrast, the incidence of dysplasia is increasing, which highlights an important need for optimal detection and management strategies.
- High-definition endoscopy and chromoendoscopy should be used wherever possible to improve dysplasia detection rate.
- Risk of dysplasia and CRC is tightly linked to burden of inflammation accumulated over the course of disease: determining surveillance interval should include assessment of multiple preceding colonoscopies for persistency and severity of inflammation.
- Each low-grade dysplastic lesion should be risk stratified based on its shape and size, as the risk of progression to CRC differs significantly between lesions.
- The relapsing and remitting nature of IBD accelerates clonal evolution in colorectal mucosa, resulting in epithelial "ageing" and "field cancerisation".
- There is a clear need for molecular biomarkers that capture this "evolvability" of colonic mucosa, as it may serve as a surrogate marker of cancer risk. Examples include measurements of clonal expansion and alterations of genetic diversity over time.

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14 The Role of Biomarkers in the Ileal Anal Pouch

Jonathan P. Segal and Ailsa L. Hart

Abstract

Restorative proctocolectomy is the procedure of choice in patients with ulcerative colitis refractory to medical therapy and in some patients with familial adenomatous polyposis. Despite overall good long-term function, pouchitis, a nonspecific inflammatory condition in the ileal pouch reservoir, can cause symptoms including increased stool frequency and fluidity, haematochezia, abdominal cramping, urgency and tenesmus, incontinence, fever and extraintestinal manifestations.

Biomarkers to determine pouchitis would ideally be predictive of pouchitis, whilst also being useful as a tool for predicting response and side effects to treatment. There is potential for both serum, faecal and microbial biomarkers which are currently unvalidated. Despite lack of validated biomarkers in pouchitis, CRP, faecal calprotectin and faecal lactoferrin are often used as an adjunct in clinical practice to help guide clinicians. These biomarkers can help predict those that will develop pouchitis and may therefore be useful as a surveillance strategy to enable prompt treatment before symptoms of pouchitis develop.

Despite their potential, no biomarker has been validated for its use in pouchitis, and the studies to date have included small numbers. With the lack of specificity for many biomarkers, thorough investigation of patients with problems with the pouch should include, clinical, biochemical, endoscopic and imaging to rule out other diagnoses that can mimic pouchitis.

The role of biomarkers in pouchitis has potential, with the development of advancing techniques such as metabonomics, metagenomics, metaproteomics and metatranscriptomics, we may be able to find better more sensitive biomarkers to predict and treat pouchitis earlier.

14.1 Introduction

14.1.1 Background to Restorative Proctocolectomy

Restorative proctocolectomy (RPC) is the procedure of choice in patients with ulcerative colitis (UC) refractory to medical therapy and in some patients with familial adenomatous polyposis (FAP). The operation was first performed by Parks and Nicholls in 1976 [1] and is suitable for

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patients with UC and FAP even when mucosal dysplasia is present. It improves quality of life [2], reduces the risk of colorectal cancer and avoids the need for a stoma bag.

Although many patients have good long-term intestinal function, some experience a variety of complications. Incidence of complications following RPC varies between 21% and 52% [3–7]. Pouchitis is one of these complications and is considered a nonspecific inflammatory condition in the ileal pouch reservoir [8]. Pouchitis almost exclusively occurs in patients with underlying UC and is rarely seen in patients with FAP [9, 10]. The pathophysiology of pouchitis is still poorly understood but is thought to involve a genetic susceptibility to inflammation, change in microbiota and the immune system [11].

14.1.2 Pouchitis

The incidence of pouchitis is 20% at 1 year and up to 40% at 5 years [12]. This syndrome is clinically characterised by variable symptoms including increased stool frequency and fluidity, haematochezia, abdominal cramping, urgency and tenesmus, incontinence, fever and extraintestinal manifestations [13].

Risk factors for pouchitis include extensive UC [3, 14, 15], backwash ileitis [14], thrombocytosis [16], primary sclerosing cholangitis [17–19], seropositive perinuclear antineutrophil cytoplasmic antibodies (pANCA) [20], non-smoking status [15, 21] and use of nonsteroidal anti-inflammatory drugs (NSAID) [15, 21]. Other risk factors include genetic polymorphisms including the IL-1 receptor antagonist [22–24], NOD2/CARD15 [25] and noncarrier status of the TNF allele [24].

14.1.3 Diagnosis of Pouchitis

The diagnosis of pouchitis requires both clinical and endoscopic assessment. Currently there are no standardised universally accepted criteria for diagnosing pouchitis, but the 18-point pouch disease activity index (PDAI) is the most commonly used (Table 14.1). It is a score made up of three domains that include clinical, histological and endoscopic data. A score of ≥7 is considered diagnostic for pouchitis.

Table 14.1 Pouch Disease Activity Index (PDAI) Sandborn et al. [26]

Criteria	Score
Clinical	
Stool frequency	
Usual postoperative stool frequency	0
1–2 stools/day > postoperative usual	1
3 or more stools/day > postoperative usual	2
Rectal bleeding	
None or rare	0
Present daily	1
Fecal urgency or abdominal cramps	
None	0
Occasional	1
Usual	2
Fever (temperature >37.8 °C)	
Absent	0
Present	1
Endoscopic inflammation	
Edema	1
Granularity	1
Friability	1
Loss of vascular pattern	1
Mucous exudates	1
Ulceration	1
Acute histologic inflammation	
Polymorphic nuclear leukocyte infiltration	
Mild	1
Moderate + crypt abscess	2
Severe + crypt abscess	3
Ulceration per low-power field (mean)	
>25%	1
25–50%	2
>50%	3

14.1.4 Treatment of Pouchitis

The treatment of acute pouchitis is largely empirical with antibiotics. Ciprofloxacin and metronidazole are the most common antibiotics used with often a rapid dramatic response [27–30]. Ten to 15% of patients with pouchitis experience chronic pouchitis [31, 32] which is poorly defined but considered when symptoms persist despite 4 weeks of antibiotics or three attacks of acute

pouchitis within a year. Overall remission rates for chronic pouchitis is 70% [33] using a variety of treatments which include antibiotics [28, 34, 35], steroids [36, 37] and biologics [38–40].

14.2 The Role of Biomarkers in Pouchitis

The ideal biomarker for pouchitis should ideally be noninvasive, convenient for the patient, rapid, inexpensive, and reproducible with good sensitivity and specificity, both responsive to changes in inflammation with defined cut-offs and ranges as suggested by Sands et al. [41]. Biomarkers to determine pouchitis would ideally also be predictive of pouchitis, whilst also being useful as a tool for predicting response and side effects to treatment.

14.3 Biomarkers in Pouchitis

14.3.1 C-Reactive Protein

C-reactive protein (CRP) is a protein-based biomarker found in blood plasma which is synthesised by the liver [42], and its role is to bind to lysophosphatidylcholine which is expressed on the surface of dead or dying cells in order to activate the complement system via the C1Q complex [43]. CRP level therefore rises in response to inflammation.

Lu et al. [44] recorded CRP levels in 83 pouch patients. These included patients with a normal pouch (n = 7), active pouchitis (n = 6), chronic antibiotic-refractory pouchitis (n = 18), Crohn's disease of the pouch (n = 23), cuffitis (n = 13) (inflammation of the retained rectum), irritable pouch syndrome (n = 10) and surgery-related complications (n = 11). They found that the CRP significantly correlated with PDAI endoscopy subscores of the pouch body (P = 0.006) and afferent limb (P = 0.03). They suggested that a CRP cut-off of 0.7 g/dL (7 mg/L) gave a sensitivity of 69.7% and specificity of 63.6% for differentiating inflammation from those pouches that were not inflamed.

14.3.2 Alpha-1 Antitrypsin

Human alpha-1 antitrypsin (AAT) is a 52-kDa acute phase protein synthesised primarily by the liver but also by neutrophils, monocytes and macrophages [45]. It is made by Paneth cells in the small bowel epithelium and in colonic metaplasia. It has a protective role and acts as a serine protease inhibitor protecting the damage done by inflammatory proteases [46, 47].

14.3.2.1 Faecal Alpha-1 Antitrypsin

Boerr et al. [48] measured faecal AAT in 33 pouch patients who had undergone RPC for UC. They reported, in those patients with active pouchitis, that there was a threefold higher mean faecal AAT concentration than patients in remission and in those who never had pouchitis. They did not report a specific cut-off but suggested that faecal AAT measurements were 80% sensitive and 97% specific for active pouchitis. They also found that the AAT measurement correlated with histological scoring whilst suggesting that faecal AAT can determine both the presence and severity of pouchitis.

In contrast Parsi et al. [49] reported faecal AAT was unable to differentiate between, pouchitis, cuffitis, irritable pouch syndrome and patients with asymptomatic pouchitis. They evaluated 49 patients including 20 with pouchitis, 3 with cuffitis, 2 with Crohn's disease, 11 with irritable pouch syndrome and 13 asymptomatic patients. They found that faecal AAT concentrations did not differ between the subjects with inflammatory phenotypes and those without inflammation. Taken together it remains unclear as to what role faecal AAT has in determining presence of pouchitis.

14.3.2.2 Serum Alpha-1 Antitrypsin

Matolon et al. [50] compared serum AAT levels in 71 UC pouch patients which included 19 normal pouches, 30 with acute or recurrent pouchitis and 22 with chronic pouchitis. There were 10 FAP and 26 normal subjects for controls. They found that in those with established pouchitis (PDAI ≥ 7), their median serum levels of AAT were higher: 183.0 (155.1–232.0) mg/dL versus

those without inflammation 167.6 (151.0–181.0) mg/dL, $P = 0.03$ [50]. They also found that serum AAT levels correlated with both the CRP and calprotectin levels [50]. They reported that a serum AAT cut-off level of 189 mg/dL had a sensitivity of 55.6% and a specificity of 100% for pouchitis [50]. With the low sensitivity, they concluded that serum AAT was unable to differentiate between patients with and without pouchitis.

14.3.3 Faecal Lactoferrin

Lactoferrin is an iron-binding protein that is found mainly in external secretions such as breast milk and in polymorphonuclear white cells [51–53]. Lactoferrin helps protect humans against enteric pathogens and contributes to the antimicrobial properties of neutrophils [54]. Lactoferrin is released when polymorphonuclear white cells are activated and in the faeces when there is a high degree of neutrophil flux into the gastrointestinal tract [55]. Therefore, the degree of inflammation within the gastrointestinal tract will correlate with lactoferrin release.

Parsi et al. [49] studied faecal lactoferrin in 17 asymptomatic subjects with normal pouch endoscopy and histology, 13 patients with irritable pouch syndrome, 23 with pouchitis, 3 with cuffitis and 4 with Crohn's disease. They found that faecal lactoferrin concentrations were significantly higher in those with inflammation of the pouch (median, 176.0 μg/mL; interquartile range [IQR], 79.0–450.8) compared with those with irritable pouch syndrome (median, 3.1 μg/mL; IQR, 0.9–6.4) or asymptomatic subjects (median, 7.8 μg/mL; IQR, 1.4–12.9, $P < 0.001$). They reported that faecal lactoferrin, at the cut-off level of 13 μg/mL, had a sensitivity of 97% and specificity of 92% that could distinguish patients with an inflamed pouch from a non-inflamed pouch. They also reported significant correlations between faecal lactoferrin and PDAI.

Lim et al. [56] assessed the use of faecal lactoferrin using the IBD EZ VUE™ assay. They evaluated its use in 32 patients (21 healthy and 11 inflamed pouches). Whilst no cut-off was reported, they found the test to have a sensitivity of 100% and a specificity of 86% in diagnosing pouchitis. The same group published on a larger series using faecal lactoferrin as a marker of pouch inflammation. They followed up 85 patients of which 24 patients had pouchitis. They found a sensitivity of 100% and specificity of 92% for pouchitis. Furthermore, they found that the test was able to accurately predict the resolution and/or persistence of pouchitis in those treated with antibiotics [57].

Yanamoto et al. [58] studied faecal lactoferrin in 60 patients with ulcerative colitis who had undergone RPC. They found that faecal lactoferrin could help predict those who developed pouchitis 2 months before clinical symptoms. A cut-off value of 50 μg/g for lactoferrin had a sensitivity of 90% and a specificity of 86%. At the time of endoscopy, the median lactoferrin levels were significantly higher in patients with pouchitis than those without pouchitis.

In summary lactoferrin can be used to distinguish between the inflamed and non-inflamed pouch, assess the degree of inflammation of the pouch and monitor response to antibiotics and as a predictor of subsequent episodes of pouchitis.

14.3.4 Faecal Calprotectin

Faecal calprotectin (FC) is a major protein in neutrophilic granulocytes and macrophages. It is found in abundance in neutrophilic granulocytes, in which it accounts for 60% of the cytosolic fraction, as well as in monocytes and macrophages [59, 60]. It has been shown that the concentration of calprotectin is directly proportional to the intensity of the neutrophilic infiltrate in the gut mucosa [61].

Pakarinen et al. studied FC levels in 32 patients with paediatric onset of UC who underwent RPC. They found that patients with recurrent pouchitis had significantly higher FC levels (832 ± 422 μg/g) compared to those with no history of pouchitis (71 ± 50 μg/g) ($P = 0.019$). They also found that FC levels correlated with numbers of neutrophilic infiltration of the distal ileum at histology. They found that a FC cut-off of

300 ug/g gave a sensitivity of 57% and a specificity of 92%.

Johnson et al. [62] studied FC in 46 ulcerative colitis patients and 8 FAP patients who underwent RPC. They found a strong correlation between the FC concentration and the endoscopic score [r = 0.605 (0.396–0.755); two-tailed P <=0.0001]. They found that a FC cut-off to 92.5 μg/g gave a sensitivity of 90% and a specificity of 76.5%.

Yanamoto et al. [58] studied FC consecutively in 60 patients with ulcerative colitis who had undergone RPC. They took stool samples every 2 months for 12 months. In patients who developed pouchitis, they found that FC levels were elevated in the preceding 2 months before diagnosis. They found a FC cut-off value of 56 μg/g had a sensitivity of 100% and a specificity of 84% that could differentiate pouchitis from non-inflamed pouches.

Thomas et al. looked at the correlation between FC and histological findings by reporting on 8 patients with FAP and 16 UC who had undergone RPC. They found that an inflamed pouch had a significantly increased first-morning stool calprotectin concentration compared with non-inflamed pouch patients (P = 0.0002) [63]. Despite the authors suggesting that FC had both good sensitivity and specificity in distinguishing between an inflamed pouch and a non-inflamed pouch, no values for these figures were suggested.

The varying cut-off for FC identified by each of these studies indicates that no ideal cut-off for distinguishing pouchitis has as yet been identified. These data therefore confirm the large interindividual variability in FC within pouchitis that is often observed with FC in other settings in IBD.

14.3.5 Faecal Matrix Metalloprotease

Matrix Metalloproteases (MMPs) are a family of zinc-dependent endopeptidases responsible for both physiological and pathophysiological tissue remodelling. MMP-9 expression is observed primarily in leukocytes, including monocytes, macrophages and neutrophils [64]. It is highly induced in response to chemokines and cytokines, and enhanced expression has been linked to a variety of inflammatory pathologies, including IBD [65].

14.3.5.1 Faecal Matrix Metalloprotease-9

Farkas et al. [66] prospectively studied levels of faecal MMP-9 in 34 patients who underwent RPC for UC; 17 (50%) of these patients had pouchitis using the pouch disease activity index. When levels of faecal MMP-9 were compared between those with pouchitis and those without pouchitis, it was found that the median faecal MMP-9 level was significantly higher in patients with (16.9 ng/ml) versus without (1.34 ng/ml) pouchitis (p = 0.004). Using these values, they found a negative predictive value of 90% and a positive predictive value of 89%. The study also found statistically significant correlations between the faecal MMP-9 concentration and the severity of pouchitis (r = 0.526, p = 0.017). Ulisse et al. supported this finding that MMP-9 activity was found in an inflamed pouch and was reduced following treatment with antibiotics [67].

14.3.5.2 Matrix Metalloproteases 1 and 2

Stallmach et al. studied Metalloproteases 1 and 2. Biopsies were taken from 33 patients with a pouch (UC, n = 25; FAP, n = 8) and from 10 UC patients. They found that in pouchitis (n = 11), MMP-1 and MMP-2 concentrations were increased when compared with non-inflamed pouches of patients. They found the mean MMP-1 levels in inflamed pouches were 17.7 ng/mg protein v 7.8 (UC)v 7.6 (FAP), $p \leqslant 0.05$, and mean MMP-2 levels in inflamed pouches were 16.4v 9.5 (UC) v 6.3 (FAP), $p \leqslant 0.05$). The authors also found that levels of MMP-1 and MMP-2 in patients with pouchitis decreased following antibiotic treatment [68].

14.3.5.3 Matrix Metalloproteases 3 and 7

Makitalo et al. analysed MMP levels in biopsies taken from 28 patients with paediatric-onset UC: 9 had not experienced pouchitis, whereas 13 reported a single episode, and 6 had recurrent pouchitis (≥4 episodes). They found that most pouch samples showed an increased expression of MMP-3 and MMP-7 in pouchitis [69] but that MMP levels could not differentiate between those that had pouchitis and those who did not.

The mixed results associated with the MMPs suggest that the MMP-subtype is important in distinguishing presence or absence of pouchitis and further work is required to establish their role in pouchitis before they can be applied to clinical practice.

14.3.6 Faecal Pyruvate

Pyruvate kinase (PK) plays an important role in the glycolytic pathway. It is expressed by all living cells. The PKM gene produces two major alternatively spliced isoforms, an active form called PKM1 and PKM2, which can switch between an active tetrameric and an inactive dimer form. PKM2 is highly upregulated in cancer cells, and the dynamic tuning of its activity causes the transition from aerobic respiration to glycolysis [70].

PKM2 catalyses the transfer of a phosphate group from phosphoenolpyruvate (PEP) to adenosine diphosphate (ADP), yielding one molecule of pyruvate and one molecule of ATP [71]. At times of increased cell turnover, there is an upregulation in glycolytic enzymes [71].

Johnson et al. [72] analysed faecal samples from 54 patients who had undergone RPC for UC (46 patients) and FAP (8 patients). They found that there were statistically significant differences when comparing the faecal PK concentrations of non-inflamed and inflamed pouches as defined by an endoscopic score of ≤2 and ≥3, respectively ($P < 0.0001$). They also reported a strong correlation between the PK concentration and the endoscopic score [$r = 0.56$ (0.34–0.72), $P < 0.0001$]. When using a cut-off value of 3.7 U/ml, they reported a sensitivity of 73% and specificity of 74%.

Walkowiak et al. [73] also looked at PK in 27 patients with restorative proctocolectomy (18 UC and 9 FAP). They further supported that furthermore, faecal PK concentrations were higher in pouch patients with PDAI ≥ 7 (211.2 ± 31.7 versus 9.7 ± 3.3 U/ml, $p < 0.00001$) but did not provide specific cut-off values that could help distinguish between pouchitis and non-inflamed pouches.

These data suggest that faecal PK may be of benefit as a biomarker that is able to distinguish the inflamed from the non-inflamed pouch, but further work is required to establish a cut-off.

14.3.7 Glycoprotein 2

Glycoprotein 2 is a gene that encodes an integral membrane protein that is secreted from intracellular zymogen granules and associates with the plasma membrane via glycosylphosphatidylinositol (GPI) linkage [74].

Werner et al. analysed 42 patients who had undergone RPC including a normal pouch ($N = 10$), recurrent acute pouchitis ($N = 13$), chronic pouchitis ($N = 13$) and FAP ($N = 5$). They found that anti-GP2 was elevated in both serum and faecal samples of patients with inflamed compared to those with non-inflamed pouches ($p < 0.05$, respectively). Furthermore, GP2 itself was more abundant in the mucosa of patients with chronic pouchitis [75]. Despite these findings, a suggested cut-off value with sensitivities and specificities for its use as a biomarker was not reported.

14.3.8 Microbiota as a Predictor of Pouchitis

It has been demonstrated that imbalance or dysbiosis of the natural gut microbiota is associated with inflammation [76], with a decrease in bacterial diversity, or richness, being the most consistent finding in relation to disease activity [77–80]. Specifically, key changes have been identified in

inflammatory bowel disease (IBD) to include a reduction in *Faecalibacterium prausnitzii* [81] and increase in Enterobacteriaceae [82, 83]. Difficulties however remain regarding unpicking whether dysbiosis is cause or effect of the inflammation found in IBD. The responsiveness of pouchitis to antibiotics suggests that the gut microbiota plays a key role in the development of pouchitis and therefore may have a potential role as a biomarker.

In an analysis of patients with UC prior to ileoanal pouch formation, it was found that a predominance of *Ruminococcus gnavus*, *Bacteroides vulgatus* and *Clostridium perfringens* and absence of *Blautia* and *Roseburia* organisms can be predictive of pouchitis [84]. The latter study was the first to suggest that certain patterns in the microbiota can predict those who get pouchitis and those that do not. Building further on this concept of the microbiota as a biomarker, Reshef et al. highlighted that *Bacteroides*, *Faecalibacterium*, *Roseburia*, *Coprococcus* and *Ruminococcus* were negatively correlated with the PDAI suggesting that these microbiota could be associated with severity of pouch inflammation [85] and therefore in the future form the basis of biomarkers.

14.3.9 Cytokines

Studies have highlighted elevated production of pro-inflammatory cytokines such as TNF-alpha, interferon-gamma, IL-1, IL-6 and the chemokine IL-8 in pouchitis relative to normal pouches [86–88, 67]; however, their use as biomarkers has yet to be fully established.

14.4 Biomarkers for Pouchitis Summary

Biomarker	Biofluid used	Cut-off	Sensitivity (%)	Specificity (%)	Used in clinical practice
CRP	Serum	0.7 g/dL	69.7	63.6	Yes
Alpha-1 antitrypsin	Faeces	189 mg/dL	55.6	100	No
Lactoferrin	Faeces	13–50ug/g	90–97	86–92	Yes
Calprotectin	Faeces	56–300 μg/g	90–100	67–84	Yes
Pyruvate	Faeces	3.7 U/ml	73	74	No

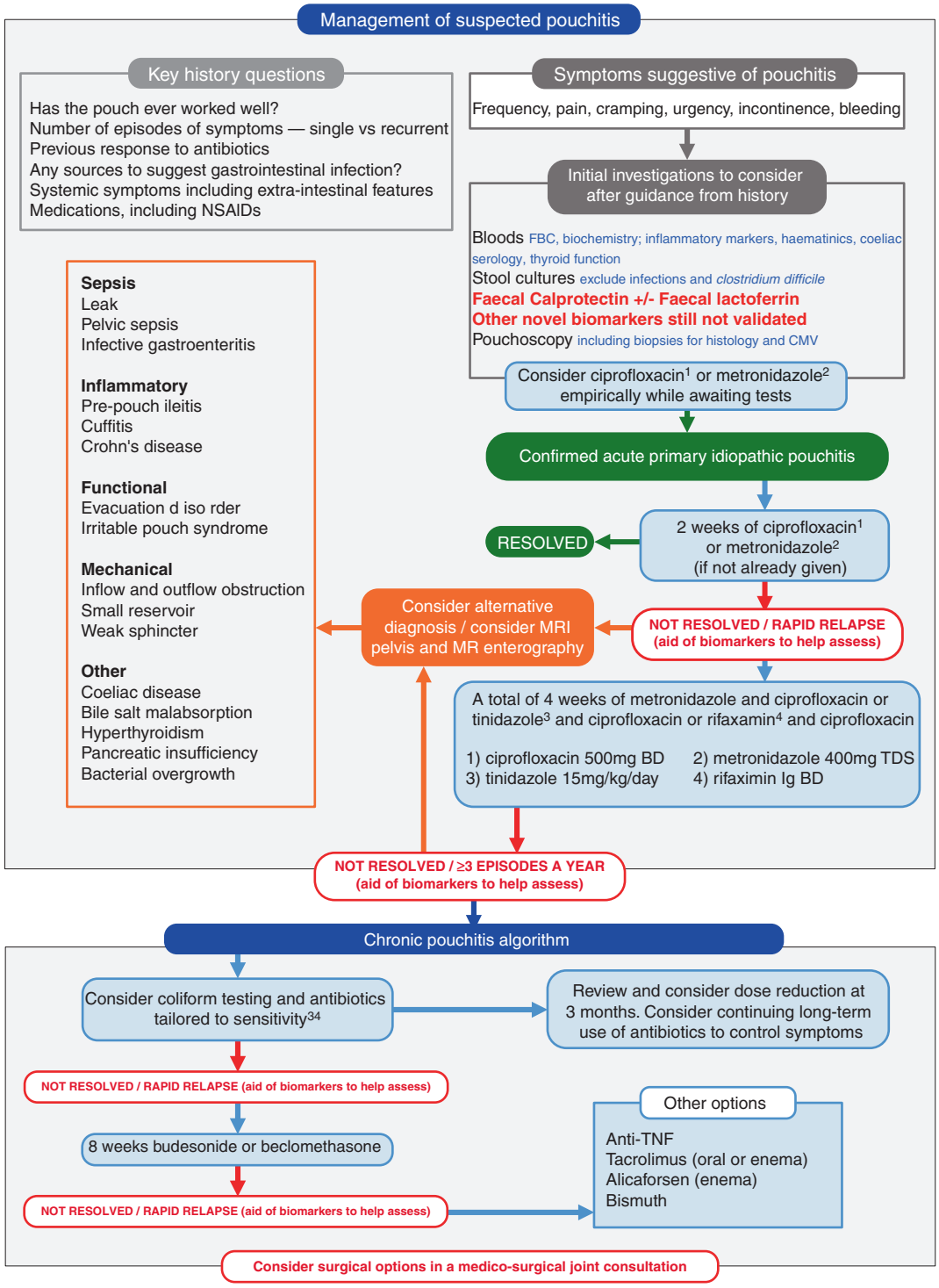
14.5 Conclusion

As yet no biomarker has been validated for its use in pouchitis, and the studies to date have included small numbers. With the lack of specificity for many biomarkers, thorough investigation of patients with problems with the pouch should include clinical, biochemical, endoscopic and imaging to rule out other diagnoses that can mimic pouchitis.

Nonetheless biomarkers may in the future be used as adjuncts to help with the diagnosis of pouchitis. Faecal calprotectin and faecal lactoferrin are highly sensitive tests with reasonable specificity. Faecal lactoferrin and faecal calprotectin can be used to predict those that will develop pouchitis and may therefore be useful as a surveillance strategy to enable prompt treatment before symptoms of pouchitis develop.

As yet the use of other biomarkers lacks the required sensitivity and specificity to be used in clinical practice. However, with the development of advancing techniques such as metabonomics, metagenomics, metaproteomics and metatranscriptomics, we may be able to find better more sensitive biomarkers to predict and treat pouchitis earlier.

14.6 Below Is a Suggested Investigation and Treatment Algorithm by Segal et al. [33]



Summary Points

- Pouchitis can cause significant morbidity to patients.
- If we can predict those patients that will develop pouchitis, this will enable prompt treatment and often relief of symptoms.
- Currently there are no validated biomarkers for pouchitis.
- CRP, faecal calprotectin and faecal lactoferrin are used in clinical practice as an adjunct to both assess degree of pouchitis and potentially predict pouchitis, but this remains unvalidated.
- Advancing techniques such as metabonomics, metagenomics, metaproteomics and metatranscriptomics may help provide new biomarkers in the future.

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Part IV

Clinical Algorithms Incorporating Predictive and Prognostic Biomarkers: Specialised Scenarios

15 Extra-intestinal Manifestations in Inflammatory Bowel Disease: Diagnosis and Management

Ramesh Paramsothy and Peter Irving

Abstract

Inflammatory bowel disease is a systemic disorder that not only affects the gastrointestinal tract but can also be associated with extra-intestinal manifestations involving multiple organs. These most commonly include articular (axial and appendicular), dermatologic or ophthalmic involvement but can also more rarely include the renal and pulmonary systems. The clinical course can either mirror intestinal disease activity or be independent of it. Currently there is a need for effective biomarkers in this area as there are none available. While biomarkers are used for related rheumatological conditions, they are not effective as diagnostic or prognostic markers for extra-intestinal manifestations of IBD. Management of extra-intestinal manifestations may involve treating the underlying condition with some resolving as the bowel inflammation improves. Others may require treatment aimed specifically at the extra-intestinal manifestation. In refractory cases the best evidence tends to be for steroids and anti-TNF agents although other biologics and immunosuppressants may also play a role.

15.1 Introduction

Ulcerative colitis (UC) and Crohn's disease, while predominantly enteric conditions, are nevertheless systemic disorders that can involve regions outside the gastrointestinal tract. Inflammatory bowel disease (IBD)-driven symptoms involving other organ systems are referred to as extra-intestinal manifestations (EIM). The most common sites involved are the joints, skin and eyes, although other organs systems can also be involved including the renal tract and respiratory system. The frequency of EIM reported in the literature ranges from 6% [1] up to 43% [2] and tends to be more prevalent in Crohn's disease [2]. EIM can arise prior to the development of gastrointestinal symptoms and diagnosis of IBD [3]. In addition, the development of one EIM has been shown to increase the risk of a second system being involved [4, 5]. The relationship between the activity of EIM and that of the underlying disease varies, with some mirroring IBD activity while others run an independent course. In this chapter we will discuss the diagnosis of the common EIM, the data regarding biomarkers where it is available and the current therapeutic options. This chapter does not address PSC (see

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Chap. 16) nor extra-intestinal complications that can arise from treatments.

15.2 Arthropathy

Enteropathic arthropathies are the most common extra-intestinal manifestation of IBD [2]. They consist of both peripheral arthritis and axial disease.

15.2.1 Peripheral Arthritis

Peripheral arthritis has been reported to occur in 10–20% of IBD patients [6]. It is often considered in terms of two subdivisions – pauciarthritis (Type 1) and polyarthritis (Type 2). Pauciarthritis tends to involve fewer than five joints and has an asymmetrical distribution [7]. Larger joints, especially of the lower limbs, tend to be involved [8]. Polyarthritis affects multiple joints (>5) with the smaller joints being predominantly affected in a symmetrical distribution [7].

The diagnosis of enteropathic arthritis is made on clinical grounds, although imaging can be particularly helpful to identify destructive changes. Serological markers like rheumatoid factor and anti-cyclic citrullinated peptide (anti-CCP) have a role in rheumatoid arthritis and many other inflammatory arthropathies. In enteropathic arthritis, however, they do not distinguish between IBD patients with or without the condition [9]. Some studies have postulated potential genetic markers, with HLA class II allele DRB1*0103 shown to predispose to Type 1 arthritis and HLA-B44 being strongly associated with Type 2 arthritis [10], though this requires further evaluation.

Type 1 arthritis generally mirrors intestinal activity and presents with acute self-limiting episodes [7]. Treatment of the underlying IBD is the mainstay of treatment alongside analgesia [11]. Intra-articular steroid injections can be used in severe cases. For Type 2 arthritis, longer-term treatment is usually required with sulfasalazine, often used first line [11, 12]. Immunomodulators like methotrexate and to a lesser extent azathioprine have some efficacy for peripheral arthritis [12]. Anti-TNF therapy is the mainstay of treatment for severe disease, with demonstrated efficacy for the management of enteropathic arthritis in a systematic review [13]. Vedolizumab has been shown to have an effect on arthritis in some studies despite having a gut-specific mechanism of action with 45% of patients having complete remission of arthritis at 54 weeks in a French open-label cohort [14]. This effect was associated with clinical remission of IBD (OR 1.89) and institution of therapy within 3.5 months of arthritic symptoms (OR 1.99) [14].

15.2.2 Axial Arthritis

Axial involvement is less common than peripheral arthritis and often runs independent of intestinal IBD activity [15]. It can be further subdivided into sacroileitis and spondylitis with prevalence rates of up to 20% and 12%, respectively, in IBD [15].

Sacroileitis is often asymptomatic, and most patients do not develop ankylosing spondylitis. These patients are predominantly HLA-B27 negative. Imaging with X-ray or magnetic resonance imaging shows sclerosis with those who demonstrate bilateral disease having an increased likelihood of developing progressive disease [8].

Ankylosing spondylitis has a higher prevalence amongst IBD patients than the general population [16]. It is characterised by morning stiffness and pain exacerbated by rest and is progressive with vertebral fusion and loss of mobility. HLA-B27 is a key genetic marker [17], and although the majority of IBD patients (50–80%) with spondylitis are HLA-B27 positive, this is lower than the 94% seen in idiopathic ankylosing spondylitis [16].

The management of axial arthropathy associated with IBD involves non-pharmacological as well as pharmacological interventions. Intensive physiotherapy and exercise are of benefit in patients with ankylosing spondylitis [18], and while NSAIDS are first-line therapy for axial disease, their benefits need to be weighed against the risk of exacerbating IBD. Low-dose NSAIDs (aspirin <= 325 mg/day, ibuprofen <=200 mg/day,

naproxen <=220 mg/day) have been demonstrated not to increase the risk of IBD flares [19]. While immunomodulators have limited efficacy in axial disease [17, 18], anti-TNF therapies are the treatment of choice in patients refractory to or intolerant of NSAIDs [20]. Limited evidence suggests that vedolizumab may have similar efficacy in axial disease as in peripheral arthritis [14].

15.3 Skin

The two main skin manifestations in IBD are erythema nodosum and pyoderma gangrenosum. Both conditions are diagnosed clinically with biopsies predominantly used to exclude differential diagnoses [21]. Given the directly accessible and observable nature of skin lesions, there is no role for biomarkers in the diagnosis and monitoring of the cutaneous manifestations of IBD. In terms of potential prognostic markers, dendritic cells with expression of skin-homing markers may have a role in cutaneous EIM as well as being associated with more severe Crohn's disease [22].

15.3.1 Erythema Nodosum

Erythema nodosum (EN) occurs in approximately 4% of IBD patients [6] and usually presents acutely with raised, tender, red or violet nodules. The nodules are warm on palpation and occur most commonly on the extensor surface of the lower extremities [21]. EN is more prevalent in patients with Crohn's disease than UC, especially those with isolated colonic involvement (OR 4.7) [23]. It is associated with female gender patients with a history of pyoderma gangrenosum and a history of eye or joint involvement [23].

Erythema nodosum parallels bowel disease activity and typically responds to treatment of the underlying IBD. Supportive measures include rest, leg elevation, compression stockings and simple analgesia. More severe cases require exclusion of other diagnoses like sarcoidosis, Behcet's disease or infection. Anti-TNF agents are effective in treating erythema nodosum [13], though are rarely started solely for this purpose [24].

15.3.2 Pyoderma Gangrenosum

Pyoderma gangrenosum (PG) is a debilitating but relatively rare EIM having a prevalence of 0.75% in IBD [23]. It is often preceded by trauma and typically commences as a nodule that develops into a burrowing ulcer with irregular edges. Deep ulcers can contain purulent material that is sterile on culture [25]. It can be solitary or multiple, unilateral or bilateral and vary in size. While it can occur anywhere on the body, PG occurs of equal frequency in Crohn's disease and UC and is associated with more complex disease [23].

There is no correlation between PG and intestinal disease activity. Multidisciplinary input is often required with wound care specialist and stoma nurse involvement. Wound care includes barrier creams and moisture retentive dressings [21]. Surgical intervention tends to worsen lesions, and debridement should be avoided. Of the medical therapies, infliximab has the strongest evidence of efficacy; in a randomised controlled trial in IBD patients with PG, 67% responded and 22% went into remission [26]. Other therapies include oral prednisone, azathioprine, cyclosporine and tacrolimus although the evidence for their efficacy is weaker and infliximab is generally preferred if there is no response to oral steroids [20]. Case reports suggest ustekinumab may be effective for treating PG [27, 28] although higher doses may be required for severe lesions [29]. There are limited data regarding vedolizumab and cutaneous extra-intestinal manifestations. In one study a single case of PG did not respond to vedolizumab, while one of the two cases of erythema nodosum had complete remission [24].

15.4 Ocular

The major ocular extra-intestinal manifestations are episcleritis, scleritis and uveitis with a combined prevalence in IBD of about 4% [30].

Episcleritis is the most common ocular EIM and is due to engorgement of vessels within the episclera, which lies below the conjunctiva [31]. This results in erythema that involves just a portion of the eye [31] with associated mild-moderate tenderness. There is no associated visual change or photophobia. Scleritis is rarer and involves the deeper scleral vessels. This presents with a purple sclera, and pain is a more prominent feature and of greater severity [25]. Patients with possible scleritis should be referred to an ophthalmologist urgently as they can develop scleral thinning and visual deficits. A cotton-tip applicator and topical phenylephrine help distinguish scleritis from episcleritis [31, 32]; the vessels in the episclera are more mobile when using a cotton-tip applicator unlike those in the sclera, and in patients with episcleritis, topical phenylephrine will result in blanching which does not occur in scleritis.

Uveitis involves the iris and most commonly affects the anterior chamber [32]. Patients develop redness, photophobia and pain, and left untreated the condition can progress to blindness. If there is any suspicion of this diagnosis, an urgent ophthalmology referral is required for prompt diagnosis using a slit lamp [33].

Patients with other EIM are more likely to have ocular involvement (OR 4.8) with the risk increasing with multiple EIMs (OR 14.7) [30]. The conditions with the strongest associations with ocular EIM are peripheral arthritis for Crohn's disease and PG for UC. Neither definite genetic nor serological markers have been found to associate with ocular EIM, although some genetic markers of interest have been identified [30]. In addition, acute anterior uveitis has an association with ankylosing spondylitis and inflammatory bowel disease amongst HLA-B27-positive patients [34].

Episcleritis tends to mirror intestinal disease activity and resolves with treatment of the underlying IBD. If required, treatment tends to be topical with artificial tears and cold compresses [35]. Topical steroids can be applied if these measures are insufficient, and oral steroids may be used in refractory cases [32]. Scleritis can parallel IBD activity but is also seen in quiescent disease. Due to the risk of complications, it requires more aggressive treatment. NSAIDs are effective, but their use needs to be balanced against the risk of exacerbating the underlying bowel disease. Oral steroids are often used alongside azathioprine or methotrexate, while rituximab and mycophenolate are also effective for scleritis [32]. There is a scarcity of data on anti-TNF therapy for episcleritis or scleritis [33].

Uveitis runs an independent course to intestinal activity. Sulfasalazine has been described as having a role in preventing relapses [36]. Flares tend to respond to topical or oral steroids. Infliximab is generally effective for acute anterior uveitis flares refractory to steroids [37], while adalimumab has been shown to reduce uveitis attacks in patients with ankylosing spondylitis [38]. Anti-TNF agents have been approved for intermediate and posterior uveitis that is refractory to steroids [36]. There is a lack of data on the effectiveness of anti-integrin or anti-IL12/23 inhibitors in the treatment of uveitis.

15.5 Pulmonary Manifestations

Pulmonary disease is a relatively uncommon extra-intestinal IBD manifestation though the true prevalence may be underreported as 40% of IBD patients in one study had abnormal pulmonary function tests compared to <5% in control patients [39]. Pulmonary disease is more prevalent in female IBD patients and tends to occur in patients with other EIM [40]. There is a wide range of pulmonary manifestations associated with IBD, which can be divided anatomically into airway and interstitial disease. Airway disease is predominantly bronchiectasis and chronic bronchitis with the upper airways being less commonly affected [40]. Interstitial disease includes cryptogenic organising pneumonia. It can be difficult to distinguish extra-intestinal manifestations of IBD from drug-induced pulmonary disease due to agents like methotrexate.

Diagnosis of pulmonary manifestations of IBD is generally through a combination of imaging and exclusion of other, more common infec-

tive and drug-induced causes. High-resolution CT is the preferred modality and is able to identify small airway disease [8]. When performed, lung biopsy can show non-specific inflammation, fibrosis and granulomatous bronchiolitis [16].

Pulmonary manifestations tend to run an independent disease course to bowel activity [41]. In managing pulmonary disease in IBD, potential causative drugs including methotrexate should be ceased. Inhaled steroids tend to be sufficient for large airway involvement [20], while oral steroids may be required if there is parenchymal involvement or resistance to inhaled steroids. Intravenous steroids are generally reserved for severe disease or subglottic involvement [16]. In case reports, infliximab has been shown to be highly effective in Crohn's disease patients with pulmonary manifestations [40, 41].

15.6 Renal Manifestations

Renal sequelae of IBD include nephrolithiasis and rarely amyloidosis. In addition, fistulisation can occur to the renal tract as well as interstitial nephritis secondary to aminosalicylate medications. There are reports of glomerulonephritis in IBD patients, but uncertainty exists as to whether this is coincidental or an extra-intestinal manifestation [16].

Renal stones tend to be oxalate stones although urate stones can be seen. This is postulated to be due to increased oxalate absorption resulting from fat malabsorption causing reduced free calcium [16], although low urinary magnesium and citrate levels may also play a role [42]. Hyperoxaluria tends to be more prevalent in Crohn's disease than ulcerative colitis [42]. Prior gastrointestinal surgery increases the risk of stone development [8]. The treatment is similar to renal stones from other causes, namely, fluids, alkalinisation of urine and supplemental citrate [43].

Amyloidosis is a rare but serious extra-intestinal manifestation of IBD. This is more commonly seen in patients with Crohn's disease, especially ileocolonic disease [44]. Initially it presents with proteinuria that then can progress to nephrotic syndrome and renal failure [43]. It carries a high mortality with 59% of patients dying in one series [44], and there are limited data on treatment options. Surgery does not appear to change the course of amyloidosis. Azathioprine and colchicine potentially delay the progression of amyloidosis [45], and both infliximab [45] and an elemental diet [46] have been reported as being effective in case reports.

15.7 Conclusion

Extra-intestinal manifestations are common in IBD and affect many organ systems. The clinical course varies with some following intestinal disease activity, while others remain independent. Currently there are no useful diagnostic or prognostic biomarkers available for extra-intestinal manifestations. There are however some potential markers on the horizon that require further evaluation before being ready for routine clinical practice. Therapeutic agents used for EIM predominantly overlap with medications for underlying IBD (Table 15.1). Further data are needed to clarify the role and efficacy of the new biologic agents.

Table 15.1 Therapies for extra-intestinal manifestations

	EN	PG	Type 1 arthritis	Type 2 arthritis	Axial disease	Episcleritis	Scleritis	Uveitis
Treat IBD	++		++			++	+	
Immunomodulators	+	+	+	+			+	+
Steroids	+	+	+	+		++	++	++
Anti TNF	++	+++	+	++	++			++
Vedolizumab	+			+	+			
Ustekinumab		+						

Summary Points

- Extra-intestinal manifestations (EIMs) occur frequently in IBD with the most commonly involved sites being the joint, skin and eyes.
- The clinical course of EIM can either mirror the intestinal disease activity or progress independently.
- There are currently no effective biomarkers available that reliably and specifically correlate with EIM from particular organ systems independent of gastrointestinal disease activity.
- Some EIMs are dependent and driven by the underlying bowel disease and resolve upon its control, while others require specific treatment. In refractory cases the best evidence for EIM tends to be for steroids and anti-TNF agents.

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Primary Sclerosing Cholangitis Overlapping with IBD

16

João Sabino, Joren tenHove, and Joana Torres

Abstract

Primary sclerosing cholangitis is a chronic and progressive cholestatic disease, characterized by inflammation and fibrosis of the intrahepatic and/or extrahepatic ducts, that may result in liver cirrhosis and eventually end-stage liver disease. No medical treatment is available, and liver transplantation remains the only curative option, albeit with an elevated recurrence rate. Having a diagnosis of inflammatory bowel disease is the strongest risk factor for PSC development, since 70% of patients with PSC have underlying IBD, most frequently ulcerative colitis. For unknown reasons, the coexistence of PSC with IBD seems to modify the IBD phenotype and disease course. PSC-IBD patients typically have extensive colonic involvement, albeit with mild inflammatory activity and symptoms, rectal sparing, backwash ileitis, and increased risk of developing pouchitis after proctocolectomy. Furthermore, some studies suggest that there may exist an inverse relationship between PSC disease severity and IBD activity. Importantly, patients with PSC-IBD present a very high risk of developing colorectal neoplasia, usually located in the right colon, requiring routine endoscopic surveillance (preferably using chromoendoscopy) every year, starting from the moment PSC is diagnosed.

No specific biomarker for diagnosing PSC exists. For prognostic purposes, the most commonly used important surrogate endpoints are alkaline phosphatase, bilirubin, transient elastography, and histology. No biomarker has proven to be accurate in diagnosing any of PSC's complications such as cholangiocarcinoma or colorectal neoplasia, and therefore surveillance is paramount. The management of the IBD follows the same approach as for patients with IBD alone. Close articulation with a specialized hepatologist is warranted when considering treatment options for PSC and for correct follow-up of the patient.

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16.1 Introduction

Primary sclerosing cholangitis (PSC) is a chronic and progressive cholestatic disease, characterized by inflammation, fibrosis, and stricturing of the intrahepatic and/or extrahepatic biliary ducts [1, 2]. PSC is associated with a significant risk of hepatobiliary and colorectal neoplasia [3–5] and with a considerable morbidity and mortality, representing a significant healthcare burden [6].

In Western countries, the reported incidence of PSC ranges from 0.07 to 1.3 per 10^5/year, and the prevalence ranges around 8.5–13.6 per 100.000 person-years [7, 8]. The major risk factor for developing PSC is having a diagnosis of IBD. Around 71% of PSC patients have concomitant IBD, most frequently ulcerative colitis (UC) in 80% of cases, followed by Crohn's disease in 15.5% of cases and IBD-unclassified in 4.2% [9]. Conversely, in patients with established IBD, PSC is present in 3–8% of all patients with UC and in around 3% of patients with CD [10]. Although both diseases run distinct courses, patients with PSC-IBD present a distinctive phenotype (Table 16.1), offering interesting insights into disease pathogenesis [11].

Table 16.1 Main phenotypic features of PSC-IBD

Summary of clinical features associated with PSC-IBD phenotype
More frequent in UC patients, especially in males [9]
Typically mildly symptomatic or asymptomatic pancolitis or extensive colitis [11, 118, 119]
Rectal sparing and backwash ileitis more frequent
Right-sided inflammation (endoscopic and histological) [35]
Increased risk of developing colorectal neoplasia [64]
Colorectal neoplasia more frequently located in the right colon [64]
Increased risk of pouchitis after proctocolectomy [120]
Increased risk of PSC recurrence after liver transplantation in patients with intact colons at the time of transplantation [54]
Increased risk of LT or hepatobiliary malignancy in PSC-UC patients [9]

16.2 Pathogenesis

Several factors play a role in the pathogenesis of PSC (Fig. 16.1). The available evidence points toward a complex interaction between genetic, immunologic, and environmental factors.

16.2.1 Genetics

Epidemiologic studies provided the first clue of the importance of genetics in PSC. In fact, having a first-degree relative with PSC increases the risk of developing the disease by four-fold [12]. Genome-wide association studies (GWAS) have allowed for the identification of 23 genetic risk loci associated with PSC [13]. The majority of these genetic risk loci play an important role in the immune system, such as the HLA complex, *IL2*, or *PRDX5*, suggesting that PSC may be an immune-mediated disorder. Furthermore, there is some overlap between some genetic risk loci of PSC and IBD. However, the known genetic defects only explain less than 10% of PSC disease liability [14], pinpointing the possible importance of the environment in the pathogenesis of the disease.

16.2.2 Microbiota Interactions

Several studies have now reported on the gut microbiome of patients with PSC [15–17]. These initial studies have described associations between some bacterial genera and the diagnosis of PSC without addressing possible causation mechanisms. It has been hypothesized that bacteria and/or bacterial products translocate through the intestinal mucosa and are transported to the liver, hereby inducing inflammation. In fact, bacteria and fungi are more frequently found in the bile ducts of patients with PSC, as compared to patients with other cholestatic liver diseases [18, 19]. Moreover, addition of metronidazole to ursodeoxycholic acid (UDCA) showed some

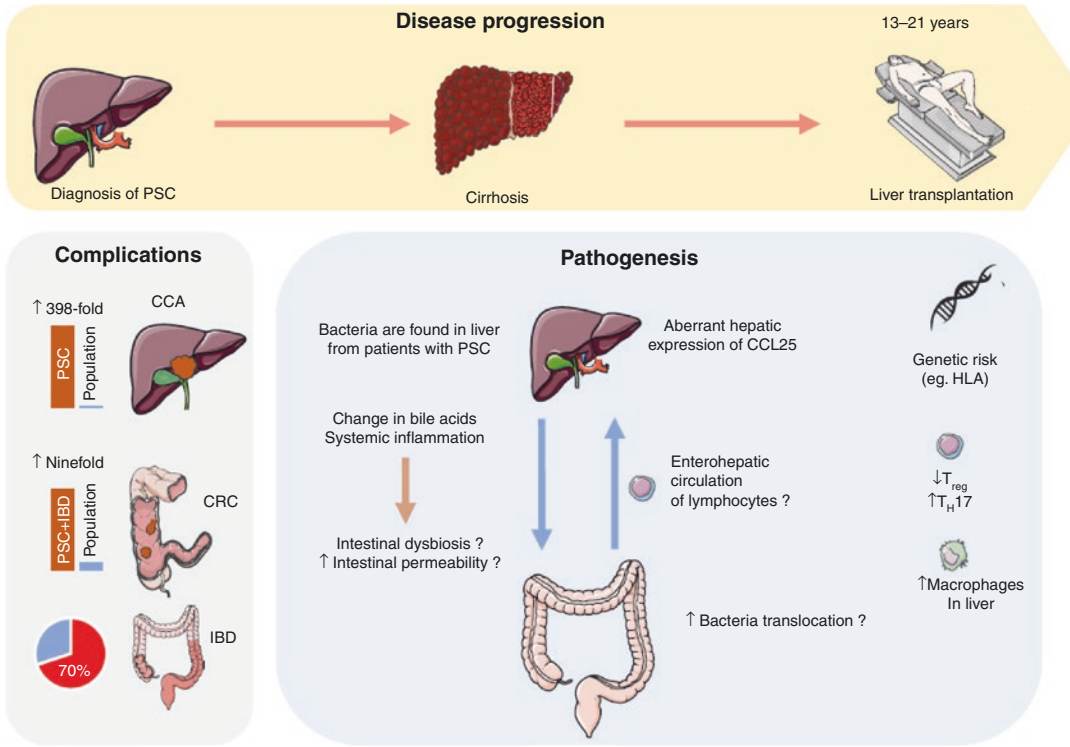

Fig. 16.1 Primary sclerosing cholangitis: disease progression, associated complications and pathogenesis. The *natural disease progression* of PSC is the development of cirrhosis and evolution to end-stage liver disease with the need for liver transplantation. Patients with PSC are at *higher risk of cholangiocarcinoma, colorectal carcinoma (CRC), and IBD*. The *pathogenesis of PSC is unclear*; however, genetic risk factors and immunological disturbances have been reported. Patients with PSC have lower T_{reg} and higher T_H17 activity. Moreover, an aberrant hepatic expression of CCL25, usually associated with the bowel, is present in patients with PSC. In these patients, bacteria and fungi are found in liver explants. Possible changes in bile acid profile and the persistent systemic inflammation might induce intestinal dysbiosis and an increase in intestinal permeability promoting bacterial translocation. CCA cholangiocarcinoma, CRC colorectal carcinoma, IBD inflammatory bowel diseases. (Source: PhD thesis João Sabino, 2017, KU Leuven, Belgium). This figure was made with Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License; https://smart.servier.com

beneficial effects in biochemical test results and liver histology [20]. Multidrug resistance gene 2 knockout ($Mdr2^{-/-}$) mice, a murine model for PSC, exhibited a more severe phenotype when maintained under germ-free conditions [21]. However, NOD.c3c4 mice, a murine model for biliary inflammation, exhibit a less severe phenotype when maintained in germ-free conditions [22]. These contradictory findings probably result from the different murine models used and the different intestinal microbiota of these mice, highlighting the complex interaction between the intestinal microbiota and PSC.

16.2.3 Gut Lymphocyte Homing

Activated T cells expressing CCR9 and α4β7 markers specifically migrate to the gut. This gut-specific homing of lymphocytes relies on the interaction between these markers and the intestinal expression of CCL25 and MADCAM-1 [23]. Interestingly, patients with PSC have an aberrant hepatic expression of CCL25, allowing the migration of gut-homing lymphocytes to the liver [24]. The enterohepatic circulation of lymphocytes may explain the interaction between the colonic immune system triggered by dysbiotic

intestinal microbiota and biliary inflammation. This theory is further supported by the finding of memory T cells with common clonal origin in both the gut and the liver of patients with PSC-IBD [25].

16.2.4 Environment

The impact of the environmental factors on the pathogenesis of PSC is unclear. Smoking has been repeatedly associated with lower risk for PSC [26–28]. Coffee consumption also seems to be associated with a lower risk for PSC [26].

16.3 PSC-IBD: Distinctive Clinical and Phenotypic Features

Both sex and all age groups can be affected, but PSC is more common in men (65.5%), and the mean age of diagnosis is 38.5 ± 15.5 years [9]. Patients with PSC-IBD tend to have a PSC diagnosis at a younger age when compared with PSC-alone controls (mean age 33.6 ± 17.2 years versus 58.9 ± 18.2 years; $p < 0.001$) [29]. Some studies indicate that the mean age for IBD diagnosis is higher among PSC-IBD patients compared with IBD controls [10, 11].

16.3.1 The Impact of the PSC on the IBD

PSC-IBD patients typically have extensive colonic involvement, irrespective of the IBD subtype. In a population-based cohort, pancolitis was observed in 83% of PSC-UC patients [30], although lower rates have also been reported [10]. In PSC-CD colonic involvement is the most often reported (37–82%), followed by ileocolic (22–58%), and rarely isolated ileal involvement (2–5%) [31]. Ulcerative proctitis or Crohn's ileitis is very rarely associated with concomitant PSC [30, 32]. The frequency of rectal sparing ranges from 6% to 66% (versus 2–25% in IBD without PSC), and backwash ileitis has been reported in 5–46% of patients (as compared to 3–24% in UC without PSC) [31].

Despite the higher prevalence of extensive colitis, the intestinal inflammation in PSC-IBD patients is usually quiescent leading to mild symptoms and milder disease course [29, 33]. Typically, the endoscopic and histologic inflammatory activity is highest in the right colon and lowest toward the distal colon [29, 31, 34, 35], and on histopathology, the colonic inflammation is mild [33, 36].

There may exist an inverse relationship between PSC disease severity and IBD activity. PSC-IBD patients with more severe liver disease requiring OLT (orthotopic liver transplantation) have less severe UC, with fewer flares and lower steroids and immunosuppressive requirements [37]. In contrast, those not requiring OLT, and therefore with presumably less aggressive liver disease, showed an increased need for intestinal surgery and more frequent colorectal neoplasia [37]. These data are supported by a recent study where patients with long-standing IBD were screened with magnetic resonance cholangiopancreatography (MRCP) for PSC [38]. Those with subclinical PSC were found to have a higher risk of IBD disease progression (with extensive colitis, persistent symptoms, and even colectomy). Although not universally confirmed, studies have reported that IBD may worsen after OLT in approximately 30% of patients [2, 39–41]. De novo IBD after OLT has also been reported, and it may develop in 14–30% of PSC patients up to 10 years after transplantation [42].

16.3.2 The Impact of IBD on the PSC

The effect of the IBD on the PSC phenotype is less well defined. Combined intrahepatic and extrahepatic biliary involvement has been described to be more common in PSC-IBD patients compared to PSC patients alone (81.5% vs 46.2%, $p < 0.05$) [43], but not universally confirmed [9, 44, 45]. Some studies have suggested that there is an increased prevalence of small-duct PSC in PSC-CD patients as compared to

PSC-UC [46]. PSC-UC is more often associated with large-duct PSC as compared to other phenotypes such as small-duct PSC (sdPSC) or PSC associated with autoimmune hepatitis (AIH) (frequency of UC in patients with classical PSC: 58.1% vs 33.5% in sdPSC and vs 47.7% in PSC/AIH; $p < 0.001$ for both comparisons) [9].

Conflicting data existed on the impact of concomitant IBD on liver-related outcomes [32, 47–49]; however, a recent large multicentric study showed that PSC-UC is associated with a greater risk of progressing to OLT or death by 56% in comparison to PSC-CD and by 15% in comparison to PSC alone. Likewise, patients with PSC-UC had a 45% and 35% higher risk of developing hepatobiliary malignancy as compared to PSC-CD and PSC alone, respectively [9]. It has been postulated that the more benign phenotype of PSC-CD may be explained by the increased prevalence of small-duct PSC; however, in a retrospective study, even large duct PSC-CD patients had less liver-related morbidity and mortality as compared to PSC-UC patients and PSC alone [46, 50].

Patients in whom colectomy occurs before PSC is diagnosed have a lower risk of OLT or death (HR 0.71, 95%CI 0.53–0.95), as opposed to those with colon in situ at the time of PSC diagnosis [51]. Additionally, several publications have suggested that an intact colon at the time of liver transplant is a strong predictor of PSC recurrence in the allograft [52–54], although not universally confirmed [55].

Altogether these data suggest that PSC severity may have a "protective" effect on UC's activity and, on the other hand, that colonic disease may have the opposite effect in the liver disease.

16.4 Diagnosis

PSC may be diagnosed many years after proctocolectomy for colitis, and conversely IBD can appear many years after the initial diagnosis of PSC or even after OLT [33]. Nowadays most patients have their diagnosis made in the setting of asymptomatic altered liver biochemistry, usually with a cholestatic liver pattern; the typical diagnostic hallmarks of fever, itching, and jaundice are rarely seen [2]. Recurrent episodes of bacterial cholangitis can also be a part of the clinical presentation and develop in about 10–15% of patients during the course of the disease [10, 56].

In patients with known IBD and persistent biochemical markers of cholestasis not otherwise explained, the presence of PSC should be excluded by cross-sectional abdominal imaging, preferably MRCP (magnetic resonance cholangiopancreatography). The use of endoscopic retrograde cholangiopancreatography (ERCP) for diagnostic purposes should be reserved only for those in whom MRCP and/or liver biopsy is contraindicated or equivocal [57]. The diagnosis of PSC is based on the findings of diffuse multifocal strictures and dilations in the intrahepatic and/or extrahepatic biliary tree [2] (Fig. 16.2), after exclusion of causes of secondary sclerosing cholangitis [58] (e.g., HIV-induced cholangiopathy, sarcoidosis, etc.). IgG4 disease is an important differential diagnosis; patients with autoimmune pancreatitis type 1 have an

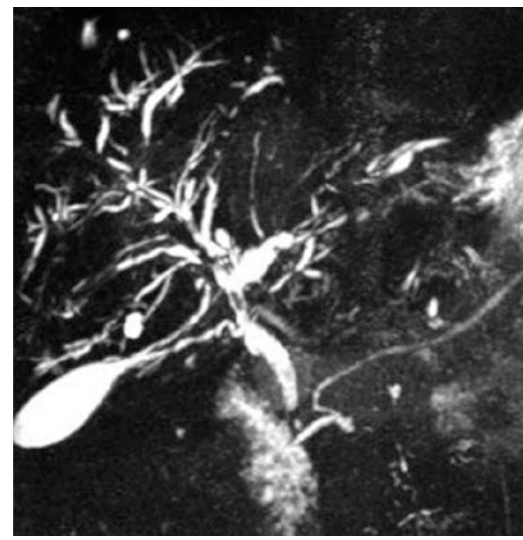

Fig. 16.2 Magnetic resonance cholangiopancreatography depicting the typical findings of primary sclerosing cholangitis. Distortion and ubiquitary dilation of the intrahepatic biliary tree are seen; multiple segmental strictures are intercalated with segments of normal caliber or slightly dilated (beading). (Courtesy of Afonso Gonçalves, MD)

increased prevalence of IBD and may have evidence of sclerosing cholangitis associated with IgG4; however clinical course and management is very different [58]. Around 10% of PSC patients may present elevated IgG4 serum levels in the absence of other IgG4 disease criteria [58]. Liver biopsy is indicated in patients with a suspicion of small-duct PSC or PSC-AIH overlap syndrome [59] [high serum aminotransferase levels, positive antinuclear antibodies (ANA), or smooth-muscle antibodies (SMA)] [59]. Histology may be non-specific and similar to that of primary biliary cholangitis; the typical finding of fibrous obliteration of small bile ducts with concentric replacement by connective tissue ("onion-skin" appearance) is only found in 25% of cases.

Patients with a confirmed diagnosis of PSC, without known IBD, should undergo colonoscopy with biopsies to exclude concomitant IBD or neoplasia [2], even in the absence of symptoms. Biopsies from each segment are mandatory, even if there is normal or slightly altered endoscopic appearance [31]. Although no evidence-based guidelines are available, if the index colonoscopy is negative for IBD, a repeat colonoscopy every 3–5 years should be performed to monitor for possible onset of IBD [10, 11].

16.5 Neoplasia Risk in PSC-IBD

16.5.1 Hepatobiliary Neoplasia

PSC is associated with an increased risk of hepatobiliary malignancy, especially of cholangiocarcinoma (CCA) [60]. This increased CCA risk is irrespective of concurrent IBD diagnosis, although prolonged duration of IBD may be associated with a further increased risk [61]. Risk estimations for CCA vary, but highest estimates reach up to a 20% cumulative 30-year risk for PSC patients [9, 62], while the risk of hepatocellular carcinoma or gallbladder carcinoma is far lower [9].

Some centers perform annual imaging studies (either MRCP or ultrasound) together with a serum CA 19-9 for the early detection of CCA [60, 63], although there are no evidence-based recommendations [59]. When a suspicion of CCA is raised, ERCP with ductal sampling (brush cytology or endobiliary biopsies) is recommended.

16.5.2 Colorectal Neoplasia

The increased risk of advanced colorectal neoplasia (aCRN) (colorectal cancer and/or high-grade dysplasia) in PSC-IBD patients is well-established [64] (Fig. 16.3). A meta-analysis analyzing 11 studies performed up until the year 2001 found an increased risk of aCRN, with an odds ratio 4.79 (95%CI, 3.58, 6.41) for PSC-UC patients as compared to non-PSC UC patients [65]. These results have been corroborated by more recent reports, with a currently estimated odds ratio of 3.2 for aCRN development (PSC-IBD vs IBD only) [66, 67]. Accompanying 10-year and 20-year risks of CRC for PSC-IBD patients are estimated at 14% and 31% [4]. A striking observation is the fact that CRC in PSC-IBD patients is more often found in the proximal colon [68], possibly due to more extensive subclinical inflammation [35].

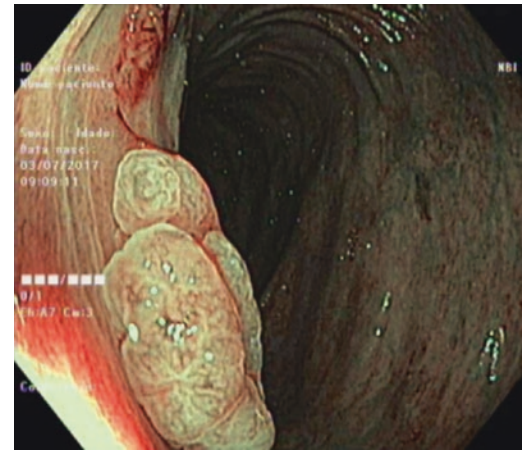

Fig. 16.3 Flat lesion in the ascending colon (Paris classification 0-IIA + Is) of a PSC-IBD patient measuring around 20 mm. Endoscopic resection revealed a low-grade dysplasia adenomatous lesion. In the image NBI is being used to contrast the mucosa

All major guidelines consider PSC-IBD patients to be a group for high risk of developing aCRN, and thus, routine colonoscopic surveillance (preferably using chromoendoscopy) every year, starting from the moment PSC is diagnosed, is advised [69, 70]. Although the difference in progression rates between PSC-IBD and non-PSC IBD patients has not been extensively studied [71], a recent meta-analysis, and a study published in the abstract form, suggests more rapid development from low-grade dysplasia to aCRN in PSC-IBD [72, 73]. Additionally, the endoscopic morphology of dysplastic lesions is thought to be more difficult to detect in PSC patients, possibly due to a flatter phenotype or the not uncommonly observed presence of extensive inflammation [35, 74]. Several studies have shown that, despite their negligible yield in routine IBD surveillance, random biopsies may still have value in PSC-IBD patients [75, 76].

16.6 Biomarkers in PSC-IBD

At this moment, the most important surrogate endpoints for PSC are alkaline phosphatase (ALP), transient elastography (TE, "fibroscan"), and histology [77]. These endpoints are to be applied in clinical trials, despite relatively little supporting evidence, whereas other clinically useful diagnostic or prognostic biomarkers are still under investigation.

Prognostic modeling has allowed physicians to estimate survival with greater accuracy, with the Amsterdam and Mayo risk scoring systems being the most commonly used ones [78, 79]. A promising role is implied for TE, which uses liver stiffness measurements to assess the degree of fibrosis. To this day, no single noninvasive biomarker has shown durable accuracy in diagnosing PSC, CCA, or CRC. Below, several noninvasive biomarker candidates are highlighted.

Alkaline phosphatase (ALP) is the most commonly measured abnormal biochemical marker in PSC. Across a number of studies, although different cutoffs/classifications for ALP levels were used, ALP was associated with prognosis, and a reduction in ALP was associated with better survival [80–82]. Measuring ALP in the individual patient is less straightforward, since the levels of ALP may fluctuate over the disease course. An elevated *serum bilirubin level* has been shown to predict worse prognosis in a number of reports. As a consequence, bilirubin has been integrated in a number of clinical scoring systems for PSC [83–86]. Bilirubin elevations predominantly occur in a late stage of the disease or in case of cholangitis or a stricture.

CA19-9 has been the most extensively studied biomarker in the support of CCA diagnosis and to monitor tumor progression, but diagnostic accuracy is suboptimal [87, 88]. Notably, CA19-9 makes up a small part of a larger diagnostic workup, which includes imaging, biliary brush cytology, and histology.

A limited amount of studies has been performed on the value of *microRNAs* as biomarkers in PSC or PSC-related complications [89]. In one study miR-200c was confirmed to be differentially expressed in PSC patients' healthy controls, and miR-483-5p and miR-194 were confirmed to be differentially expressed in CCA patients [90].

The inflammatory markers *calprotectin* (in bile) and *IL-8* have been reported to predict clinical outcomes of PSC but did not outperform the Mayo risk score [91]. In two reports, an increase in *antiglycoprotein 2* (anti-GP2) levels helped identify patients with more severe PSC phenotype, in addition to being associated with CCA [92]. The usefulness of a range of other noninvasive markers, such as vascular adhesion protein-1 (VAP-1) or PR3-ANCA, has been investigated as biomarkers in PSC patients [93]. Up to 80–90% PSC patients present a positive ANCA. Some studies have suggested that ANCA-positive patients have a lower risk for cholangiocarcinoma and a younger age at diagnosis when compared with ANCA-negative PSC patients [94].

16.6.1 Other Biomarkers (CCA)

Several noninvasive screening methods have been investigated, such as a four-methylated

gene panel on biliary brushings (CDO1, CNRIP1, SEPT9, VIM) [95], as well as protein biomarkers in serum extracellular vesicles (AMPN, FCN1, NEP, PIGR, VNN1) [89, 96]. In addition, bile has also been investigated as a potential fluid to be used in CCA screening. Among the potential biomarkers being studied are peptide-based screening method in bile, able to identify 80% of CCA associated with PSC [97], and a panel of volatile organic compounds (VOCs) in bile [98].

16.6.2 Other Biomarkers (Colorectal Neoplasia)

A stool assay of exfoliated DNA markers (vimentin, EYA4, BMP3, NDRG4) has been under investigation, although further studies are awaited within this specific patient group [99].

16.7 The Management of PSC-IBD

Patients with PSC-IBD should be managed according to the general IBD guidelines. However, as noted earlier, there are several differences in the IBD disease phenotype of PSC patients (pancolitis, rectal sparing, mild symptoms) that may lead to different management decisions. Close articulation with a specialized hepatologist is warranted when considering treatment options for PSC, such as *UDCA*. When somewhere in the course of PSC-IBD, a colectomy is necessary, both an ileal pouch-anal anastomosis (IPAA) or pancolectomy and ileostomy can be performed. In case of a colectomy with ileostomy, there is a risk of parastomal varices. In addition, this procedure often results in a rectal remnant remaining in situ, which is at risk of developing rectal stump cancer [100]. Therefore, endoscopic rectal stump surveillance should be performed. There is a higher risk of developing pouchitis after IPAA, affecting 14–90% of cases (versus 33% in patients with conventional IBD) [10, 11, 31, 101]. Nonetheless, the incidence of pouch failure in PSC-IBD seems to be similar to IBD-alone patients [31, 102].

There are no effective medical therapies for PSC, and the progressive nature of disease often results in liver cirrhosis and eventually end-stage liver disease [2], requiring orthotopic liver transplantation (OLT) [2]. OLT or death usually takes place within 13.2–21.3 years after the initial diagnose of PSC, depending on severity of disease [3]. The therapeutic approach to PSC is the same, whether there is concomitant IBD or not. *Ursodeoxycholic acid (UDCA)* is used extensively, leading to an improvement in liver biochemistry results, but not on important liver-related outcomes [103]. Experimental and animal model studies have suggested a possible suppressive effect of UDCA on colonic tumor formation [104]. Results on a possible chemopreventive effect of UDCA are conflicting, and a majority of studies did not incorporate findings on dosage and treatment duration. A recent meta-analysis found a significant chemopreventive effect of UDCA on the risk of aCRN [105]. Specifically, the risk of all colorectal neoplasia was decreased for low-dose (8–15 mg/kg/day) UDCA use. Notably, a recent study found an increased risk of CRC for patients treated with high doses of UDCA (28–30 mg/kg/day) [106]. While high-dose UDCA as a chemoprotective agent or as a maintenance treatment in PSC is discouraged, many practitioners will still contemplate its use in lower doses (20 mg/kg) albeit further evidence is required [107].

Vedolizumab, a biologic drug approved for the treatment of IBD, blocks gut leukocyte trafficking by preventing the α4ß7 subunit from binding to mucosal addressin cell adhesion molecule-1 (MAdCAM-1). Aberrant expression of these gut adhesion molecules in PSC has opened the possibility of exploring vedolizumab as a potential therapy [108], although no RCT results are available. The potential role of microbiota in disease pathogenesis is further supported by positive results from trials exploring the use of *antibiotics* in the treatment of

PSC. Vancomycin, particularly, has shown promising results [109], and further trials are ongoing.

16.7.1 Treating IBD Patients After Liver Transplantation

OLT is the only curative option for patients with PSC, although PSC can recur in roughly 25% of the transplanted patients [110]. Although not unanimously shown, a range of studies suggests a persistence or increase in IBD activity after OLT [39, 40, 111]. In addition, IBD colitis should be differentiated from transplant-related bowel complications (CMV colitis and mycophenolate colitis). A recent study reported overall favorable outcomes for IBD patients after solid organ transplantation [112], but the amount of data to guide management remains limited. Unfortunately, the evidence on colonic neoplasia risk after transplantation is scarce. While early reports may have suggested an increase in CRC risk post-OLT, more recent evidence did not find such an effect [113–116]. Interestingly, a recent study comparing dysplasia progression rates between transplanted and non-transplanted patients found a longer time to progression of LGD in patients who had been transplanted [117].

16.8 Conclusions

PSC-IBD remains a puzzling phenotype. Epidemiological studies looking at the specific clinical features and disease course have provided very interesting clues into this phenotype, even if limited in many cases by small sample size, retrospective nature, and referral center source. Nevertheless, it is evident that PSC-IBD represents an excellent paradigm of the cross talk between the liver and the colon. Translational research directed into this phenotype could provide important pieces of information that could potentially lead to the development of new strategies for colonic and liver inflammation.

Summary Points

- PSC associated with IBD is a rare occurrence that requires special attention due to the elevated risk of malignant complications and poor liver outcomes.
- PSC-IBD patients present a distinctive IBD phenotype.
- The elevated risk of colorectal neoplasia in PSC-IBD mandates yearly colonoscopy starting at the time of the PSC diagnosis and independent of the IBD duration.
- The medical management of the IBD follows the same approach as IBD in general, but close articulation with the hepatologist is recommended.

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Pregnancy and IBD

17

Emma Flanagan and Sally Bell

Abstract

This chapter examines the role of biomarkers in the assessment of inflammatory bowel disease (IBD) during pregnancy. The pregnant state is unique as it requires consideration of the wellbeing of both mother and foetus. It is now established through multiple studies that active IBD can lead to adverse pregnancy outcomes. Hence, accurate monitoring of disease activity during pregnancy is imperative. However, evaluating disease activity during pregnancy is complicated as the physiological adaptations that occur during pregnancy may affect gastrointestinal symptoms and interpretation of available biomarkers. In addition, several methods of assessing IBD bring safety concerns regarding potential risks to the foetus, including endoscopy and computed tomography (CT) imaging.

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17.1 Introduction

Inflammatory bowel disease (IBD) is a chronic disease that commonly affects women in their peak childbearing years. The majority of women with IBD who are taking maintenance therapy will require medication throughout pregnancy, and most IBD medications are thought to be less harmful to pregnancy outcome than the risk of disease flare during pregnancy [1].

IBD, particularly if active, can lead to adverse pregnancy outcomes including spontaneous abortion, preterm birth and low birth weight [2–4]. Women with IBD who become pregnant when their disease is active are more likely to experience ongoing active disease during pregnancy than those who become pregnant when their disease is in remission [5].

A prospective European cohort study among pregnant women with IBD who were mostly in remission at conception showed that women with Crohn's disease (CD) had a similar disease course during pregnancy when compared to their respective age- and disease-matched non-pregnant cohorts, whereas pregnant women with ulcerative colitis (UC) had a higher risk of relapse during the first and second trimesters of their pregnancy than non-pregnant women with UC [6]. Similar data for larger cohorts of women with active disease during pregnancy have not been reported.

In light of the potential variability in disease activity during pregnancy and the known adverse

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impact of active disease on pregnancy outcomes, pre-pregnancy disease activity status should be measured, ideally to confirm established remission, but importantly to serve as a baseline from which to monitor individual disease activity throughout pregnancy. Once pregnant, monitoring of disease activity in each trimester is essential. However, evaluating disease activity during pregnancy can be challenging, as the available methods are either precluded due to possible risk to the foetus or are not validated during pregnancy [7].

Endoscopy is considered to be the gold standard for the diagnosis of IBD; however, it is not without significant risk [8]. This becomes even more meaningful during pregnancy when the wellbeing of both the patient and the developing foetus must be considered. Hence, a combination of available biomarkers including serological, faecal and radiological methods should also be used to best assess disease activity in IBD. The interpretation, utility and safety of standard markers of inflammation used in IBD are impacted by pregnancy; the evidence for this and consequent recommendations will be discussed in this chapter.

17.2 Serum Biomarkers in Pregnancy

17.2.1 Physiology of Serum Biomarkers in IBD

Serum biomarkers of inflammation such as C-reactive protein (CRP) are often used to aid in monitoring the disease course in IBD [9]. CRP is an acute phase-reactant protein that is produced by hepatocytes primarily in response to the inflammatory cytokine interleukin-6 (IL-6).

CRP has been shown to correlate with endoscopic disease activity in both CD and UC; however, there is a stronger association with histologic findings in CD [10]. It must be noted, however, that CRP is not specific to inflammation of the bowel and levels can indeed be raised in the setting of alternative inflammatory states. Moreover, when using CRP as a serologic biomarker of inflammation in IBD, it is important to consider that active IBD does not always manifest with biochemical evidence in the form of an elevated CRP [11].

17.2.2 Physiology of Serum Biomarkers During Pregnancy

The values of serum biomarkers such as CRP may be altered in healthy pregnant women compared to non-pregnant women secondary to the physiological changes that take place in pregnancy.

During normal pregnancy, there are complex shifts in maternal inflammatory responses [12, 13]. Some studies have shown increased levels of cytokines such as IL-6 in pregnancy, and likewise, CRP can be elevated slightly during normal pregnancy [14, 15]. Moreover, elevated CRP levels have also been associated with maternal obesity and obstetric complications such as pre-eclampsia and preterm labour [16–18].

Laboratory blood tests were measured during pregnancy in a study by Klajnbard et al., including 391 healthy Caucasian women with uncomplicated pregnancies [19]. Suggested reference intervals during pregnancy are reported based upon results on the 2.5–97.5 percentiles. In this study, the CRP concentrations were largely stable throughout pregnancy but were higher than the standard non-pregnant reference range; the suggested reference interval for CRP at 35–42 weeks gestation was 10–210 nmol/L (1.05–22.05 mg/L) [19].

Similarly, other serological biomarkers used to monitor IBD can be affected by physiological changes in normal pregnancy. Albumin may be low in normal pregnancy and mild anaemia is normal in the pregnant state.

In the same study by Klajnbard et al., haemoglobin (Hb) values were stable during pregnancy but were slightly lower than the standard reference range. The suggested reference interval for Hb in iron replete pregnant women at 35–42 weeks gestation was 110–147 g/L [19].

Additionally, this study demonstrated that albumin levels were lower during pregnancy than non-pregnant reference intervals and decreased slightly as pregnancy progressed, with reference intervals for albumin at 35–42 weeks gestation being 30.0–

39.8 g/L [19]. Another study measuring laboratory markers in 52 normal pregnancies showed that albumin levels can decrease further during pregnancy, with reference intervals calculated as 23.1–33.8 g/L at 34–38 weeks gestation [20].

17.2.3 Interpreting Biomarkers During Pregnancy

Serum biomarkers including CRP can be useful for monitoring IBD in the non-pregnant patient. However, particular care needs to be taken when interpreting serum biomarkers during pregnancy due to physiological adaptations, which can simultaneously affect these markers.

In addition, serum levels of biomarkers of inflammation such as CRP may be affected by other pertinent factors such as maternal body mass index or pregnancy complications. Consideration given to these limitations is necessary during pregnancy when using serum biomarkers to monitor disease activity and ideally such biomarkers should be combined with other non-invasive markers to improve accuracy.

While it is important not to rely solely on serum biomarkers during pregnancy for assessment of disease activity in IBD, blood laboratory tests are readily available. Serial measurements of Hb, albumin and CRP should be performed at preconception and throughout pregnancy and integrated with other parameters to monitor disease activity over time on an individual patient basis.

17.3 Faecal Biomarkers

17.3.1 Faecal Calprotectin

Faecal calprotectin is a protein produced by neutrophils and is a useful non-invasive faecal biomarker for monitoring disease activity in IBD. An elevated faecal calprotectin level has consistently been shown to correlate with endoscopic disease activity in IBD and can be predictive of relapse, while a normal faecal calprotectin reflects mucosal healing [21].

Data related to the use of faecal calprotectin in pregnancy are somewhat limited. A number of small studies have demonstrated that, unlike serum biomarkers such as CRP, faecal calprotectin can be useful to detect disease relapse and does not appear to be affected by physiological changes during pregnancy.

In the prospective multicentre ERA study, it was demonstrated in a subset of 46 pregnant patients with IBD that an elevated faecal calprotectin (above 250 mg/g) correlated with active disease according to the Physicians Global Assessment during pregnancy [22]. In another study including 75 pregnant patients with IBD, faecal calprotectin had an overall specificity of 80.7% for detecting disease activity, but an elevated faecal calprotectin did not accurately predict disease relapse [23].

More information is needed regarding faecal calprotectin levels in pregnancy and further studies are ongoing; however, it represents a simple, repeatable biomarker that can be followed during pregnancy and the peri-partum period.

17.3.2 Faecal Lactoferrin

Faecal lactoferrin is also a protein produced by neutrophils that has been shown to reflect endoscopic disease activity in IBD [24].

Data related to the use of faecal lactoferrin in pregnancy are very limited. Recent evidence from the MECONIUM (Exploring MEChanisms Of disease traNsmission In Utero through the Microbiome) study has shown that it may be useful in monitoring disease activity in IBD during pregnancy. These data include faecal lactoferrin concentrations during pregnancy from 76 patients with IBD and 175 controls showing that faecal lactoferrin was higher in patients with IBD than controls during each trimester of pregnancy [25].

17.3.3 Use of Faecal Inflammatory Markers in Pregnancy

Faecal calprotectin appears to be accurate in detecting relapse during pregnancy. Faecal cal-

protectin is a non-invasive biomarker that can be utilised as a monitoring tool for disease activity and can be performed preconception and then in each trimester or when there is a suspected flare in pregnant patients with IBD. It is likely that faecal lactoferrin will be similarly helpful in monitoring individual disease activity in pregnancy.

17.4 Imaging Considerations in IBD During Pregnancy

17.4.1 Indications for Imaging in IBD

Cross-sectional imaging including magnetic resonance imaging (MRI), computed tomography (CT) imaging and bowel ultrasonography may be necessary as non-invasive markers to evaluate disease activity and in particular to investigate extra-luminal complications in patients with IBD. Imaging in IBD is especially useful in Crohn's disease in order to measure small bowel disease extent and activity, as well as assess for the presence of strictures, intra-abdominal fistulae and abscesses when there is clinical suspicion. Traditionally, cross-sectional imaging is less useful in the assessment of ulcerative colitis where the disease can be accessed with colonoscopy.

Bowel ultrasonography, magnetic resonance imaging (MRI) and computed tomography (CT) have been shown to have similar diagnostic accuracy for imaging in IBD [26, 27]. The choice of imaging modality in non-pregnant patients is dependent on clinical urgency and accessibility, but MRI or ultrasound is preferred over CT for assessment of IBD due to the lack of radiation exposure.

17.4.2 Efficacy and Safety Considerations of Imaging Options in Pregnancy

17.4.2.1 Computed Tomography (CT)

Abdominal and pelvic CT is associated with significant ionising radiation and thus should only be performed during pregnancy in the rare circumstance when the possible risk of misdiagnosis is greater than the potential risks associated with radiation exposure.

The potential effects of radiation exposure on the foetus include an increased risk of malformations, neurodevelopmental abnormalities and carcinogenesis. The risk of malformations is dependent on the timing and dose of radiation exposure, with the foetus being most susceptible during the period of major organogenesis and early foetal development (2–15 weeks gestation), while it is thought that doses above 100 mGy of radiation may induce malformations based on animal data [28, 29]. Similarly, for neurodevelopmental effects the most sensitive period is 8–15 weeks gestation and associated with radiation doses of at least 100 mGy [28, 29]. The foetal dose exposure from abdominal and pelvic CT is less than this threshold. In the setting of foetal exposure to radiation from CT scanning, termination of pregnancy is not indicated; closer ultrasound monitoring is undertaken for congenital anomalies. There is no dose threshold relating to risk of childhood cancer, for which the risk increases with increasing doses of radiation but remains low overall [30]. It is not currently recommended that neonates exposed to radiation from CT in utero undergo longer-term follow-up.

17.4.2.2 Magnetic Resonance Imaging (MRI)

The safety of MR enterography in pregnancy has not been definitively established. In particular, there is uncertainty regarding the contrast medium gadolinium.

While MR imaging does not involve any radiation exposure, there have been theoretical concerns regarding the exposure to the electromagnetic fields such as potential effects on cell proliferation or foetal hearing. However, there have not been any reported adverse effects on the human foetus linked to MRI exposure, and MRI is considered safe during pregnancy [30]. Studies involving gadolinium exposure in pregnancy are extremely limited, and as such contrast-enhanced MR imaging is only recommended when considered crucial for the diagnosis [30].

Although the optimal MR enterography protocol includes administration of gadolinium, MR enterography can be accurately performed without gadolinium. Typically, MR enterography is used to assess Crohn's disease activity by incorporating a number of findings including contrast enhancement. However, other parameters are evaluated such as bowel wall thickness and hyperintense signal on T2-weighted images as well as extra-luminal complications [31].

In a small case series including nine pregnant patients with known or suspected Crohn's disease, MR enterography utilising a modified protocol without gadolinium demonstrated reliable diagnostic information and impacted clinical management [32].

17.4.2.3 Bowel Ultrasonography

In non-pregnant patients with IBD, ultrasound is increasingly being recognised as an accurate method to assess luminal disease activity of the small and large bowel as well extra-luminal complications, particularly in patients with Crohn's disease. The precise role of bowel ultrasonography for monitoring of IBD in pregnancy has not yet been substantiated; however, this is an emerging area of investigation.

Bowel ultrasonography is a non-invasive imaging modality that does not require radiation and is notionally an ideal imaging modality in pregnancy [33]. However, views of the bowel can be impeded by the foetus in late pregnancy, and thus, currently, bowel ultrasonography is thought to be useful for assessing IBD during the first two trimesters of pregnancy.

In our experience, adequate assessment of the colon and terminal ileum can generally be obtained with ultrasound up to 24 weeks gestation [34]. Beyond 24 weeks, bowel ultrasound provides good views of the left colon, but the remainder of the colon and terminal ileum can be difficult to assess with confidence [34]. In the setting of a flare of left-sided colonic IBD during pregnancy, bowel ultrasonography may be a useful, non-invasive alternative to undertaking a flexible sigmoidoscopy. In patients with Crohn's disease affecting the terminal ileum, ultrasound is a valuable imaging tool up to around 24 weeks gestation.

17.4.3 Imaging Recommendations During Pregnancy

Cross-sectional imaging may be indicated in pregnancy to investigate a suspected flare of IBD or possible extra-intestinal complications of disease. Imaging results should be used to complement other biomarkers of disease activity in pregnancy. During pregnancy, MRI without gadolinium or bowel ultrasonography are preferred if available.

17.5 Endoscopy for IBD in Pregnancy

17.5.1 Indications for Endoscopy for IBD in Pregnancy

Colonoscopy is generally considered the gold standard for assessing luminal disease activity in patients with IBD. Hence, endoscopy may be indicated if the findings will alter the management of IBD during pregnancy.

For instance, endoscopy is indicated if there is suspected severe disease activity or if after initiating therapy for a disease flare, there is ongoing clinical and biomarker evidence of disease activity.

17.5.2 Safety Considerations in Pregnancy

Potential foetal safety concerns include adverse effects of anaesthetic medications and risk of hypotension or hypoxia. However, limited data exists regarding the safety of endoscopy in pregnant women and in particular in pregnant women with IBD, and much of the published data is retrospective or case series in nature.

Procedural precautions must be observed when performing endoscopy during pregnancy

to minimise risk to the patient and foetus. This includes using the minimum dose of sedating medication possible and standard monitoring, including pulse oximetry and positioning to avoid maternal hypotension [35]. For patients in the second or third trimesters, endoscopic procedures should be performed with the patient in the left lateral position to prevent vascular compression.

On the basis of limited human data, the latest American Society for Gastrointestinal Endoscopy guidelines for endoscopy during pregnancy recommend sedation with narcotic analgesia such as low-dose fentanyl [35], and if this is inadequate, small doses of midazolam may be used [36]. If deeper sedation is required, propofol may be administered by a trained specialist in anaesthesia [36].

A recent Swedish population-based cohort study reported no increased risk of stillbirth or congenital malformation associated with any endoscopy during pregnancy, but did report an increased risk of preterm birth or small for gestational age [37]. However, although the risk of adverse pregnancy outcomes associated with endoscopy remained rare overall, this study was based on registry data and was not able to take into account indication for endoscopy or disease activity, which can affect pregnancy outcome [37].

In relation to endoscopy in pregnant patients with IBD specifically, a systematic review and small prospective cohort study by De Lima et al. concluded that lower gastrointestinal endoscopy appears to be of low risk based on the limited available data in this field [38, 39]. The prospective study, including 42 pregnant patients with IBD, demonstrated no increase in adverse outcomes for the mother or the newborn relating to endoscopy when compared to controls matched based on age, medication and disease activity [38]. In this study, 42 patients underwent 47 lower gastrointestinal endoscopies (35 sigmoidoscopies, 12 colonoscopies) during pregnancy; in 48.9% of these patients, no sedation was used, in 19.1% fentanyl only was used, in 6.4% midazolam only and midazolam was not used as sedation in the first trimester [38].

17.5.3 Recommendations

Endoscopy for IBD can be performed during pregnancy if there is a strong indication such as acute severe UC or failure to respond to escalation of therapy. If clinically indicated, endoscopy should not be delayed in pregnant patients. Whenever possible, endoscopy during pregnancy should be in the form of flexible sigmoidoscopy (for patients with distal colitis) and can be performed without sedation, rather than colonoscopy (which is generally reserved for patients in whom the terminal ileum cannot be otherwise satisfactorily assessed).

17.6 Tools Predictive for Disease Flare and Future Directions

Currently, we advocate for the utilisation of serological biomarkers during pregnancy with cautious interpretation and in combination with faecal calprotectin, which is able to detect disease relapse during pregnancy.

It is likely that faecal calprotectin may be useful not only in accurately detecting disease flare during pregnancy but also in predicting disease flare during pregnancy and the post-partum period. This has not yet been demonstrated in available studies to date.

One small study has suggested that another faecal biomarker, faecal lactoferrin, may also be useful in the future to monitor disease activity during pregnancy. We await further data regarding correlation of faecal lactoferrin with disease activity during each trimester of pregnancy.

Bowel ultrasonography is likely to be effective in detecting and predicting disease relapse in pregnancy, but there is a paucity of data relating to bowel ultrasonography in IBD during pregnancy. Practically, it is likely that views of the bowel may be obscured after week 24 of gestation. A number of studies are currently being conducted to monitor the usefulness and accuracy of bowel ultrasonography for IBD during pregnancy.

Further prospective data relating to endoscopy in IBD patients during pregnancy will help to inform the potential risk associated with endoscopic procedures in the future.

17.7 Case Studies

Case 1

A 29-year-old female presents to outpatient IBD clinic currently 18 weeks pregnant with recently diagnosed Crohn's disease of the terminal ileum

- *Background*: Diagnosed with Crohn's disease 2 months prior to pregnancy with colonoscopy demonstrating severe ulceration of the ileocaecal valve, terminal ileum unable to be intubated due to stenosis; MR enterography at diagnosis showed thickening of the terminal ileum over 15 cm with mild proximal small bowel distension. Had declined to commence a thiopurine at diagnosis due to concerns regarding medication in future pregnancy.
- *Presentation*: On review in clinic at 18 weeks gestation, reports colicky abdominal pain, bloating, occasional nausea and vomiting.
- *Assessment of disease activity performed with available biomarkers*:
  - Serum biomarkers: Hb 127 g/L, albumin 34 g/L, CRP 10 mg/L
  - Faecal calprotectin: 310ug/g
  - Intestinal ultrasound: moderately active inflammation of the terminal ileum over 10 cm with associated luminal narrowing and proximal small bowel distension of 2 cm
- *Outcome*: Active Crohn's disease treated with course of weaning prednisolone with improvement in biomarkers (repeat CRP <5 mg/L; faecal calprotectin 90 ug/g; no active inflammation of terminal ileum on intestinal ultrasound).
- *Learning point: A suite of objective, non-invasive tests including intestinal ultrasonography is useful to identify active disease in pregnancy.*

Case 2

A 25-year-old female currently 22 weeks pregnant with left-sided UC admitted to hospital with severe disease flare

- *Background*: Chronic active left-sided UC. Colonoscopy 4 months prior to (unplanned) pregnancy showed moderate colitis to descending colon. Maintenance therapy 5 mg/kg infliximab 8-weekly and maximal dose of 5-ASA, previously intolerant to thiopurine. Had trialed 1 week of high-dose oral prednisolone prior to admission without resolution of colitis symptoms.
- *Presentation*: On admission reports increased bowel frequency up to 10 bowel actions daily with blood, associated urgency.
- *Assessment of disease activity performed with available biomarkers*:
  - Serum biomarkers: Hb 87 g/L, albumin 24 g/L, CRP 28 mg/L
  - Faecal calprotectin: 2700ug/g (culture negative)
  - Flexible sigmoidoscopy performed: Mayo 2 colitis to descending colon (CMV negative)
- *Outcome*: Obstetric ultrasound as inpatient showed normal foetal appearance. Active colitis treated with IV hydrocortisone and additional dose of 10 mg/kg infliximab then weaning course of prednisolone and infliximab increased to 10 mg/kg dose 6-weekly.
- *Learning point: Flexible sigmoidoscopy is indicated during pregnancy in cases where there is clinical or biomarker evidence of severe disease activity that has not responded to escalation of therapy in order to perform direct evaluation of mucosal inflammation and exclude CMV infection.*

Case 3

A 34-year-old female with pan-UC reviewed regularly in outpatient IBD clinic during pregnancy

- *Background*: Diagnosed age 14 with pan-colitis on colonoscopy, treated initially with oral steroids then remained in remission on maintenance dose 5-ASA therapy.
- *Preconception disease assessment*:
  - Serum biomarkers normal (CRP <5 mg/L)
  - Faecal calprotectin normal (<50ug/g)
  - Colonoscopy: no active inflammation
- *Trimester 1 review in IBD clinic* – remains clinically well:
  - 11 weeks pregnant, no symptoms of colitis
  - Serum biomarkers: normal (CRP <5 mg/L)
  - Faecal calprotectin: 193ug/g
  - 5-ASA increased to maximal dose due to calprotectin result, which was elevated compared to patient's previous level
- *Trimester 2 review in IBD clinic* – moderate flare of colitis:
  - 23 weeks pregnant, increased frequency with up to 8 bowel actions daily with blood, associated urgency
  - Serum biomarkers: Hb 129, albumin 29 g/L, CRP 12 mg/L
  - Faecal calprotectin: 458ug/g (culture negative)
  - Intestinal ultrasound performed: moderately active colitis from sigmoid to mid-transverse colon
  - Disease flare managed with addition of weaning course of oral steroids and topical 5-ASA/steroid therapy with clinical improvement
- *Trimester 3 review in IBD clinic* – improved:
  - 31 weeks pregnant, no symptoms of colitis
  - Serum biomarkers: Hb 124, albumin 29 g/L, CRP 10 mg/L
  - Faecal calprotectin: 221ug/g
  - Intestinal ultrasound performed: adequate views of left colon from proximal sigmoid with no active colitis seen
  - Maximal dose 5-ASA oral and topical therapy continued throughout pregnancy
- *Normal vaginal delivery at term*, healthy baby; reviewed concurrently in high-risk obstetric clinic due to active disease.
- *Planned for review at 6 weeks post-partum* with repeat biomarkers.
- *Learning point: Preconception disease activity status should be measured with a combination of available biomarkers to confirm established remission and to serve as a baseline from which to monitor individual disease activity in each trimester of pregnancy and post-partum.*

17.8 Therapeutic Drug Monitoring in Pregnancy and Exposed Infants

Infliximab and adalimumab are immunoglobulin G1 (IgG1) anti-TNF monoclonal antibodies, which are used both to induce and maintain remission in IBD. Available data has shown that IBD patients exposed to anti-TNF therapy during pregnancy do not have increased rates of adverse pregnancy outcomes [40–42]. Infliximab and adalimumab are transferred across the placenta in the second and third trimesters of pregnancy [43].

Our group has shown in the ERA study, which measured drug levels in infants following intrauterine exposure to anti-TNF medications, that infant drug levels at birth were inversely related to the time from last intrapartum dose of anti-TNF therapy [44]. Clearance

occurred by 6 months in all adalimumab-exposed neonates and by 12 months in infliximab-exposed babies [44]. The presence of anti-TNF antibodies may allow replication of live vaccines, with a death from disseminated BCG reported in an infliximab-exposed infant [45]. Hence, infants exposed to anti-TNF medications in utero should not be administered any live vaccinations until 12 months of age. Routine therapeutic drug monitoring at birth in exposed infants is not currently indicated as a predictive biomarker to assess the timing of live vaccinations as the most important live vaccinations are not administered until 12 months in most countries (such as the vaccine against measles, mumps and rubella). Similarly, breastfeeding is considered safe with anti-TNF agents, and while very low levels of anti-TNF medication may be detected in the breast milk, this does not alter neonatal levels. Therefore, monitoring of levels in breast milk or infants is not required.

17.9 Summary

Due to the increased risk of adverse pregnancy outcomes associated with active IBD, safe and reliable biomarkers are necessary in order to accurately guide management and ensure optimal pregnancy outcomes. During pregnancy, there is no single ideal biomarker for both detecting and predicting disease activity, and thus we need to use a number of tools and interpret blood biomarker results in the context of the normal ranges for each trimester of pregnancy in IBD (Table 17.1).

Effective monitoring of IBD during pregnancy should integrate all available non-invasive biomarkers of disease activity, and this includes establishment of disease activity preconception using the same tools so that serial measurement during pregnancy is meaningful. Pregnant patients with IBD should undergo assessment of serological biomarkers (CRP, Hb, albumin) and faecal calprotectin at least once per trimester and more regularly if they have active disease during pregnancy. If imaging is required, this should ideally be in the form of bowel ultrasonography or MR enterography without gadolinium. Exposure to radiation through CT should be avoided if possible but could be considered in cases of significant intra-abdominal sepsis where bowel ultrasound and MR are not available, as an alternative to surgery. Endoscopy should only be performed if clinically necessary such as acute severe UC and is generally considered safe in pregnancy with appropriate monitoring and precautions regarding patient positioning and minimal anaesthetic.

Table 17.1 Summary of available biomarkers for IBD in pregnancy

Biomarker	Considerations and recommendations
Serum inflammatory markers	Values altered in normal pregnancy; interpret with caution
Faecal calprotectin	Limited data; helpful to detect relapse
Radiographic imaging	Radiation risk with CT; avoid if possible Limited data regarding gadolinium; MRI without contrast recommended Bowel ultrasound safe; views restricted in late pregnancy
Endoscopy	Limited safety data in pregnancy; perform if strong indication

Summary Points

- IBD commonly affects women in their peak childbearing years.
- Active IBD can lead to adverse pregnancy outcomes.
- Assessing disease activity during pregnancy is challenging as available methods may present potential risks to the foetus or are not substantiated during pregnancy.
- Serum biomarkers such as CRP are affected by normal physiological adaptations during pregnancy.
- Faecal biomarkers including calprotectin are useful for detecting disease relapse in pregnancy but data are limited.

- There are foetal safety concerns regarding the use of both CT and MRI in pregnancy due to radiation exposure associated with CT and the contrast medium gadolinium used in the standard small bowel MRI protocol.
- Bowel ultrasonography is safe in pregnancy; however, views of the bowel can be impeded by the foetus in late pregnancy.
- Safety data concerning endoscopy for IBD during pregnancy are limited.

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Nutrition in IBD

18

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Abstract

Malnutrition in inflammatory bowel disease (IBD) is related to reduced oral intake, malabsorption and catabolic stress due to inflammatory burden ultimately leading to loss of body form, composition and function. Malnutrition persists even when disease is in remission, and it is associated with increase in mortality, prolonged hospitalisations, post-operative complications, poor quality of life and greater health-care burden. There are multiple proxies of malnutrition, ranging from a simple bedside anthropometry and biochemistry to a more detailed body composition analysis. Anthropometry and biochemistry are practical and low-cost pragmatic malnutrition screening tools; however they cannot discriminate key body composition changes such as loss of lean body mass (LBM) or fat-free mass (FFM) and mesenteric fat deposition (MFD). These key body composition changes contribute to the higher inflammatory burden, poor therapeutic response to anti-TNFs and increased risk for intestinal surgery.

Treatments targets for IBD have also evolved with increasing emphasis on using therapies capable of inducing mucosal healing. Exclusive enteral nutrition (EEN) is the most well-established therapeutic diet in CD capable of inducing mucosal healing rates compared to conventional steroids. Concomitant use of partial enteral nutrition is also associated with reduction in loss of response to infliximab. There is also growing interest in anti-inflammatory exclusion diets to maintain remission, but robust endpoints like endoscopic remission are missing. Multiple serum and faecal biomarker studies have demonstrated anti-inflammatory effects of enteral diet, but the exact mechanism of action remains elusive. Modulation of microbiota and metabolomic changes following dietary elimination studies, more specifically in Crohn's disease, have been tested in many recent studies; however these shifts do not establish a cause and effect relationship and may simply reflect functional gut adaptations due to changes in dietary substrates. As our understanding of the relationship between diet, nutrition and gut health evolves, we expect to see major advances in the role of dietary patterns and constituents in the development, treatment, cure and finally prevention of IBD.

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18.1 Introduction

Inflammatory bowel diseases (IBD) are chronic intestinal-inflammatory disorders, widely believed to be the result of an aberrant mucosal immune response to environmental triggers in genetically predisposed individuals. Steep rises in incidence of IBD, particularly in developing countries with a previously low incidence of IBD, indicate a strong contribution of environmental factors such as caesarean delivery, childhood antibiotics, smoking and diet [1]. Although diet is the most frequently reported risk factor associated with IBD flare by patients, there are limited prospective studies examining the role of dietary factors in contributing or perpetuating inflammation in IBD [2]. Prospective lifestyle and dietary risk cohort studies suggest a preventative role of fibre intake from fruits, vegetables and an adverse association with animal proteins and trans-unsaturated fats [3–5]. More recently, benchmark studies have linked specific dietary factors with intestinal inflammation. Food emulsifiers and saturated fats contribute to epithelial barrier dysfunction, aberrant mucosal immune responses and unhealthy microbiota profiles in animal and in vitro human intestinal ecosystem models [6–8]. These epidemiological, clinical and benchmark observations have further renewed interest in the therapeutic role of dietary interventions in IBD. Consequently diet has a multifaceted role in the management of IBD, with interventions focussed on more than correcting malnutrition including diets capable of inducing and maintaining remission. In this chapter, we briefly review biomarkers of therapeutic response to and evidence behind dietary interventions in IBD.

18.2 Malnutrition in IBD

"Malnutrition is defined as a state in which deficiency or excess of energy, protein and other nutrients causes a measurable adverse effect on body (shape, form, composition, function) and clinical outcomes" [9]. Prevalence of malnutrition in IBD ranges from 16% to 80%, depending on the study population, disease extent, activity and measuring tools. Malnutrition in CD is related to reduced oral intake, malabsorption and catabolic stress due to inflammatory burden ultimately leading to loss of body form, composition, function and adverse outcomes [10] (Fig. 18.1). It is important to appreciate that malnutrition can persist many years after diagnosis and even when disease is in remission and is associated with adverse outcomes including mortality (OR 3.49, 95% CI 2.89–4.23), hospitalisations (11.9 versus 5.8 days, $p < 0.00001$), postoperative complications, poor quality of life and health-care burden [11–16].

18.3 Surrogate Markers of Malnutrition

There are multiple proxies of malnutrition, ranging from a simple bedside anthropometry and biochemistry to a more detailed body composition analysis (Table 18.1). An ideal screening tool is one that is fast, reliable and can accurately predict adverse health outcomes. Anthropometry and biochemistry are practical low-cost malnutrition screening tools in a busy clinical environment; however, they cannot discriminate key body composition changes such as loss of lean body mass (LBM) or fat-free mass (FFM) and mesenteric fat deposition (MFD) [13]. A variety of methods can reliably predict these key body composition changes, and the choice of techniques is dictated by cost, time, availability, purpose and application (Fig. 18.2). A number of studies now suggest that these alterations in body compositions are no longer innocent bystanders but rather may contribute to inflammatory response and increase risk of intestinal resections, complicated disease course and insufficient response to anti-TNF agents (Table 18.2) [17–32].

18.4 Nutritional Interventions Associated with Disease Remission

18.4.1 Exclusive Enteral Nutrition (EEN)

EEN is the most well-established therapeutic diet in CD with reported benefits beyond nutritional recovery. The role of exclusive enteral

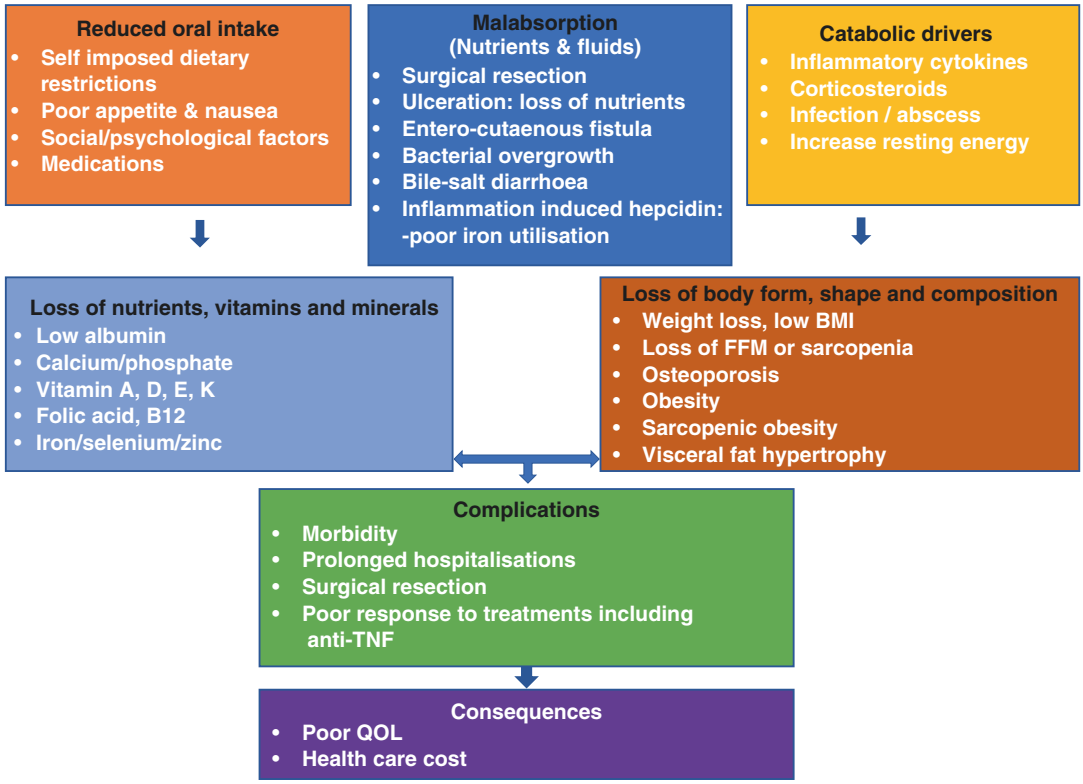


Fig. 18.1 Malnutrition in IBD

nutritional (EEN) as a primary therapy in active CD was first described four decades ago with enteric fistula closure, clinical remission and reduced surgical resection in medically refractory adults with CD [33, 34]. Over the last four decades, there have been numerous studies reporting clinical efficacy of various elemental, semi-elemental and polymeric liquid diets as a sole source of nutrition in CD. Polymeric formula has been shown to be as effective, more palatable and less likely to require nasogastric tube insertion, thus promoting better acceptance and facilitating greater weight gain [35–38]. Previous systematic reviews, combining adult and paediatric studies, suggested that EEN may be less efficacious than corticosteroids (CS) at inducing clinical remission; however, a recent paediatric meta-analysis demonstrated that both were equally effective. EEN was as effective as CS in inducing clinical remission (OR = 1.26 [95% CI 0.77, 2.05]) and normalisation of biomarkers including CRP (OR = 0.85 [95% CI 0.44, 1.67]) and faecal calprotectin (OR 2.79) [39–42]. A recent multicentre paediatric cohort study using propensity matching also reported superior clinical remission in mild-moderate CD and greater improvement in height Z scores on EEN vs. CS [43].

Clinical remission rates in adults with CD on EEN have been inferior to CS with pooled OR (0.33, 95% CI 0.21–0.53), which is likely to have been related to very high withdrawal rates (39% in one large RCT), heterogeneous definition of disease remission and shorter duration of treatment. In sub-analyses, aiming to estimate the true effect of EEN in those adhering to the EEN vs. CS, similar clinical remission rates have been observed in adults with CD (100/148, 67.5% vs. 135/172, 78%, p = NS).

In addition to avoiding the many adverse side effects of corticosteroids and permitting the uptake of vaccination schedules, the major

Table 18.1 Surrogate markers of malnutrition

Method	Components	Definition	Comments
BMI	Weight (kg)/height [m]^2	Underweight: <18.5 kg/m^2 Normal: 18.5–25 kg/m^2 Overweight > 25–29.9 kg/m^2 Obesity = 30 kg/m^2	Fast Inaccurate assessment of body composition
SGA (Subjective Global Assessment)	Unintentional weight loss Dietary intake GI symptoms > 2 weeks Functional capacity Disease type	Well nourished Mild/moderate Malnourished Severe malnourished	Accurate Time consuming
MUST (Malnutrition Universal Screening Tool) MUST-Patients (MUST-P) MUST-Health Care Professional (MUST-HCP)	BMI Unintentional weight loss Acute disease effect score	0 – Low risk 1 – Moderate risk 2 – High risk	Fast and reliable Good agreement Predicts: Hospital stay Mortality
Triceps skin fold thickness (TSST)	Estimates body fat % Using calipers	TSST normograms	Observer variability Estimates fat mass only
Hand grip strength	Estimates muscle strength and functional capacity Using dynamometer		Correlates with body cells mass May overestimate (high false positive)
Albumin/ pre-albumin	Acute phase reactant Reduced synthesis Increased losses	<3.5 g/dl	Low levels associated with rapid clearance of anti-TNF agents and poorer clinical outcomes in patients with IBD
Vitamins, minerals and micronutrients deficiency	Inadequate intake Increased losses	Vitamin A, D, E, folic acid Micronutrients Iron, zinc, calcium, copper, selenium	Common in small bowel CD Low vitamin D levels associated increased risk for surgery and hospitalisation Anaemia – most common EIM of IBD

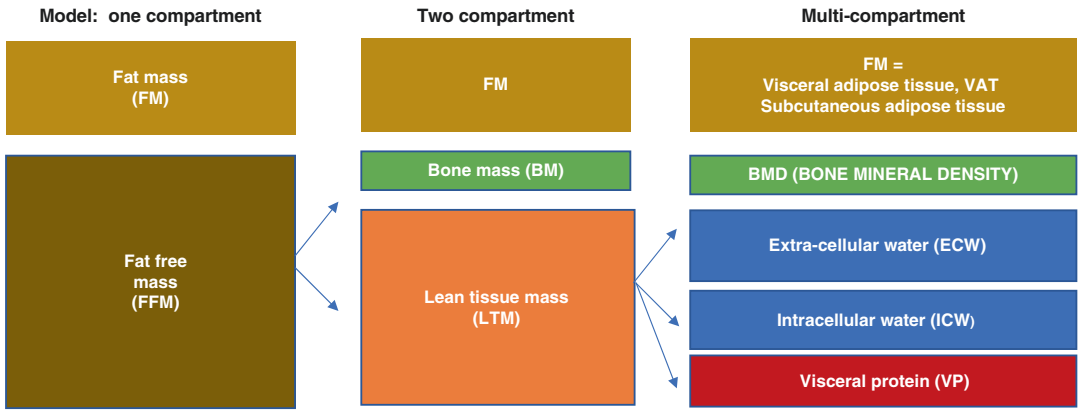
advantage for EEN is its ability to induce greater mucosal healing (MH) compared to CS (OR = 4.5 [95% CI 1.64, 12.32]) [42, 44–46]. The benefits of this early MH provided by EEN extend beyond 3 years, with greater CS and anti-TNF free biological remission rates on maintenance immunomodulators (IMs) in those with early complete mucosal healing [47].

An English study recently reported the benefits of a 4-week EEN course in adults with complicated CD waiting for surgery, with 25% avoiding surgery and safer surgery achieved in the others with a fourfold reduction in complications in those treated with EEN vs. No EEN [48].

18.5 Role of Partial Enteral Nutrition in CD

18.5.1 Maintenance Therapy in Adults with Clinical Remission

Partial enteral nutrition (PEN) plus normal eating is more acceptable than the complete exclusion of normal diet (EEN), and its role as a maintenance therapy in adults with clinical remission has been reported in several adult studies. Partial supplementation of elemental formula with normal diet



Technique	Measurements	Application	Benefits/limitations
Anthropometry Skin fold thickness (SFT)	Fat Mass (Subcutaneous adipose tissue)	Clinical	Simple and safe/inaccurate
Bioelectrical impedence (BIA)	Measures: TBW Estimates = FM, FFM, TBW	Clinical	Simple/mobile/widely used Inaccurate (impacted by hydration)
Ultrasound	FAT MASS/VAT	Research	Simple/No Radiation
Dual energy xray absorptiometry (DXA)	FM, LTM, BMD (Bone mineral density), visceral adiposity (VA)	Research	Cost/Simple/Invasive
Air displacement plethysmography or hydrodensinometry	FM, FFM	Research	Cost/impacted by hydration Water displacement is invasive
Isotope dilution technique	TBW/ECW	Research	Labour intesnsive, inaccurate and need for mass spectroscopy
Imaging QCT MRI	FM, VAT, LTM, BMD	Research	 Cost/radiation (CT) Cost/Safe/Time consuming (MRI)

Fig. 18.2 Body composition analysis

is associated with better sustained remission rates, reduced relapse and improved endoscopic response vs. unrestricted normal diet [49–51].

18.5.2 Role of PEN in Combination with Infliximab (IFX) and Following CD-Related Surgery

Loss of response to IFX is common and estimated to be 13% per patient per year secondary to anti-TNF antibody formation and increased drug clearance [52]. Loss of response to IFX can be minimised by early IFX dose optimisation based on therapeutic drug monitoring and concomitant use of immunomodulators. The addition or concomitant use of immunomodulators (thiopurines or methotrexate) during the induction phase reduces the risk of LOR, mainly due to a pharmacokinetic effect on IFX levels, but the benefits of ongoing combination therapy during the maintenance phase (>6 months) are less clear and associated with an increased safety risk, particularly with azathioprine. The role of combination therapies with other anti-TNF agents and biologic therapies is less well defined.

Concomitant use of PEN is also associated with clinical benefits during the infliximab maintenance phase with a reduction in loss of response to IFX [53, 54]. An overall increase in sustained

Table 18.2 Change in body compositions alters disease phenotype and treatment response

Body composition and measurement		
Surrogate marker	Key mechanisms and outcomes	References
Low albumin	Increase infliximab clearance and poor clinical response in IBD	Fasanmade A, *Clin Ther,* 2011 Jul [17]
	(Albumin and anti-TNF are IgG class and have common clearance pathway)	Fasanmade A, *Int J Clin Pharmacol Ther,* 2010 [18]
	Poor clinical response in UC	Arias T, *Clin Gastroenetrol Heaptol,* 2015 [19]
	Increase faecal loss of anti-TNF antibodies in severe UC	Brandse J, *Gastroenterology,* 2015 [20]
Sarcopenia	Fatigue and reduced physical performance	Van Langenberg D, *JCC,* 2014 [21]
	Osteopenia, pathological fractures, hospitalisation and reduction of mobility	Bryant R, *Aliment Pharmacol Ther,* 2015 [13]
	Correlates with UC disease activity	Zhang T, *Clin Nutri,* 2017 Dec [22]
	Associated with increased risk of colectomy	
	Predicts intestinal resection in CD	Bamba S, *Plos,* 2017 June [23]
	Associated with anti-TNF failure	Holt A, *Eur J Clin Nutri,* 2017 June [24]
	Predicts primary non-response to anti-TNFs	Ding N, *Aliment Pharmacol Ther*, 2017 [25]
Visceral adiposity	VAT in CD is associated with pro-inflammatory gene expression	Zulian A, *Gut,* 2012 [26]
	Bacterial translocation may stimulate CRP production by VAT in CD	Peyrin-Biroulet L, *Gut,* 2012 [27]
	A higher visceral-to-subcutaneous fat predicts post-operative surgical morbidity, PO disease recurrence, fistula, strictures and complicated Crohn's disease	Connelly TM, *Dig Surg,* 2014 [28]
		Ding Z, *Colorectal Dis,* 2016 [29]
		Erhayiem B, *Clin Gastroenterol Hepatol,* 2011 [30]
		Uko V, *Inflamm Bowel Dis,* 2014 [31]
	VAT volume associated with a significant increase in the risk of penetrating disease and surgery in CD	Van Der Sloot, *Inflamm Bowel Dis,* 2017 Jan [32]

clinical remission rates has been seen to extend beyond the first year (OR 2.93, $p < 0.01$) with concomitant use of PEN (600–1500 K calories/day) [55]. It is plausible that the addition of PEN improves therapeutic efficacy of IFX by reducing inflammatory load, although this is yet to be proven in prospective large studies with more stringent outcomes including calprotectin and endoscopy.

PEN with a low-fat diet has been associated with reduced endoscopic recurrence and consequently reduced need for anti-TNFs following ileal and Ileo-colonic resection, compared to an unrestricted diet in a long-term prospective adult study [56, 57]. These results need to be replicated in large prospective therapeutic dietary intervention studies in the setting of post-operative CD recurrence, exploring biomarkers, microbiota alterations and endoscopic surveillance in the intervention vs. control group.

18.5.3 Other Anti-inflammatory Exclusion Diets

The major principle behind EEN's efficacy likely relates to the complete avoidance of antigenic factors in normal diet as suggested by studies which have reported a much lower efficacy of partial enteral nutrition (50% daily calories given through EN) compared to exclusive EN (100% EN). Further evidence that remission rates are influenced by the relative contribution of EEN and normal diet comes from recent paediatric studies reporting higher clinical efficacy when 80–90% calories are received through EN

compared to 50% EN (PEN) in CD [58, 59]. The notion that consumption of certain normal dietary factors may perpetuate inflammation has led to multiple dietary intervention studies with improvement in symptoms, and in some cases biomarkers, but robust endoscopic remission outcomes are lacking (Table 18.3). In addition, there are inherent limitations in conducting and interpreting exclusion diet trials, as they cannot be blinded or placebo controlled.

18.5.4 Biomarkers of Therapeutic Response to Nutritional Therapy

EEN induced clinical remission in CD and closely parallels rapid improvement of surrogate systemic inflammatory markers such as CRP, ESR and albumin [68–71]. The contention that resolution of the systemic inflammatory response in CD during EEN occurs as a consequence of improvement in gut inflammation has been rigorously tested in several studies. Fell et al. were first to comprehensively demonstrate that improvement of endoscopic and histological CD activity closely coincides with fall of mucosal pro-inflammatory cytokines mRNA (IL-1, IFN-γ, IL-8) in children before and after 8 weeks EEN [69]. Another study in adults with active CD also demonstrated reduced mucosal pro-inflammatory cytokines IL-1β, IL-6, IL-8 and TNF-α compared with normal healthy control levels after 4 weeks EEN [45]. In addition, EEN corrects perturbed anti-inflammatory/pro-inflammatory regulatory responses as indicated by an increasing IL-1 ra (IL-1 receptor antagonist)/IL-1 β ratio in those with endoscopic and histological healing [45]. Direct anti-inflammatory effects of polymeric formula (PFA) have

Table 18.3 Therapeutic diets in inflammatory bowel disease

IBD type/diet	Main components	Results	Author
Crohn's disease CD-TREAT Cross-over RCT adults	Ordinary food-based diet similar composition to EEN vs. EEN	Increase stool pH, increase sulphide and harder stool consistency and bacterial community shifts similar between CD-TREAT and EEN	Svolos, *Gastroenterology*, Dec 2018 [60]
Crohn's disease CDED+ partial EN in children/adults	Exclude Gluten, dairy Animal fat, processed meat Emulsifiers, canned food	Remission Clinical 33/47 (70%), CRP remission = 21/20 (70%)	Sigall-Boneh R, *Inflamm Bowel Dis,* 2014 [61]
CDED+ partial EN after failure of anti-TNF		Remission week 6 = HBI < 5, 13/21 (62%)	Sigall-Boneh R, *JCC,* 2017 [62]
IBD SCD in children	Exclude Processed sugar Canned vegetables All grains Potatoes, yams, starchy foods, chickpeas, bean sprouts, and soybeans Canned/processed meats, all milk, high-lactose cheeses, commercial yogurt	Clinical improvement 7 CD	Cohen S, *JOGN,* 2014 [63]
		26 (20 CD, 6UC) – symptoms improvement	Obih, *Nutrition,* 2016 [64]
		1/7 (lleo-colonic mucosal healing)	Wahbeh G, *IPGN,* 2017 [65]
Crohn's disease EEN followed by food antigen–based diet exclusion for disease remission	A significantly enhanced immune response to rice, tomato, egg white/egg yolk and maize was observed in CD patients	CD relapsed in 12.5% of the exclusion group vs. 25% of the control	Wang G, *Clinics and Research in Hepatology and Gastroenterology,* Nov 2017 [66]
Low FODMAP diet in IBD	Fermentable sugars	Improves functional bowel symptoms in patient with IBD	Prince, *Inflamm Bowel,* 2016 [67]

been further elucidated in an in vitro experiment of intestinal epithelial cells (IEC) whereby a PFA-induced anti-inflammatory response persisted even after the separation of PFA from pro-inflammatory stimuli in a two-compartment model [72, 73]. EEN's direct anti-inflammatory properties are specific to CD as demonstrated by an in vitro study incubating elemental diet with biopsies of adult patients with CD, UC and control samples that observed an increased ratio of the anti-inflammatory/pro-inflammatory cytokines (IL-1 receptor antagonist/IL-1β) only in tissue with CD [73].

18.5.5 Faecal Calprotectin: A Reliable Biomarker of Therapeutic Response in CD to EEN

Faecal calprotectin (FC) is an established surrogate faecal marker that strongly correlates with endoscopic and histological disease activity [74]. Normalisation of FC is a dependable marker of MH, suggested by a colonoscopy study in asymptomatic adult CD patients where a normal FC reliably predicted histological remission in 85% of patients [75]. A FC ≤ 250 μg/g correlates with endoscopic remission (defined as CDEIS ≤ 3) with 94.1% sensitivity and 62.2% specificity (PPV 48.5%, NPV 96.6%) [76]. There is limited data on therapeutic efficacy and risk prediction analysis based on post-induction FC in IBD, with early normal vs. elevated (FC < 100 μg/g vs. >100 μg/g) associated with sustained 1-year clinical remission (84% vs. 38%) [77]. There are multiple paediatric studies reporting post-EEN induction drop in FC with an estimated 26% achieving FC levels under <250 μg/g (Table 18.4) [59, 78–82]. Further robust validation studies to examine quantitative drop in FC that reliably predicts early endoscopic response to EEN and clinical outcomes beyond the first year are essential.

Table 18.4 Faecal calprotectin changes following EEN induction therapy

Faecal calprotectin	Results	Study
FC < 250 mcgm/gm of stool	10/45 (EEN), 26/62 (anti-TNF)	Lee [59]
	14/44 (EEN)	Gerasimidis [78]
	19/76 (CS) vs. 6/30 (EEN)	Frivolt [79]
	3/17 (PF/EF)	Grogan [80]
	7/21 (EEN)	Levine [81]
	4/14l (EEN)	Grover [82]
	Overall: 44/171 = 26%	

18.6 Microbiome and Metabolome Changes on Therapeutic Diets: Biomarkers of Therapeutic Response

18.6.1 Microbiome in CD

There is a large body of evidence that "dysbiosis" contributes to the pathogenesis of IBD. The human microbiota is characterised by four major phyla: *Firmicutes*, *Bacteroidetes*, *Proteobacteria* and *Actinobacteria*. CD is characterised by a reduction in overall biodiversity. Many studies consistently report an overall reduction in *Firmicutes* and increase in *Proteobacteria* [83] in CD. A recent large European cohort study employing high-throughput DNA sequencing confirmed stool microbiota signatures highly specific to adult CD, with predominant loss of beneficial *Faecalibacterium*, *Christensenellaceae*, *Methanobrevibacter* and *Oscillospira* and a gain of pathobionts

Fusobacterium and *Escherichia* [84]. On the other hand, in treatment-naïve children with CD [85], the mucosa-associated microbiota (MAM) was characterised by an abundance of "pathobionts" including *Enterobacteriaceae*, *Pasteurellaceae*, *Fusobacteriaceae* and *Veillonellaceae* and, to a lesser degree, a reduction in symbionts such as *Bifidobacteriaceae*, *Erysipelotrichales* and *Clostridales*. There are significant variations across studies that report the specific microbiota changes in CD which reflects the differences in methodology (gradient gel electrophoresis to more recent next-generation sequencing), sample size and nature (i.e. stool vs. mucosa), disease activity (active vs. inactive) and other confounding factors including treatments and disease duration (new vs. established CD).

18.6.2 Clinical and Experimental Evidence of Microbiome Modulation with EEN

Modification of the intestinal microbiota is proposed as a therapeutic mechanism of action of EEN in CD. Previous investigations relying on culture-driven methods failed to confirm microbiota modification following EEN [86, 87]. However, the availability of culture-free techniques like PCR/DGGE and PCR/TGGE has made it possible to further investigate this putative mechanism [88]. Most recent paediatric studies have utilised next-generation sequencing targeting 16S rRNA [89–97]. Multiple studies exploring the stool microbiota during EEN in paediatric CD have reported reduction in bacterial alpha-diversity, loss of *Firmicutes*, *Bacteroides*/*Prevotella* and increase in *Bacteroidetes* (Table 18.5). Another consistent report is of a greater reduction in biodiversity in those with the greatest improvement in clinical disease activity scores.

These alterations of the gut microbiota in response to EEN include the depletion of specific bacteria such as *Faecalibacterium prausnitzii*, which is widely considered to be beneficial for its anti-inflammatory properties [89, 93, 100], although not all studies report a depletion of this beneficial species after EEN [90, 95].

The mucosa-associated microbiome (MAM) is now accepted as distinct from the faecal microbiome [85, 101], and there are currently no published studies correlating post-EEN induction endoscopic disease activity scores and parallel changes in MAM. A single case report described the ileal microbiome changes pre- and post-EEN in a newly diagnosed child with CD, compared to a single healthy control [96]. The pre-EEN MAM showed reduced microbial diversity, increased levels of *Proteobacteria* and lower levels of *Bacteroidetes* species compared to the healthy controls, and these changes corrected post-EEN to become more similar to healthy controls. However, a recent observational study examining longitudinal mucosa-associated microbiome (MAM) changes at diagnosis and after completion of EEN from eight treatment-naïve children diagnosed with CD suggests a significant reduction in MAM following EEN. Although previous studies indicated reduction in stool microbial diversity post-EEN to be a crucial step associated with clinical remission, this is the first study to document significant reduction in mucosal microbial diversity to be associated with complete mucosal healing [97].

In summary, while EEN promotes MH in many patients with CD, there are conflicting results arising from limited studies of the changes in stool microbiota and MAM during EEN and a lack of published microbiome studies paired with endoscopic mucosal assessment data.

18.6.3 Metabolomic Changes on EEN and Structured Diet

Alterations of the gut microbial metabolic functional activity on EEN have been reported in recent studies. Reductions have been observed in faecal butyric acid, genetic expression of biotin and thiamine biosynthetic pathways. In contrast an increase in stool pH, stool sulphides and spermidine/putrescine biosynthesis are documented [94, 100]. Dunn et al. recently reported an increase in the functional metagenomics path-

Table 18.5 EEN-induced changes in stool microbiota

Author	Samples	Methods	Results (changes post EEN)
Lionetti 2005 [98]	9 CD, 5 controls	Gel electrophoresis	Reduced bacteria diversity
Leach 2008 [99]	6 CD, 7 controls	Gel electrophoresis	Reduced bacterial diversity Reduction in activity = reduction in *Bacteroides*/*Prevotella*
Gerasimidis 2014 [94]	15 CD,7 controls	16S rRNA, metagenomics	Reduced clinical activity = reduced *Bacteroidetes* Reduction in *F. prausnitzii* post EEN
Quince 2015 [100]	23 CD, 21controls	16S rRNA, metagenomics	Reduction in microbial diversity Reduced *Bifidobacterium*, *Ruminococcus*, *Faecalibacterium* Increase in *Lactococcus*
Schwerd 2016 [90]	8 CD	16S rRNA	Reduction *Bacteroidetes* Increase in *Firmicutes – Christensenellaceae*, *Ruminococcus*
Kaakoush 2015 [89]	5 CD	16S rRNA HTS	Reduction diversity = clinical improvement Clinical activity = reduction in *Lachnospiracea* and Ruminococcaceae
Dunn 2017 [92]	10 CD, 5 controls		Sustained remission (SR) = reduced diversity SR = increase in *Akkermansia*, *Lachnospiracea*, *Bacteroides* Poorly sustained remission = increase in *Proteobacteria*
Lewis 2015 [91]	22 CD	16S rRNA HTS	Decrease in *Dialister*, *Dorea*, *Streptococcus, Haemophilus* Increase in *Alistipes*
Jia 2010 [93]	20 CD	16S rRNA	Reduction in bacterial diversity Reduction in *F. prausnitzii*

ways involved in the degradation of environmental pollutants and xenobiotics and reduction in NOD-like receptor signalling in those with a poor clinical response to EEN [92].

There are only limited data on metabolomic changes during dietary interventions. In treatment-naïve children with CD, stool metabolic alterations similar to those seen in children on EEN were observed on a CD-TREAT diet, an ordinary diet with a nutritional composition identical to EEN. As we look to using more normal, less restrictive diets than EEN in CD, these observations are encouraging but preliminary. They do not provide the biological explanation of response to these dietary measures. Furthermore, a more objective endpoint of response such as mucosal or endoscopic healing is required to establish therapeutic efficacy of dietary interventions. It is also possible that such metabolic shifts may simply reflect functional gut adaptations to EEN or a structured elimination diet. To better characterise underlying functional metabolomics mechanisms associated with greater mucosal remission, endoscopic assessment and biopsies should be obtained before and after dietary interventions and on normal diet.

18.6.4 MicroRNA: New Biomarker of Nutritional Response

MicroRNAs (miRNAs) are a class of endogenous, small, noncoding RNAs that play a key role in programming cellular responses to environmental stresses by regulating gene expression [102]. miRNAs are remarkably stable so serve as excellent biomarkers. Specific miRNA expression associated with multiple immune pathways are reported in IBD, including epithelial barrier function (e.g. miR-21, miR-150, miR-200b) [103–105], autophagy (e.g. miR-30c, miR-130a, miR-106b, miR-93, miR-196) [106–108] and nuclear factor kappa B pathway (miR-146a, miR-146b, miR-122, miR-132, miR-126) [109, 110]. miRNA expression is impacted by macro- and micronutrient; hence miRNA profiling can pro-

vide valuable clues underpinning therapeutic mechanisms [111].

In an adult study, ileal mucosal miRNA expression profiles were measured before and after EEN induction, and results were correlated with Crohn's clinical disease activity index (CDAI) and CRP levels. Mucosal hsa-let-7b-5p was upregulated after EEN and this positively correlated with serum CRP levels. Lack of correlation of ileal mucosal miRNA expression profiles with harder endpoints like faecal calprotectin and endoscopically defined mucosal healing are required to establish them as therapeutic biomarkers [112].

18.6.5 Nutrigenomics

Nutrigenomics is the emerging field of study exploring effects of diet on gene expression, structure and function of proteins and metabolites on human health. The role of dietary interventions in IBD to better understand each individual's response to these treatments and resulting change in genetic expression remains an area of significant interest and will pave the way for individualised dietary prescriptions.

However, to our knowledge no prognostic or predictive biomarkers of response to dietary interventions for IBD have been identified.

In conclusion, the role of diet in management of IBD remains a high research priority. Future dietary therapeutic studies will need to employ composite clinical endpoints such as mucosal and endoscopic remission to measure treatment success and correlate these with composite biophysical endpoints including microbiologic, metabolic, genomic, cytokine and nutrigenomic signatures.

18.7 Future Directions

The future of research into diet and IBD is exciting, and some key research priorities have recently been summarised in an excellent summary compiled by the D-ECCO working group [113]. As our understanding of the relationship between diet, nutrition and gut health evolves, we expect to see major advances in the role of dietary patterns and constituents in the development, treatment, cure and finally prevention of IBD. We expect these to be individualised and related to the genetic and transcriptomic signatures of each disease phenotype and each individual. These aims require a longitudinal systems biology approach starting with more targeted studies of the impact of diet on disease activity in particular improving intestinal inflammation and sustaining mucosal remission. Moreover there is a need for functional assessment and outcome measures to evaluate efficacy of nutritional intervention.

Summary box of key points is required:

Summary Points

- Loss of lean body mass (LBM) and mesenteric fat deposition (MFD) can contribute to higher inflammatory burden, poor therapeutic response to anti-TNFs and greater risk for intestinal surgery.
- Exclusive enteral nutrition (EEN) is the most well-established therapeutic diet in CD capable of inducing mucosal healing rates compared to conventional steroids.
- Efficacy of dietary interventions can only be accurately measured by including composite clinical endpoints like mucosal and endoscopic remission.
- A targeted longitudinal systems biology approach combining microbiologic, metabolic, genomic, cytokine and nutrigenomic signatures is required to better characterise therapeutic role of diet in IBD.

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19 Therapeutic Drug Monitoring in Inflammatory Bowel Disease: Optimising Therapeutic Effectiveness of Thiopurines

Ashish Srinivasan, Peter De Cruz, and Daniel van Langenberg

Abstract

Azathioprine (AZA) and 6-mercaptopurine (6-MP) remain important therapeutics in the management of Crohn's disease (CD) and ulcerative colitis (UC). Despite their clinical effectiveness, thiopurines present clinicians with several challenges including their narrow therapeutic index and risk of adverse reactions. These factors account for high rates of discontinuation, underscoring the importance of optimal dosing strategies geared towards maximising clinical effectiveness and minimising intolerances.

There are a number of methods to optimise therapy including measuring thiopurine-S-methyltransferase (TPMT) genotype or phenotype, manipulating metabolism through the addition of allopurinol in shunters, and splitting the thiopurine dose to reduce adverse effects. Furthermore, 6-MP has been established as a safe and effective alternative to AZA intolerance, while thioguanine presents an alternative in patients intolerant of either AZA or 6-MP.

Understanding their pharmacokinetic profile and acknowledging inter-patient variations also remain important, particularly given thiopurine metabolite levels have been shown to correlate poorly with dose. Despite the lack of high-quality, supportive data, there is sufficient evidence to suggest that targeting therapeutic 6-thioguanine nucleotide (6-TGN) levels in the setting of active disease is worthwhile, particularly given that sub-therapeutic levels expose patients to side effects without comparative effectiveness. However, based on current evidence, recommending proactive thiopurine metabolite monitoring and optimisation relative to standard weight-based dosing remains uncertain. Thiopurines have also been shown to be useful when used in combination with antitumour necrosis factor-α (anti-TNF) agents, although optimal dosing and 6-TGN in this context remain to be clearly defined. Metabolite testing also plays an important role in evaluating suboptimal response, poor adherence, and/or identifying the cause of suspected toxicities, all of which provide valuable information to direct clinical decision-making.

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This chapter will concentrate on thiopurine optimisation in inflammatory bowel disease (IBD), with a particular focus on aspects of thiopurine metabolite monitoring to guide clinical decision-making.

19.1 Thiopurines

19.1.1 Thiopurines in IBD

Thiopurines play an important role as steroid-sparing agents capable of both inducing and maintaining remission across both Crohn's disease (CD) and ulcerative colitis (UC), particularly given that up to 50% of patients develop steroid-dependent or refractory disease despite first-line therapy [1]. While more supportive data exists for their use in CD, studies have demonstrated thiopurine efficacy in maintaining steroid-free remission in steroid-dependent UC patients [2, 3]. Furthermore, thiopurines tend to be introduced earlier in CD than UC given the lack of evidence for aminosalicylate use in CD [4]. Studies such as the Study of Biologic and Immunomodulator Naive Patients in Crohn's Disease (SONIC) have also highlighted the benefit of thiopurine co-therapy alongside anti-TNF therapy in optimising treatment outcomes [5].

Despite their clinical effectiveness, thiopurines present clinicians with several challenges including their narrow therapeutic index and risk of adverse reactions. Although up to 60% of IBD patients are prescribed thiopurines at some point, these factors account for high rates of discontinuation within 5 years of initiation [6–8].

19.1.2 Thiopurine Pharmacology

19.1.2.1 Metabolism

Thiopurine metabolism reflects a complex intracellular process through which the prodrug azathioprine is rapidly absorbed after oral ingestion and converted to 6-mercaptopurine (6-MP) via non-enzymatic pathways. Subsequent metabolism of 6-MP to its active thioguanine nucleotides (TGNs) metabolite requires multiple enzymes, chief amongst them thiopurine-S-methyltransferase (TPMT) which catalyses the methylation of 6-MP to an inactive metabolite 6-methylmercaptopurine (6-MMP), xanthine oxidase (XO) which catalyses 6-MP to inactive 6-thiouric acid (6-TU), and hypoxanthine-guanine phosphoribosyltransferase (HPRT) which converts 6-MP into the active metabolite 6-TGN (Fig. 19.1) [9–11].

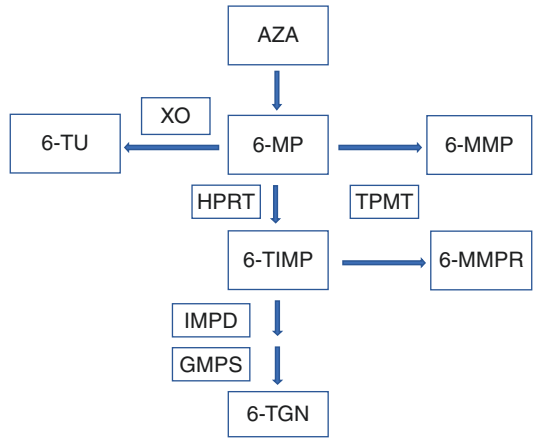

Fig. 19.1 Thiopurine metabolism. *AZA* azathioprine; *6-MP* mercaptopurine; *XO* xanthine oxidase; *6-TU* thiouric acid; *TPMT* thiopurine-S-methyltransferase; *6-MMP* 6-methyl mercaptopurine; *HPRT* hypoxanthine-guanine-phosphoribosyltransferase; *6-TIMP* 6-thioinosine 5-mono 6-methyl-mercaptopurine ribonucleotides phosphate; *6-MMPR* 6-methyl-mercaptopurine ribonucleotides; *IMPD* inosine monophosphate dehydrogenase; *GMPS* guanosine monophosphate synthetase; *6-TGN* 6-thioguanine

19.1.2.2 Mechanism of Actions

Thiopurines primarily exert their immunosuppressive effects through their active 6-TGN metabolite, which acts to inhibit purine and protein synthesis in lymphocytes [9, 12, 13]. Both AZA and 6-MP have also been shown to inhibit B and T lymphocyte proliferation, resulting in diminished production of plasma cells and cytotoxic T lymphocytes.

19.1.3 Pharmacogenomics

Altered thiopurine metabolism has been associated with variations in clinical effectiveness, with TPMT genotype/phenotype exemplifying a test predictive of different clinical outcomes across a wide population.

19.1.3.1 TPMT Genotype and Phenotype

Genetic polymorphisms in TPMT enzyme activity are inherited and can be both qualitatively and quantitatively measured. Low and intermediate TPMT enzyme activity is noted in 0.3% and 11.1% of individuals, respectively, with about 15% considered rapid metabolisers owing to increased TPMT activity [14, 15]. Low and intermediate TPMT enzyme activity leads to preferential production of the active 6-TGN metabolite, while high TPMT enzyme activity tends to shunt 6-MP towards preferential 6-MMP production. Evaluating TPMT phenotype rather than genotype offers the advantage of being able to detect all 'high-risk' completely deficient patients which may not be accounted for owing to rare or variant genotypes.

Generally, thiopurines are avoided in those with low (homozygous genotype) TPMT activity owing to risks of myelotoxicity, although therapeutic 6-TGN concentrations can still be achieved while minimising myelotoxicity in those with intermediate (heterogeneous genotype) TPMT enzyme activity by starting at lower doses [16, 17]. Conversely, patients with high TPMT enzyme activity often remain treatment refractory despite supranormal thiopurine doses, with hypermethylation occurring in those with high TPMT activity, predisposing to 6-MMP-induced complications such as hepatotoxicity associated with MMP:TGN ratios above 11 [18]. Accordingly, screening TPMT genotype or phenotype prior to AZA or 6-MP prescription has been used to predict those at greater risk of toxicity.

However, randomised control data evaluating the merits of routine pretreatment TMPT testing relative to empiric weight-based dosing has not demonstrated significant advantages in achieving clinical remission (RR, 1.03; 95% CI, 0.84–1.27) or preventing complications such as myelotoxicity (RR, 0.94; 95% CI, 0.59–1.50) and treatment cessation (RR, 1.09; 95% CI, 0.94–1.27) [19–21]. Conversely, routine TPMT testing was associated with an 89% risk reduction in cytotoxic adverse effects amongst those with intermediate or low TMPT activity, highlighting the potential role of pretreatment testing in identifying patients with an abnormal TPMT profile [19–21].

19.1.3.2 Additional Factors

Nevertheless, it remains that the majority of patients with adverse reactions to AZA and 6-MP have normal TPMT activity, with comorbidities, co-therapeutics, and other anomalies in thiopurine metabolism attributed [22, 23]. Other genetic polymorphisms associated with inosine triphosphate pyrophosphatase (ITPA) and NUDT15 have also been implicated in variant thiopurine metabolism, and their role in thiopurine-induced myelosuppression requires further elucidation in population-based studies before they can be recommended in routine clinical practice [24, 25].

19.1.4 Dosing

The molecular weight of 6-MP is 55% that of AZA, and 88% of AZA is converted to 6-MP, accounting for the 0.5 dosing conversion factor used when dosing 6-MP (1–1.5 mg/kg) relative to AZA (2–2.5 mg/kg) by weight [26, 27]. Clinical studies have also demonstrated that thiopurines have a relatively slow onset of action, with a therapeutic response typically realised after a minimum of 8–12 weeks, although some patients may take longer [28]. Traditionally AZA and 6-MP have been commenced at low

Table 19.1 Therapeutic and toxic metabolite levels for azathioprine and 6-mercaptopurine

	Thiopurine metabolites	Reference range
Therapeutic	6-TGN	230–450 pmol/8 × 10^8 RBC [18, 23, 30, 31, 33]
	6-MMP/6-TGN ratio	5–25
Toxic	6-TGN	>450 pmol/8 × 10^8 RBC [41]
	6-MMP	>5700 pmol/8 × 10^8 RBC [23, 41, 42]

AZA azathioprine, *6-MP* 6-mercaptopurine, *RBC* red blood cells

doses with stepwise up-titration given toxicity concerns, albeit at the potential cost of delaying time to therapeutic response. Others have suggested routine testing of TPMT activity may prove cost-effective ($7,142 vs $3,861) given this might facilitate more rapid dose up-titration, thereby achieving a faster time to initial response (22.41 vs 18.96 weeks) [29].

19.1.5 Thiopurine Metabolite Monitoring

Target 6-TGN concentrations between 230 pmol/8 × 10^8 red blood cells (RBCs) and 450 pmol/8 × 10^8 RBCs for thiopurine monotherapy have been promulgated, with higher risk of myelotoxicity associated with 6-TGN concentrations above 400 pmol/8 × 10^8 (Table 19.1). For instance, a 2013 meta-analysis concluded 6-TGN concentrations above 230–260 pmol/8 × 10^8 RBCs were more frequently associated with clinical remission relative to those with levels below this threshold [18, 23, 30–33]. Despite this proposed therapeutic window, studies have demonstrated poor correlations between target 6-TGN concentrations with both clinical response and weight-based dosing. Hence despite the appeal of dosing to a therapeutic threshold to achieve treatment targets, this approach remains inconclusive. Nevertheless, metabolite testing also plays an important role in evaluating suboptimal response, poor adherence, and identifying the cause of suspected toxicities. For instance, studies have indicated thiopurine non-response and toxicity to be associated with high 6-MMP concentrations, particularly above 5700 pmol/8 × 10^8 RBCs, or with elevated 6-MMP/6-TGN ratios, reiterating the need for routine monitoring of liver biochemistry [23, 34].

The practice of reactive thiopurine metabolite testing in response to active IBD is supported by retrospective studies and society guidelines, with favourable responses noted following algorithm-driven thiopurine dose optimisation to achieve 'therapeutic' metabolites (i.e. within the proposed therapeutic window) relative to algorithm-discordant intervention (RR, 5.15; 95% CI, 1.82–14.56) [35, 36]. While pooled analysis across two small trials demonstrated a higher proportion of clinical remission at week 16 using thiopurine metabolite guided dosing (42%) compared to standard weight based dosing (31.6%), these findings did not reach statistical significance (RR, 1.44; 95% CI, 0.59–3.52) [37–39]. There were also comparable rates of serious adverse events requiring therapy discontinuation using both approaches (RR, 1.20; 95% CI, 0.50–2.91). Furthermore, optimal 6-TGN concentrations have not been established when thiopurines are used in combination with anti-TNF therapy, although preliminary studies have suggested that thiopurine dose reduction, with resultant lower metabolite levels, may not adversely impact infliximab trough levels [40]. Based on current evidence, recommending proactive thiopurine metabolite monitoring and optimisation relative to standard weight-based dosing remains uncertain.

19.1.6 Alternatives to Thiopurine Metabolite Monitoring

Mean corpuscular volume (MCV) of peripheral RBCs has also been suggested as a surrogate measure of TGN levels, with an MCV increment of 7 or more associated with increased likelihood of steroid-free remission, mucosal healing, and infliximab concentrations of 3 mcg/ml or more on post hoc analysis of SONIC patients [43]. Since thiopurine treatment requires routine blood monitoring, there is ample opportunity to monitor peripheral MCV as a proxy for thiopurine metabolite testing.

19.1.7 Therapeutic Manipulation of Thiopurine Metabolites

19.1.7.1 Allopurinol

A subset of patients treated with AZA or 6-MP preferentially metabolise 6-MP into 6-MMP compared to 6-TGN. Dose escalation with the target of therapeutic 6-TGN levels can place these patients at risk of 6-MMP-related toxicities, particularly hepatotoxicity [18]. Co-prescription of allopurinol 100 mg together with a 25–50% dose reduction in thiopurine therapy represents an effective optimisation strategy by preferentially redirecting 6-MP metabolism towards 6-TGN rather than 6-MMP production by utilising allopurinol-mediated xanthine-oxidase inhibition. This strategy has proven safe and effective across both CD and UC, with demonstrated reductions in corticosteroid use, adverse effects, and improvements in disease activity indices [44, 45]. Although the benefit of first-line combination allopurinol-thiopurine relative to standard thiopurine monotherapy remains unclear, we remain hopeful that upcoming studies will address this unanswered but clinically important question.

19.1.7.2 Aminosalicylates

Aminosalicylates cause in vitro inhibition of TPMT [46]. When co-prescribed with AZA or 6-MP, aminosalicylates alter thiopurine metabolism by increasing 6-TGN production without significant deviation in 6-MMP concentrations although the precise mechanism through which this occurs is uncertain [47, 48]. This remains particularly relevant in ulcerative colitis, where thiopurines are commonly used in conjunction with aminosalicylates, although there is no evidence to suggest that their concurrent use with thiopurines results in superior disease outcomes [49].

19.1.8 Overcoming Intolerance

19.1.8.1 Split-Dose Administration of Thiopurines

While dose reduction can obviously mitigate many side effects including those related to high 6-MMP levels, this can also compromise therapeutic effectiveness via lower 6-TGN levels. Alternatively, splitting the daily thiopurine dose appears to reduce 6-MMP levels and side effects without adversely affecting disease activity given therapeutic 6-TGN levels are still maintained [50]. This approach also potentially avoids additional side effects associated with alternatives such as introduction of allopurinol or escalation to biologic therapy.

19.1.8.2 Use of 6-MP in Patients Who Are Intolerant of AZA

Studies have demonstrated that 6-MP can be used safely and effectively as an alternative in patients who demonstrate intolerance to AZA, although patients who stopped AZA secondary to hepatotoxicity and pancreatitis were less likely to tolerate 6-MP [51]. The success of this switch varies considerably, and given similarities between AZA and 6-MP, many reactions occur with both drugs.

19.1.8.3 Thioguanine

In those unable to tolerate either AZA or 6-MP, 6-thioguanine (6-TG) has been proposed as a possible alternative [52]. Its use has been tempered by the putative risk of hepatic nodular regenerative hyperplasia, especially with higher doses; however, data are conflicting [53, 54]. Although 6-TG has the advantage of bypassing several metabolic steps implicated in toxicity of AZA and 6-MP, the absence of formal dose-ranging studies and a defined relationship between 6-TGN levels (often far higher than that seen with AZA or 6-MP) and 6-TG response is lacking [41, 54].

19.1.9 De-escalation of Thiopurine Monotherapy

Re-evaluating the need for ongoing therapy remains important, particularly given that duration of thiopurines prescription has been linked to risks of rare albeit serious complications such as lymphoma. Evidence supporting dose reduction of thiopurine monotherapy in the setting of clinical remission is lacking, and similarly, thiopurine discontinuation has also been associated with high rates of clinical relapse. Trials have demonstrated

higher rates of relapse after azathioprine cessation relative to ongoing therapy ranging from 16.5% to 53% and approaching 60% at 12 months following discontinuation across CD and UC, respectively [55–59]. This suggests that the decision to continue or discontinue thiopurines needs to be individualised based on relative risks and benefits, although, once the decision to stop therapy has been made, gradual dose reduction rather than abruptly stopping thiopurine monotherapy therapy has not been shown to reduce relapse. Conversely, thiopurine dose reduction when used in combination with infliximab did not appear to significantly impact infliximab drug levels and antidrug antibodies in a single underpowered study with 12 months of follow-up, highlighting that these findings need to be reproduced across larger cohorts [40].

19.1.10 Combination Therapy: Thiopurine and Anti-TNF

As per the SONIC study, the combination of AZA and infliximab achieved higher rates of steroid-free remission and mucosal healing in moderate to severe CD than monotherapy with either agent alone in biologic-naïve patients. Interestingly, combination adalimumab therapy did not demonstrate similar findings, although many believe these findings are applicable across the anti-TNF class [60, 61]. Similar findings in UC were also noted in the UC SUCCESS trial, albeit over a shorter duration of follow-up [62]. Nonetheless, caution must be exercised given the potential risks associated with combination immunosuppression, such as increased risk of lymphoma and opportunistic infections [63, 64]. Moreover it remains unclear as to whether the benefit of this therapeutic combination persists beyond 12 months, suggesting the need for ongoing thiopurine co-therapy may be reassessed sooner than for thiopurine monotherapy [65, 66].

19.2 Conclusions

Thiopurines remain an important therapy in the management of both CD and UC with proven effectiveness as monotherapy or combination therapy with aminosalicylates and/or anti-TNF agents, particularly infliximab. Thiopurine optimisation strategies remain important in improving clinical effectiveness and reducing rates of discontinuation attributable to toxicity. There are a number of methods to optimise therapy including measuring TPMT genotype or phenotype, manipulating thiopurines metabolism through the addition of allopurinol in shunters, and splitting thiopurine doses to reduce adverse effects. Understanding their pharmacokinetic profile and acknowledging inter-patient variations is important, particularly given metabolite levels correlate poorly with thiopurine doses. Despite the lack of high-quality supportive data, there is sufficient evidence to suggest that targeting therapeutic 6-TGN levels in the setting of active disease is worthwhile, particularly given that subtherapeutic levels expose patients to side effects without comparative effectiveness (Table 19.2).

Summary Points

- Thiopurines are capable of inducing and maintaining remission in Crohn's disease and ulcerative colitis.
- Pretreatment screening using TPMT genotype or phenotype remains useful in identifying those at high risk of toxicity.
- Targeting therapeutic 6-TGN levels in the setting of active disease is worthwhile.
- Metabolite testing plays an important role in evaluating suboptimal response, poor adherence, and identifying the cause of suspected toxicities.
- Thiopurine metabolism can be manipulated through the addition of allopurinol or dose splitting.
- Inter-patient variations in thiopurine metabolism are common, particularly given metabolite levels correlate poorly with thiopurine doses.
- Thiopurine co-prescription alongside anti-TNF therapy has been associated with improved treatment outcomes.

Table 19.2 A practical approach to the interpretation of thiopurine metabolites

Patient subgroup	6-TGN	6-MMP	Suggested intervention(s)
Low/intermediate TPMT activity	↑↑	↓	Consider low-dose AZA or 6-MP (e.g. intermediate) Consider alternate IM or escalation to biologic (e.g. low)
Thiopurine overdose Thiopurine refractory disease	↑↑	↑↑	Stop and/or consider lower dose AZA or 6-MP Consider alternate IM or escalation to biologic
Poor compliance	↓↓	↓↓	Patient education Reiterate importance of compliance
Suboptimal dosing	↓	↓	Increase thiopurine dose
Pharmacologic-resistant disease (e.g. high TPMT activity)	↓	↑↑	Consider allopurinol with low dose AZA or 6-MP Consider alternate IM or escalation to biologic
Preferential 6-MMP producer Hypermethylator	↓	↑↑	Consider allopurinol with low dose AZA or 6-MP Consider dose splitting

TPMT thiopurine-S-methyltransferase, *AZA* azathioprine, *6-MP* 6-mercaptopurine, *IM* immunomodulator

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20 Therapeutic Drug Monitoring in Inflammatory Bowel Disease: Optimising Therapeutic Effectiveness of Biologics

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Abstract

Biologic therapies have proven effective in inducing and maintaining remission across both Crohn's disease (CD) and ulcerative colitis (UC). Treatment algorithms incorporating therapeutic drug monitoring (TDM), including assessment of drug levels and antidrug antibodies (ADA), have been advocated to improve outcomes.

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The utility of TDM is increasingly recognised; however, there has been much debate regarding the benefits of a reactive versus proactive approach to measuring drug levels to guide intervention. Several studies have documented favourable clinical, biochemical and endoscopic outcomes with higher drug levels. Although TDM targets to achieve clinical remission during maintenance anti-tumour necrosis factor-α (anti-TNF) therapy are well developed, an evidence-based approach to utilise TDM to assess and intervene following anti-TNF induction is lacking. Furthermore, studies have documented that higher drug levels should be targeted to achieve more stringent endpoints such as mucosal healing, with higher and/or accelerated biologic dosing strategies demonstrating improved outcomes across clinically aggressive inflammatory bowel disease (IBD) subtypes such as perianal fistulising CD and acute severe UC.

Despite their efficacy, a proportion of patients will demonstrate primary non-response (PNR) and secondary loss of response (SLOR) to biologic therapy, with immunogenicity emerging as an important cause. Understanding the mechanism through which biologic loss of response occurs remains important in directing subsequent therapeutic decisions such as dose escalation, switching biologic agents or classes and considering the addition of immunomodulator

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co-therapy. Preliminary data also reassuringly suggests that the principles of anti-TNF TDM are broadly applicable across newer agents such as vedolizumab and ustekinumab. As treat-to-target algorithms continue to evolve, they should strive to integrate induction and maintenance TDM targets specific to both the disease phenotype and target endpoints.

This chapter will concentrate on aspects of TDM in biologics within IBD, with a particular focus on infliximab and adalimumab given the vast majority of currently published data exists for these agents.

20.1 Biologics

20.1.1 Biologics in IBD

Biologic therapies have demonstrated themselves to be highly effective drugs in the management of both Crohn's disease (CD) and ulcerative colitis (UC). Although anti-tumour necrosis-α (anti-TNF) agents including adalimumab and infliximab were amongst the first biologics shown to induce and maintain remission in inflammatory bowel disease (IBD), several agents including ustekinumab (anti-interleukin (IL)12/23), vedolizumab (anti-α4β7 integrin) and tofacitinib (Janus kinase (JAK) inhibitor) have demonstrated similar efficacy, albeit via different mechanisms [1–6]. This chapter however will focus on adalimumab and infliximab given the vast majority of currently published data exists for these agents. Despite the efficacy of anti-TNF agents in IBD, up to 30% of patients exhibit primary non-response (PNR), with up to a further 50% demonstrating features of secondary loss of response (SLOR) within 12 months, warranting surgery, anti-TNF dose escalation, switching biologic agents or classes, and adding immunomodulator co-therapy [7–9]. Addressing mechanisms underlying both PNL and SLOR remains of critical clinical importance, with therapeutic drug monitoring (TDM) emerging as a useful tool in guiding subsequent therapeutic interventions.

Overall there is much evidence to support measuring anti-TNF drug levels to guide treatment decisions, with several studies documenting favourable clinical, biochemical and endoscopic outcomes with higher drug levels [10–13]. Conversely, unfavourable outcomes such as treatment failure and drug discontinuation are described in association with low or undetectable drug levels. While such findings support a TDM-driven dosing strategy, the lack of a well-defined therapeutic range that correlates with desired clinical endpoints remains a challenge.

20.1.2 Reactive Versus Proactive TDM

Although the utility of TDM is increasingly recognised, there has been much debate regarding the relative benefits of a reactive versus proactive approach to measuring anti-TNF drug trough levels to guide intervention. Variability in disease activity at the time of TDM represents one of the inherent inconsistences when attempting to compare outcomes across reactive and proactive TDM cohorts. The actual benefit of optimising therapy in clinically asymptomatic patients remains unclear, and there is uncertainty as to how best to manage low-titre antidrug antibodies (ADAs) in this setting. These factors have been implicated in necessitating more frequent dose escalation and earlier biologic substitution when employing a proactive TDM approach. Another unresolved issue pertains to the optimal timing of TDM, particularly given the associated cost of the test and any resultant treatment changes. A pragmatic strategy likely lies somewhere in between an 'all or nothing' reactive or proactive approach, with a recent retrospective study demonstrating that proactive infliximab monitoring following initial reactive TDM was associated with improved drug persistence and fewer IBD-related hospitalisations than reactive TDM alone [14]. Such an approach may also more effectively focus intensive proactive monitoring on the patient subset most likely to benefit.

20.1.3 Clinical Application of TDM in IBD

20.1.3.1 Target Anti-TNF Concentrations

Although anti-TNF drug concentrations are typically measured at trough prior to the subsequent maintenance dose, whether this actually best predicts therapeutic response relative to levels measured at other time points is unclear [15, 16]. Target trough levels of 3–7 mcg/ml and 5–10 mcg/ml have been consistently proposed across maintenance infliximab and adalimumab therapy in IBD, respectively [17–25]. These thresholds were largely derived from retrospective data evaluating clinical response and/or remission to maintenance anti-TNF therapy, although subsequent studies have suggested higher drug levels appear to be required to achieve more stringent endpoints such as endoscopic remission with infliximab (6–10 mcg/ml) and adalimumab (8–12 mcg/ml) [26].

Moreover, higher infliximab trough levels have been linked with higher healing rates of perianal fistula in CD. For instance, Yarur et al. concluded that an infliximab trough threshold of ≥10.1 mcg/ml within 4 weeks of endoscopic evaluation and following at least 24 weeks of infliximab therapy was associated with fistula healing, defined as the absence of documented fistula drainage without a seton at endoscopic examination [27]. Interestingly, this study also suggested that levels in excess of 20 mcg/ml were associated with fistula healing across some patients [27]. Indeed, others have demonstrated that thresholds of ≥9.25 mcg/ml (week 2) and ≥7.25 mcg/ml (week 6) during infliximab induction were useful predictors of week 14 fistula response [28]. Similar to perianal fistulising CD, other IBD subtypes with a high degree of inflammatory burden such as acute severe UC have seen many apply higher and/or accelerated dosing strategies to increase anti-TNF drug levels and potentially achieve improved outcomes, though this requires further prospective evaluation [29, 30].

Hence, it remains uncertain whether target anti-TNF trough concentrations are comparable for achieving endpoints across both UC and CD, but also within each disease, where phenotype (e.g. CD: penetrating, stricturing, non-stricturing non-penetrating; UC: acute severe, disease extent) may also represent an important factor. Thus, individualised strategies based on patient, disease and drug characteristics with particular regard to thresholds specific to the target clinical endpoint (e.g. clinical vs endoscopic vs histologic remission) are required.

20.1.3.2 Measuring Drug Levels and Antidrug Antibodies (ADAs)

Clinically useful assays to quantify anti-TNF levels and ADAs include the enzyme-linked immunosorbent assay (ELISA), the homogeneous mobility shift assay (HMSA), the electrochemi-Luminescence assay (ECLIA) and the radioimmunoassay (RIA) [31–33]. Reassuringly, despite their varied methodology, these assays generally result in comparable clinical interventions based on similar drug levels quantified [33–35]. However, the measurement of ADA tends to be far more variable as most assays are not equipped to detect ADAs in the presence of measurable drug, with studies demonstrating dose-dependent assay interference in the presence of high-affinity neutralising ADAs across multiple assays [11, 13, 36]. At present, there is insufficient evidence to support the use of more expensive drug-tolerant assays such as the HMSA to detect ADAs, particularly given that identifying ADAs in the presence of therapeutic drug levels has yet to be proven clinically useful, and there remains a lack of consensus regarding the clinical significance of different antibody titres. There is also emerging evidence to suggest point-of-care anti-TNF assays present an opportunity for quick and reliable drug level results to inform personalised and proactive dosing decisions, although recent publications have highlighted that therapeutic thresholds specific to point-of-care assays may need to be further developed [37, 38].

20.1.3.3 Role of TDM During Clinically Active Disease

Numerous studies have demonstrated that clinical symptoms correlate poorly with objective disease activity in IBD, highlighting the need to use

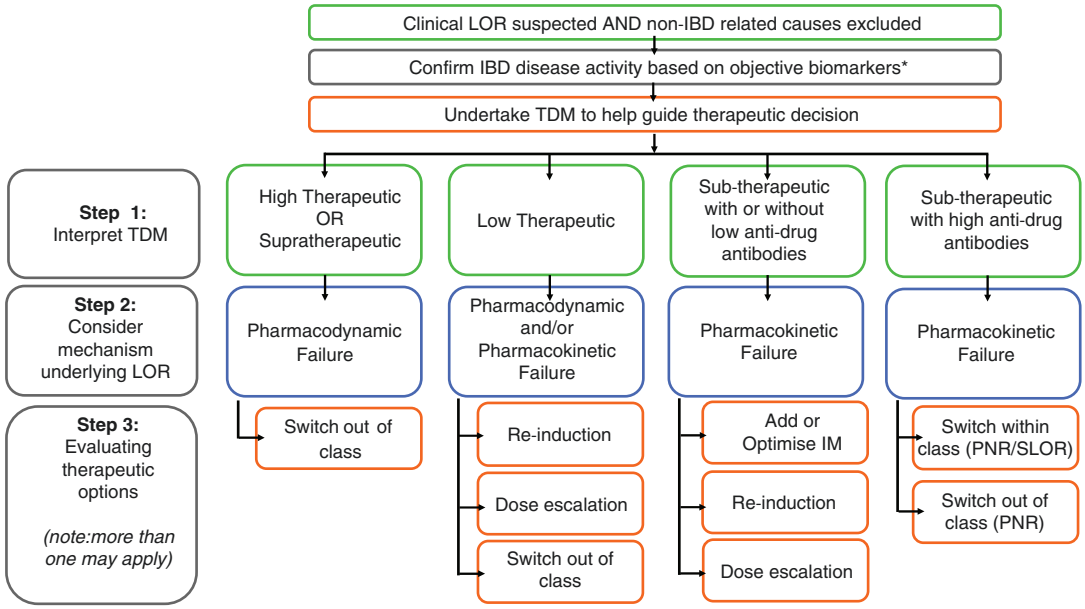

Fig. 20.1 A practical approach to managing loss of response (LOR) to anti-TNF therapy using therapeutic drug monitoring (TDM). *Objective biomarkers refer to assessments of C-reactive protein (CRP), faecal calprotectin, radiologic activity and/or endoscopic activity; *LOR* loss of response, *TDM* therapeutic drug monitoring, *PNR* primary non-response, *SLOR* secondary loss of response, *IM* immunomodulator

objective markers of inflammation in therapeutic decision-making [39–46]. Although TDM represents a useful tool to guide therapeutic intervention in the setting of objectively verified SLOR, relatively few studies have illuminated the role of TDM in PNR. Yet discerning the aetiology of PNR and SLOR remains critical for subsequent treatment decisions with TDM proving particularly useful in this context, helping to differentiate between anti-TNF refractory pharmacodynamic failure, relative to pharmacokinetic failure secondary to subtherapeutic anti-TNF concentrations (Fig. 20.1) [20, 21, 47–51].

Pharmacokinetic Failure

Anti-TNF drug clearance is multifactorial, with decreased clearance attributed to elevated body mass index (BMI), male sex, genetic polymorphisms, increased inflammatory burden and ADAs [52–55]. As large molecule foreign antigens, biologics such as adalimumab and infliximab can induce immunogenicity leading to subtherapeutic or undetectable drug levels, and immune-mediated infusion reactions. Immunogenicity remains an important concept in the context of biologic TDM, particularly as a potential driver of unfavourable treatment outcomes such as PNR, SLOR, drug cessation and lack of endoscopic remission.

ADA may be transient or persistent, neutralising or non-neutralising in nature. Transient ADAs are of little clinical significance, while persistent ADAs have a propensity to negatively influence treatment outcomes [56–58]. Neutralising ADAs reduce the activity of biologics by binding the (Fab′)2 region of anti-TNF, while non-neutralising ADAs do not preclude anti-TNF agents from binding their target molecules [59, 60]. Yet it is often impossible to discriminate between different types of ADAs, thus underscoring the need to exercise caution in their interpretation. Baert et al. demonstrated that ADA titres >8 ug/ml to infliximab were associated with a reduced time to disease relapse relative to those with lower titres, while other studies have suggested that the presence of ADA was also associated with an increased risk of hypersensitivity reactions [61–63].

The presence of ADAs in the setting of subtherapeutic drug levels helps differentiate between

immune and non-immune-mediated pharmacokinetic failure. Objective evidence for active disease in association with subtherapeutic drug levels without detectable ADAs is suggestive of non-immune-mediated pharmacokinetic failure and has been shown to be responsive to anti-TNF dose escalation or adding immunomodulator co-therapy [41, 42, 50, 51]. For instance, addition of azathioprine or methotrexate has each been shown to increase anti-TNF drug levels and suppress ADA formation. Multiple mechanisms have been proposed, including reducing inflammatory load, increased clearance of monoclonal antibodies via the reticuloendothelial system, direct suppression of ADA production and reduction of circulating and tissue anti-TNF levels [41, 54, 64–66]. Hence at least in the case of anti-TNF biologics, starting or optimising immunomodulator co-therapy when switching or escalating therapy remains an important aspect of addressing immunogenicity and suppressing current and/or future ADA formation [50, 65, 66].

Conversely, the combination of high ADA titres and subtherapeutic drug levels is indicative of immune-mediated pharmacokinetic failure with evidence suggesting that adding an immunomodulator or intensifying anti-TNF therapy is unlikely to overcome persistently high ADAs and re-establish therapeutic anti-TNF concentrations [50, 67]. The efficacy of switching to an alternate anti-TNF in this setting has been established from infliximab to adalimumab and vice versa, with the likelihood of success correlating with the prior degree of anti-TNF response achieved, as reflected by steroid-free mucosal healing [49, 51, 68–70]. The relative merits of dose escalation and switching both within and across biologic classes, has not been established in immune-mediated PNR.

Pharmacodynamic Failure

Objectively confirmed active disease despite therapeutic anti-TNF drug levels is suggestive of pharmacodynamic failure, although it remains important to ensure that levels are benchmarked against target levels specific to the IBD phenotype and target endpoint. This remains particularly important in the context of perianal fistulising CD with emerging data suggesting that infliximab levels above conventional TDM thresholds may be required to achieve stringent endpoints such as fistula healing [27].

Pharmacodynamic failure may be suggestive of anti-TNF resistance related to molecular polymorphisms in the setting of PNR, while deferred SLOR may be mediated by non-TNF-mediated inflammatory pathways [16, 71–75]. Theoretically therefore, persisting with or dose escalating the same anti-TNF agent in the case of true PNR is unlikely to prove successful nor is switching to an alternate anti-TNF agent, though supportive data are limited [50, 71–73]. In contrast, dose escalation of the same anti-TNF or switching within class to another anti-TNF has been deemed to be effective strategies to address SLOR due to pharmacodynamic failure, despite studies suggesting that switching to another class is associated with significantly improved outcomes [41, 50, 71].

20.1.4 Dosing Strategies to Recapture Response

20.1.4.1 Dose Interval Shortening and Dose Escalation of Anti-TNF Therapy

While there is evidence to support shortening of the dose interval as a successful dose escalation strategy, with up to two-thirds of patients regaining response in studies with short-term follow-up, doubling the baseline anti-TNF dose rather than shortening the interval between doses has also been shown to be effective [1, 11, 51, 76–80]. Further, the ECCO consensus guidelines concluded that increasing the dose of anti-TNF and shortening the dosing interval are equivalent strategies [EL4] [81].

Infliximab dose escalation typically involves shortening the dosing interval from 8-weekly to 4/6-weekly, or alternatively, increasing the infliximab dose from 5 mg/kg to 10 mg/kg while maintaining the 8-weekly dosing interval. Studies have demonstrated that halving the infliximab infusion interval is probably not superior to doubling the dose in both CD and UC, although cost and patient convenience may favour the dose-doubling strategy [82, 83]. Dose interval shortening to 6-weekly infliximab has also been found to be at least as effective as

dose-doubling or halving the interval to 4-weekly, especially when symptoms re-emerge 5–7 weeks post-infusion [80]. With adalimumab, shortening the dosing interval to weekly doses of 40 mg, or alternatively increasing the fortnightly dose of adalimumab to 80 mg, has been found to be safe and effective in overcoming SLOR [84, 85]. Thus, the chosen intervention typically is based on local practice, anecdotal experience and resource availability.

20.1.4.2 Anti-TNF Reinduction

Amidst growing scrutiny regarding the cost and resource implications of anti-TNF therapy, there is increasing awareness of the need to minimise unnecessary or excessive periods of dose escalation [86]. Although the optimal duration of dose escalation following SLOR remains unclear, observational studies have demonstrated that patients can recapture response using the more cost- and resource-effective strategy of fixed-duration dose escalation [87]. Anti-TNF reinduction represents one such model of fixed-duration dose escalation, advocating for short-term dose escalation in a manner identical to adalimumab and infliximab induction (i.e. readministering adalimumab 160/80 mg at weeks 0, 2 or infliximab 5 mg/kg at weeks 0, 2 and 6, respectively). The rationale for reinduction is that SLOR may represent a transient phenomenon that can be successfully overcome by a short-term increase in serum anti-TNF drug levels.

A recent retrospective study comparing anti-TNF dose interval shortening and reinduction to address SLOR in CD showed that there was no significant difference in rates of treatment failure at 12 and 24 months using either approach [88]. The study also demonstrated that first-line reinduction had no impact on the efficacy of subsequent dose interval shortening, although this subset of patients was small. Hence, reinduction might be considered as a first-line intervention in the setting of SLOR to anti-TNF therapy in CD, reserving more expensive dose escalation strategies such as ongoing dose interval shortening for those who fail to respond; however, this approach requires prospective evaluation. Data regarding the efficacy of anti-TNF reinduction in UC is lacking, and the role of reinduction in the setting of PNR has also not been established.

20.1.4.3 Addition of Thiopurine or Methotrexate Therapy

Reducing immunogenicity is imperative in the setting of SLOR, particularly in light of significant reductions in treatment response in the presence of ADA (RR 0.43, 95%CI 0.3–0.63) relative to those without, in patients treated with adalimumab (RR 0.40) and infliximab (RR 0.37) [89]. The SONIC study first demonstrated the therapeutic benefits of combination thiopurine and infliximab therapy versus either alone as part of a top-down strategy in the management of CD, while the UC-SUCCESS study later showed that combination therapy more effectively achieved steroid-free remission than monotherapy across a UC cohort [13, 90]. The COMMIT trial evaluated the combination of parental methotrexate and infliximab in CD, and although combination therapy was not associated with improved clinical efficacy relative to infliximab monotherapy, patients were significantly less likely to develop ADA with combination therapy [91]. The relative decrease in ADAs appears of similar magnitude when using infliximab in combination with either methotrexate or thiopurines, although studies directly addressing this question are lacking [13, 91, 92, 93].

Although the mechanism underlying the reduction of ADA production remains poorly understood, the addition of immunomodulator co-therapy perhaps indirectly increases serum infliximab levels when used in combination with biologic therapy. A recent prospective study by Roblin et al. even suggested that dose reduced (1–1.25 mg/kg) relative to full dose (2.5 mg/kg) azathioprine may be of comparable effectiveness in maintaining infliximab trough concentrations or ADA production, although this study was relatively underpowered, with only 12 months of follow-up [94].

Studies have also demonstrated that rates of ADA production are lower in adalimumab-treated patients relative to those treatment with infliximab, perhaps suggesting that adalimumab is of less immunogenic potential [2, 78, 95, 96]. Although adalimumab combination therapy has been associated with reducing immunogenicity and higher trough levels, the clinical effectiveness of combination therapy has been inconsistent, with larger trials such as CHARM (CD) and

ULTRA (UC) not demonstrating a significant clinical benefit [1, 89, 97–99].

20.1.5 Role of TDM During Biologic De-escalation

Clinical relapse confers significant morbidity for IBD patients, emphasising the importance of an individualised approach to treatment de-escalation that considers the risk of relapse on the basis of patient, disease and treatment characteristics. Anti-TNF drug levels represent a useful adjunct to inform decisions regarding anti-TNF de-escalation or cessation. However, a patient in apparent clinical remission with subtherapeutic levels alone cannot guarantee successful de-escalation given many other factors including prolonged steroid-free remission, prior surgical history, smoking status and inflammatory biomarkers, and endoscopic remission should also be considered [100–103]. Furthermore, subtherapeutic drug levels in this setting might otherwise be explained by imminent LOR, inter-patient drug variability with some exhibiting a lower anti-TNF level at which remission is maintained, sufficient concentration at other periods during the dosing cycle or non-anti-TNF-dependent remission [104].

20.1.6 Newer Biologics

Vedolizumab drug levels in UC patients have been associated with higher rates of clinical and endoscopic remission, with a further study showing that vedolizumab levels less than 19 mcg/ml were predictive of future SLOR and need for subsequent dose escalation. Median serum vedolizumab levels as early as week 6 have been demonstrated to be higher in clinical remission than active disease (40.2 vs 29.7 ug/ml, $p = 0.05$) and higher in those achieving a normalised compared to elevated CRP (21.8 vs 11.9 ug/ml, $p = 0.0006$). Studies have documented the development of immunogenicity across 1–4% of CD and UC patients treated with vedolizumab [4, 5, 105, 106]. Although ADAs to vedolizumab have been detected during both induction and maintenance phases, their presence was not associated with clinical outcomes [107].

Optimal ustekinumab levels have not yet been established, although higher trough levels have been associated with improved clinical remission, endoscopic response (>4.5 mcg/ml) and CRP normalisation (>5 mcg/ml) [108, 109]. Nevertheless, larger studies are required to ascertain optimal ustekinumab drug levels. Initial studies in moderate-severe Crohn's disease suggested that most (81%) patients had undetectable ustekinumab levels at week 36, yet few (0.7%, 3/427) patients developed ADA to ustekinumab, implying that serum drug concentrations did not have a marked influence on ADA production [110].

Although more data are required to establish the immunogenic potential of newer biologics, the potential discrepancies in immunogenicity across biologic classes may represent an important consideration in future therapeutic decision-making.

20.2 Conclusion

Despite the proven clinical utility of TDM, widespread use is limited by availability, expense and timeliness of testing. The advent of rapid point-of-care assays promises to allow clinicians to make 'real-time' dose adjustments based on pretreatment trough levels. A proactive approach to TDM is typically more expensive, resource intensive, and leads to more frequent dose escalation, perhaps suggesting that such an approach may be appropriate at well-resourced centres across patient cohorts at high risk of disease-related complications or with limited therapeutic alternatives should current therapy fail. Despite its theoretical appeal, advocating for proactive rather than reactive TDM cannot yet be justified, based on published studies to date.

While there are well-developed TDM targets to achieve clinical remission during maintenance anti-TNF therapy, dosing targets pertaining to fistula healing, endoscopic remission and mucosal healing remain less developed. Conversely, there is a lack of concrete, evidence-based TDM approach to assess and intervene following anti-TNF induction, especially given that this represents a critical time point to alter dosage or choice of therapy, thus pre-emptively reducing the likeli-

hood of future SLOR and early ADA development [25, 111]. Preliminary data also indicates that the principles of anti-TNF TDM are broadly applicable to newer biologic agents such as vedolizumab and ustekinumab.

Although it has long been acknowledged that IBD is a heterogeneous disease, data is beginning to emerge in support of TDM targets specific to disease phenotypes and aspirational treatment endpoints. Hence, as treat-to-target algorithms continue to evolve, they will likely strive towards integrating induction and maintenance TDM targets specific to both the disease phenotype and aspirational target endpoints such as endoscopic healing, mucosal healing, fistula healing and patient-reported outcomes.

Summary Points

- Treatment algorithms incorporating TDM have been proposed to improve outcomes.
- There are well-developed TDM targets to achieve clinical remission during maintenance adalimumab and infliximab therapy.
- An evidence-based approach to assess and utilise TDM following anti-TNF induction is lacking.
- TDM targets specific to treatment endpoints such as endoscopic, mucosal and fistula healing are evolving.
- Despite its appeal, advocating for proactive rather than reactive TDM approach cannot yet be justified.
- Mechanisms underlying loss of response remain important in directing therapeutic intervention.
- Immunogenicity has emerged as an important concept in understanding and managing loss of response.
- Treat-to-target algorithms should strive to incorporate induction and maintenance TDM targets specific to both the disease phenotype and aspirational target endpoints.
- Principles of anti-TNF TDM are broadly applicable to newer biologic agents.

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21 Drug Toxicity: Personalising IBD Therapeutics – The Use of Genetic Biomarkers to Reduce Drug Toxicity

Gareth Walker and Tariq Ahmad

Abstract

Adverse drug reactions (ADRs) result in morbidity and mortality as well as placing a significant financial burden on health-care resources. In Europe and North America, they are responsible for approximately 6% of all hospital admissions. Patients with inflammatory bowel disease (IBD) commonly experience adverse drug reactions. Whilst the explosion of new IBD therapies has improved patient outcomes, ADRs remain a significant challenge and for many drugs the major cause of discontinuation. Most ADRs cannot currently be reliably predicted prior to starting treatment, and therefore clinical and laboratory monitoring, which is expensive and inconvenient for patients, is recommended for the duration of treatment. An ability to accurately predict an individual's risk of developing an ADR prior to treatment may allow the dose to be altered or the drug avoided in at-risk individuals. Pharmacogenetic biomarkers are particularly attractive for the purpose of predicting ADRs as they are present at diagnosis and unaffected by disease phenotype, disease activity or other treatments. Discovery of these biomarkers has been made possible by the increasing availability of reliable, robust and cheap high throughput genotyping and sequencing platforms. In this chapter, we describe several inflammatory bowel disease ADR pharmacogenetic biomarkers, as well as the process of biomarker discovery and the barriers which hinder the successful 'bench to the bedside' translation of these tests into routine clinical care.

21.1 Personalising IBD Therapeutics: The Use of Genetic Biomarkers to Reduce Drug Toxicity

21.1.1 Clinical Significance of ADRs in IBD

An adverse drug reaction (ADR) is defined as an appreciably harmful or unpleasant reaction resulting from the use of a medicinal product; adverse effects usually predict hazard from future administration and warrant prevention, or specific treatment, or alteration of the dosage regimen or withdrawal of the product [1]. ADRs result in morbidity and mortality as well as placing a significant financial burden on health-care resources. In Europe (EU) and North America, they are responsible for between 3.5% and 6.5% of all hospital admissions [2–4], with a further 10% of ADRs occurring during the subsequent

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hospital stay [2]. ADRs are thought to contribute to the deaths of approximately 197,000 EU and 159,000 US citizens annually, which places ADRs as one of the top ten causes of death [2, 4–6]. It is estimated that EU countries spend 15–20% of their health-care budgets dealing with the consequences of ADRs and in the UK alone this exceeds £500 million per year [4, 6].

21.1.2 Types of ADRs

Traditionally ADRs have been classified as Type A and Type B. Type A reactions (80% of ADRs) are predictable through the known pharmacological mode of action of the drug and are often a consequence of an exaggerated on-target effect. They have a strong dose relationship such that a dose reduction will usually lead to resolution of sequelae (e.g. oral iron and gastrointestinal side effects). Type B reactions (20% of ADRs) are off-target interactions, often associated with a high mortality and require drug cessation. These ADRs were previously thought to be idiopathic and independent of drug dose; however, recent studies have shown that they actually have a complex dose relationship [6] and many are predictable through knowledge of the underlying immunological and genetic aetiology (e.g. thiopurine-induced pancreatitis [7]). They are often referred to as 'allergic' or 'hypersensitivity' reactions because they involve complex interactions of multiple components of the host's adaptive immune system including IgE antibodies, drug-specific T-cells and immune complexes [8–10]. Indeed, the same drug may activate many different arms of the immune system via different pathways [11]. A common theme to many hypersensitivity ADRs is the activation of T-lymphocytes, which occurs in some cases exclusively in patients with a specific human leukocyte antigen (HLA) type. Candidate gene studies, as well as hypothesis-free genome-wide association studies (GWAS), have shown a number of HLA associations with Type B ADRs, for example, abacavir hypersensitivity [HLA-B*57:01] [12], carbamazepine hypersensitivity in Caucasians and Japanese [HLA-A*31:01] [13, 14] and carbamazepine-induced Stevens-Johnson syndrome in Han Chinese [HLA-B*15:02] [15]. However, these studies have shown that the carriage of specific HLA genotype is often not sufficient, nor indeed necessary, to cause an ADR in patients exposed to a particular drug. This suggests that other factors such as regulatory T-cells (T-reg), the cytokine milieu and danger signals caused by tissue damage also may also contribute to the development of ADRs [16].

21.1.3 Pharmacogenetic Biomarkers of ADRs

Predictive biomarkers allow the identification of individuals who are more likely to respond to a particular therapy. This response could be a symptomatic benefit, improved survival or an ADR. Predictive biomarkers of ADRs may direct drug avoidance, dose reduction or enhanced monitoring in at-risk individuals. Pharmacogenetic biomarkers are particularly attractive for the purpose of predicting ADRs as they are present at diagnosis and are unaffected by disease phenotype, disease activity and other drug therapies. Pharmacogenetic biomarker discovery has been made possible by the increasing availability of reliable, cheap high throughput genotyping and sequencing platforms. This has led to a rapid expansion in the number of publications reporting pharmacogenetic associations, although very few have reached clinical practice. The first step towards implementation is independent replication, and many claimed biomarkers have fallen at this first hurdle. In this review we highlight the most promising examples of pharmacogenetic associations for drugs used in patients with inflammatory bowel disease (IBD).

21.1.4 Biomarker Discovery

The essential requirements of an ADR pharmacogenetic biomarker discovery study include strict phenotype definitions, a robust assessment of causality and an adequate sample size. Rare idiosyncratic drug reactions are notoriously difficult to characterise due to the small number of cases

available to individual researchers. Therefore, nationwide and global collaboration is essential to build cohorts of sufficient size for hypothesis-free genome-wide pharmacogenetic studies. Recent efforts of the UK IBD pharmacogenetics network [17], the International IBD Genetics Consortium [18] and the Serious Adverse Events Consortium (iSAEC) [19] have demonstrated that collaboration can successfully deliver sufficient patient numbers to adequately power such studies. Strict phenotype definitions allow the inclusion of a homogenous population, and work by the Phenotype Standardisation Project [20] has been instrumental in the effort to address this issue. In clinical practice it is often difficult to be certain that an ADR has been caused by the drug of interest. Adjudication is an essential part of ADR pharmacogenetic studies; this process maximises the likelihood that symptoms experienced by recruited patients are due to the drug rather than other unrelated causes. Case adjudication is typically carried out by an independent panel of clinicians using a validated adjudication pathway, e.g. the Liverpool causality pathway (see Fig. 21.1) [21]. High-quality cases demonstrate a clear temporal relationship with drug administration; no other identifiable risk factors for the ADR, including the concomitant use of other drugs recognised as causing a similar ADR; and resolution of the ADR on drug withdrawal. A positive rechallenge with a second ADR developing after re-exposure to the same drug provides even stronger evidence of causality. Cases which successfully pass through this adjudication process are sent for genotyping using hypothesis-free array and/or exome sequencing or whole genome sequencing methodologies. Replication of positive findings in an independent cohort is crucial. Particular attention should be paid to minimising population stratification in the analysis of data, especially when cases and controls are recruited from populations of differing ethnic backgrounds. This confounding factor could lead researchers to assume an association with an ADR is present, when in fact this variant is simply more commonly found in patients of one particular ethnicity who are over- or under-represented in either cases or controls.

21.1.5 Overview of Gene-Drug Adverse Drug Reaction Biomarkers in IBD

21.1.5.1 Adverse Reactions to Thiopurine Drugs: Azathioprine and Mercaptopurine

The thiopurines (mercaptopurine and its prodrug azathioprine) are commonly used in patients with IBD to maintain corticosteroid-free remission, prevent postoperative recurrence and reduce the risk of immunogenicity associated with biologic therapy. 59% of CD and 33% of UC patients receive thiopurine therapy within the first 5 years of diagnosis [22]. Despite this widespread use, up to 40–50% of European IBD patients have to discontinue therapy, most commonly (~15%) because of the development of one or more ADRs [23, 24]. Thiopurine-induced ADRs include pancreatitis (4–7% prevalence) [24, 25]; liver injury (3–10%) [25–27]; myelosuppression (7%) [28]; GI side effects (1–6%) [29, 30]; and a flu-like hypersensitivity reaction (8–12%) [24, 31]. Over recent years there has been significant progress in our understanding of thiopurine metabolism (reviewed in González-Lama and Gisbert, 2015 [32]) and the mechanisms underlying ADRs.

21.1.5.2 Thiopurine-Induced Myelosuppression (TIM)

TIM may occur at any time during thiopurine treatment, and whilst most patients are asymptomatic, serious opportunistic infections may occur, especially if neutrophils fall $\leq 1.0 \times 10^9$/L, with an estimated mortality of 1% [23, 24]. In the 1980s, Weinshilboum and others recognised that TPMT activity in white Europeans followed an autosomal codominant mode of inheritance with a trimodal distribution [33, 34]. Approximately 89% of individuals possess high TPMT activity levels, 11% intermediate activity and 0.3% low activity [35]. This phenotypic observation correlates with genetic variation in the thiopurine S-methyltransferase (*TPMT*) gene, with variant alleles resulting in decreased TPMT enzyme activity and higher production of the active 6-thioguanine nucleotides (6TGNs),

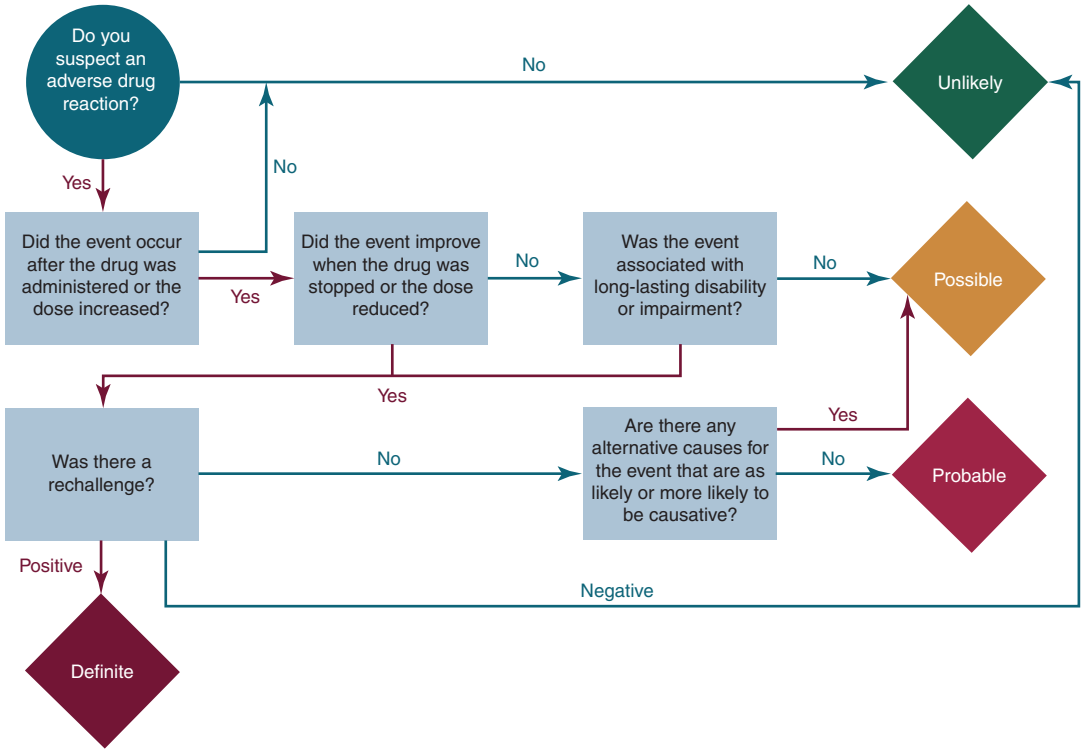

Fig. 21.1 Causality assessment tool. (Adapted version of the Liverpool adverse drug reaction causality assessment tool used in the adjudication process. Adapted from Gallagher et al. [21])

which predispose patients to bone marrow suppression [33, 34]. Pretreatment phenotyping (usually measurement of TPMT activity in red blood cells) or genotyping of *TPMT* is recommended by the UK Medicines & Healthcare products Regulatory Agency (MHRA) [36] and US Food and Drug Administration (FDA) [37] and routinely carried out prior to initiation of treatment to identify patients at risk of overproduction of 6TGNs and therefore TIM: in those with reduced TPMT activity, thiopurines are used in reduced doses or avoided altogether [38]. However, *TPMT* variants are only found in 25% of TIM cases in populations of European descent, suggesting the presence of other genetic and environmental determinants [38, 39]. In contrast, variant *TPMT* haplotypes are rare in patients of East Asian descent, a population where TIM is particularly prevalent [40–42]. Recently, Yang et al. identified a common variant in *NUDT15* associated with myelosuppression in patients of East Asian descent [43]. The exact mechanism of action of NUDT15 is still being elucidated; however, it is thought to catalyse the hydrolysis of nucleoside triphosphates. Patients with defective *NUDT15* variants therefore have excessive levels of thiopurine active metabolites (thioguanosine triphosphate [TGTP] and DNA-incorporated thioguanine [DNA-TG]) and increased host toxicity [44].

In unpublished work we have demonstrated that genetic variation in *NUDT15* is also important in patients of European descent [45]. In a case-controlled study of 961 European IBD patients, including 328 cases of TIM, we identified a novel association with a 6 bp in-frame deletion (p.Gly17_Val18del) in exon 1 of *NUDT15* and TIM (5.8% of TIM cases vs. 0.2% thiopurine-tolerant controls [odds ratio [OR] = 38, 95% CI 5–286, $P = 1.3 \times 10^{-8}$]). We also searched our data set for other non-monomorphic variants in this gene which were previously reported as associated with TIM in other cohorts (p.Arg139Cys and p.Gly17_Val18dup) and found that carriage of one or more of three coding *NUDT15* variants (including p.Gly17_Val18del) was associated with

a 27-fold increase in the odds of TIM (OR = 27 [95% CI 9–117], $P = 1.1 \times 10^{-7}$), independent of both *TPMT* genotype and thiopurine weight-adjusted dose. We estimate a number needed to genotype (NNG) of 95 (95% CI 62-143): for every 10,000 patients genotyped, 164 would test positive for a *NUDT15* variant, and of these patients, 105 would have developed TIM if they had not received an alternative treatment (PPV 64%, 95%CI 43%–100%). Genotyping 10,000 patients for *NUDT15* would prevent 105 cases of TIM, which is 95 patients genotyped for every case prevented. This figure is similar to the number needed to genotype for TPMT of approximately 100 [46], which is already widely adopted in clinical practice. Pretreatment genotyping for *NUDT15* should reduce the number of TIM cases by 14% and is currently being considered by Genomics England as a standard pretreatment assay in the UK [47].

21.1.5.3 Thiopurine-Induced Pancreatitis (TIP)

Thiopurine-induced pancreatitis is a well-recognised, idiosyncratic, dose-independent ADR with an incidence of approximately 4–7% in patients with IBD [24, 25]. This ADR most commonly occurs within the first month after commencement of therapy, and rechallenge with either AZA or MP usually leads to recurrence of symptoms. Most episodes of acute pancreatitis are mild and resolve after the discontinuation of the drug, although more severe cases can occur (with local and systemic complications of pancreatitis, including death) [48]. The pathogenesis of thiopurine-induced pancreatitis is unknown. We previously reported the first large-scale clinical and genetic analyses of thiopurine-induced pancreatitis and identified an association with a common variant (rs2647087) in the Class II HLA region which tags HLA-DRB1*07:01 [7]. In our study we estimated that the risk of developing pancreatitis amongst variant carriers was increased 2.5 times for heterozygous and five times for homozygote patients. This finding has recently been replicated in a cohort of 373 azathioprine-exposed patients from Canada [49]. In this cohort, which included 13 patients with a history of azathioprine pancreatitis, the risk was highly predictable and genotype dependent: 0.5% for wild type (A/A), 4.3% (OR = 4, 95% CI 1–36, $P = 0.044$) for heterozygous (A/C) and 14.6% (OR = 16, 95% CI 4–145, $P = 0.0001$) for homozygous variant (C/C) patients. Data from our UK study suggests that for every 1000 patients tested, 77 risk allele homozygotes will be identified, and these individuals will have a 17% risk of pancreatitis. If azathioprine/mercaptopurine are subsequently avoided in all homozygote-risk allele individuals (and we believe most clinicians would consider this reasonable), this equates to an overall number needed to genotype of 76 patients to prevent one case of pancreatitis.

21.1.5.4 Thiopurine-Induced Liver Injury (TILI)

TILI most commonly leads to an asymptomatic hepatocellular liver injury characterised by elevated transaminases (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) within the first 12 weeks of treatment or soon after dose escalation [50]. This hepatocellular liver injury generally resolves after dose reduction or drug cessation [25, 51]. Less commonly, approximately 1 in 1000 treated patients, thiopurines cause a cholestatic liver injury in association with symptoms of jaundice, fatigue and itching [51]. This ADR often is typically seen between 2 and 12 months after starting treatment and resolves after drug cessation, although some persistent cases have been described [51]. Finally, after long-term therapy thiopurines rarely lead to chronic liver injury with symptoms and signs of portal hypertension. Histologically such cases demonstrate nodular regenerative hyperplasia, sinusoidal dilatation, central congestion and injury to sinusoidal endothelial cells suggestive of veno-occlusive disease [51–53].

The enzyme, thiopurine S-methyltransferase (TPMT), inactivates thiopurines to methylated metabolites, reducing the production of the active 6-thioguanine nucleotides (6TGN). High TPMT enzyme activity may result in a greater 6-methylmercaptopurine (6MMP) production, which has been associated with liver toxicity [54, 55]. In such cases of thiopurine hypermethylation, the use of adjunctive allopurinol (a xan-

thine oxidase inhibitor) has proven effective in shunting thiopurine metabolites towards active 6TGNs without increasing 6MMP levels [56]. However, TILI may still occur in the absence of elevated 6MMP and 6TGN levels [57]. To date, there have been no hypothesis-free genome-wide association (GWAS) approaches employed to investigate the genetic basis of drug-induced liver injury. However, data from our study of over 200 patients with thiopurine-induced liver injury using GWAS and whole-exome sequencing methodologies will be published shortly.

21.1.5.5 Thiopurine-Induced Hypersensitivity Reactions (THR)

Thiopurine hypersensitivity reactions are dose independent and occur in 8–12% of patients treated with azathioprine and mercaptopurine [24, 31, 58]. Most hypersensitivity reactions are mild, presenting with a flu-like illness within the first 4 weeks of therapy, and resolve rapidly on drug withdrawal. Symptoms and signs of mild hypersensitivity reactions are poorly defined in the literature but include fever, myalgia, arthralgia, headache and fatigue often leading to drug cessation. These symptoms can be associated with an acute inflammatory response, supported by a rise in serum markers, e.g. CRP, mimicking active IBD. The mechanism of thiopurine hypersensitivity is unknown. It has been proposed that the imidazole component of azathioprine may be responsible by binding to endogenous proteins resulting in hapten formation and immune activation. This might explain why a small proportion of patients who develop flu-like illness in response to azathioprine therapy are subsequently able to tolerate mercaptopurine [59]. However, this theory must be challenged as there is no evidence to suggest that the syndrome is more common with azathioprine than mercaptopurine and a number of patients experience identical reactions to mercaptopurine rechallenge. This hypersensitivity syndrome does not appear to be associated with *TPMT* genotype and is not dose-related, suggesting an idiosyncratic mechanism [60]. An association with flu-like hypersensitivity to thiopurines and an exonic variant in *ITPA* has been described in a case-control candidate gene study; however, this finding has not been replicated [61]. Our preliminary data from a genome-wide association study suggests the presence of a genetic determinant in the Class II HLA region. Further work is underway to replicate this finding prior to publication.

21.1.5.6 Mesalazine-Induced Nephrotoxicity

5-Aminosalicylates (5-ASAs) are the most frequently prescribed class of drug to induce and maintain remission in patients with mild to moderately active ulcerative colitis. The use of these agents in maintenance therapy over decades means that long-term toxicity is an important consideration. Mesalazine-induced nephrotoxicity is rare (incidence of approximately at 0.17 cases per 100 patients per year [62]), but the consequences may be serious including the development of end-stage renal failure and the need for renal replacement therapy. As a consequence, regular monitoring of renal function for the duration of mesalazine treatment is advised by the European Crohn's and Colitis Organisation (ECCO), British National Formulary (BNF) and American Gastroenterology Society (AGA) [63–65]. Data from our previous work has shown that 5-ASA-induced nephrotoxicity may present at any age and is characterised histologically by chronic tubulointerstitial nephritis [66]. In our case-control study, median time to renal injury was 3 years, following which only 30% of our cohort fully recovered renal function, with 10% requiring permanent renal replacement therapy. A genome-wide association demonstrated association within the HLA region although this failed to reach genome-wide significance (OR = 2, 95%CI 2–3, $P = 1 \times 10^{-7}$). Limiting the association analyses to the biopsy-positive cases significantly strengthened the HLA association signal despite the smaller number of cases, with an odds ratio of 3.1 and a genome-wide significant P-value ($P = 4 \times 10^{-9}$). The high frequency of this single-nucleotide polymorphism (SNP) and the low frequency of the adverse event limit

its clinical utility, and we therefore cannot recommend its use in guiding treatment choice or monitoring intervals.

21.1.5.7 Sulphasalazine-Induced Agranulocytosis

Sulphasalazine consists of a sulphonamide antibiotic (sulphapyridine) linked via an azo bond to 5-aminosalicylic acid (5-ASA). It is rarely used in IBD, aside for maintenance treatment of UC patients suffering from IBD-associated arthropathy, having largely been replaced by 5-ASAs which have a comparatively better side effect profile. Sulphasalazine reaches the colon mostly unchanged and is split by gut bacteria at the azo linkage, releasing 5-ASA and sulphapyridine [67]. Whilst the systemic absorption of 5-ASA is limited, a positive correlation exists between serum sulphapyridine concentration and both therapeutic efficacy and toxicity [67]. The more severe adverse drug reactions include agranulocytosis, liver injury, Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). Plasma levels of sulphapyridine are influenced by common polymorphisms in genes that encode N-acetyl transferase 2 (*NAT2*) and ATP-binding cassette protein G2 (ABCG2) [68]. Allelic variation at the *NAT2* gene locus determines whether individuals are fast or slow acetylators [69] with fast acetylators having lower plasma concentrations [67, 70]. Prevalence of the slow acetylator phenotype shows marked ethnic variation: 40–70% Caucasians and African-Americans, 10–20% Japanese, >80% Egyptians and certain Jewish populations [71–73]. However, to date, studies involving low patient numbers have mostly failed to detect a relationship between *NAT2* acetylator status and drug toxicity [68, 74], and pretreatment genotyping of *NAT2* or phenotyping of acetylator status is not carried out in clinical practice.

21.1.5.8 Allopurinol-Induced Severe Cutaneous Adverse Reactions (SCAR)

Allopurinol, a commonly prescribed medication for gout and hyperuricemia, is increasingly used alongside thiopurines in order to reduce thiopurine toxicity or increase efficacy in hypermethylators [75, 76]. Up to 0.4% of patients treated with allopurinol suffer severe cutaneous adverse reaction (SCAR) with a mortality rate up to 25% including drug reaction with eosinophilia and systemic symptoms (DRESS), SJS or TEN [77]. Allopurinol-induced SCAR is strongly associated with HLA-B*58:01 carriage (OR = 165 when compared to allopurinol-tolerant controls) [78]. This allele is rare in patients of European descent with a 1% carriage rate but common in patients of Asian descent, including Han Chinese, in whom the PPV of this association is 2% and NPV 100% [79]. The clinical utility of pretreatment genetic testing for HLA-B*5801 has been demonstrated in a non-randomised trial design using historical data as control [80]. Given the high negative predictive value of the allele, especially in patients of Asian descent (>99%), CPIC states that HLA-B*58:01 testing could significantly reduce the incidence and risk for allopurinol-associated SCAR [81].

21.1.5.9 Methotrexate-Induced Mucositis, Hepatotoxicity and Haematological Toxicity

Methotrexate is a commonly used immunosuppressive agent used in the maintenance treatment of IBD. Therapy is frequently limited by side effects including mucositis, hepatotoxicity and haematological toxicity. In a meta-analysis of 14 paediatric oncology candidate gene studies of ADRs methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms were associated with hepatotoxicity and haematological toxicity and mucositis [43]. These authors concluded that 'in children with malignancy, genotyping of the MTHFR C677T polymorphism is expected to be a useful tool in reducing toxicity and improving outcome in personalized MTX therapy' [43]. This is not currently advocated by CPIC (evidence level C/D) [82].

21.1.5.10 Calcineurin-Induced Hypertension and Nephrotoxicity

The calcineurin inhibitors include ciclosporin, which is used as rescue therapy in acute severe ulcerative colitis, and tacrolimus, used to induce

and maintain remission in patients with ulcerative colitis refractory to systemic corticosteroids [83]. Dosing of ciclosporin and tacrolimus is routinely directed by therapeutic drug level monitoring because of their narrow therapeutic index and significant interindividual variability in blood concentrations. The calcineurin inhibitors are metabolised by CYP3A5, and genetic variation in this gene contributes to the pharmacokinetic variability of these drugs and the risk of developing hypertension [84, 85]. Data from the solid organ and stem cell transplantation literature suggests that CYP3A5 genotype-based dosing of tacrolimus may allow target tacrolimus levels to be achieved earlier, although whether this translates to improved efficacy or reduced toxicity is not known [86]. Using this algorithm CYP3A5 extensive (*1/*1) or intermediate (*1/*-) metabolisers are started with 1.5–2 times the standard dose. To date CYP3A5 genotype-directed dosing of calcineurin inhibitors has not been studied in patients with IBD.

21.1.5.11 Anti-TNF-Induced Skin Reactions

The use of antitumour necrosis factor (anti-TNF) drugs is associated with the development of paradoxical inflammatory skin eruptions in up to 30% of treated patients across all disease indications [87, 88]. Skin manifestations may present after many years of anti-TNF treatment and include palmoplantar pustulosis, psoriasis, psoriasiform eczema, eczema and xerosis [87]. Smoking and obesity have been identified as risk factors particularly of palmoplantar psoriasis, but these clinical factors are not currently used to stratify patients [89–92]. Initial treatment of skin lesions includes the use of topical steroids in mild to moderate cases (<5% of skin affected), but 10–40% of patients fail to respond and therefore necessitate anti-TNF drug withdrawal [87, 90]. Switching to an alternative anti-TNF drug does not lead to resolution of skin lesions suggesting a class effect for this ADR [90]. In contrast, switching out of class to ustekinumab (an antibody directed against the p40 subunit of IL-12 and IL-23, approved for use in psoriasis and Crohn's disease) has been shown to be effective in the treatment of anti-TNF-induced skin lesions refractory to topical steroids [91, 93]. Severe skin lesions cause patients with inflammatory bowel disease to discontinue anti-TNF therapy. The mechanism of anti-TNF-induced skin lesions is not well understood, but recent data suggests that the skin lesions are characterised by infiltration of interferon-γ expressing Th1 lymphocytes and IL-17A/IL-22 expressing Th17 cells, with the severity of skin lesions correlating with the density of Th12 cell infiltrates [89]. It is speculated that the Fc region of anti-TNF antibodies binds to Fc-gamma CD64 (Fc-gamma receptor I (FcγRI)) and CD16/32 (Fc-gamma receptor III/II (FcγRIII/II)) on monocytes and macrophages triggering secretion of IL-23 which drives Th17 production of IL-17 and IL-22 and the development of skin lesions [94]. A preliminary small candidate gene study has reported association with the rare IL23R variant rs11209026 (p.Arg381Gln) and severe anti-TNF-induced psoriasiform skin lesions, suggesting it might be possible to identify patients at risk of adverse skin reactions prior to treatment [89].

21.1.6 Clinical Implementation and Future Clinical Use of Pharmacogenetic Markers of ADRs

The clinical implementation of a genetic association into a pretreatment test has traditionally demanded a randomised controlled trial (RCT) to assess its clinical utility and cost-effectiveness. However, these studies are costly, require large sample sizes and often fail to deliver consistent actionable results [95]. To hold pharmacogenetic studies up to the same standards designed to assess drug efficacy may be inappropriate and may delay translation of research from bench to bedside, although clearly an appropriate balance is needed. The greater availability and falling costs of whole-genome sequencing (currently less than US$ 1500) [96, 97] means that the question is increasingly not whether to genotype but how best to utilise existing sequence data, perhaps generated at diagnosis or even at birth.

As our knowledge of gene-drug interactions increases, this information needs to be curated,

reviewed and translated into actionable prescribing guidelines for clinicians who lack knowledge and confidence of pharmacogenetic testing. This crucial work is being supported by bodies such as Clinical Pharmacogenetics Implementation Consortium (CPIC) [82] and Pharmacogenetics and Pharmacogenomics Knowledge Base (PharmGKB) [98]. There is a need to integrate genetic data into electronic patient systems to help physicians chose and deliver the right drug at the right dose first time for individual patients. A number of genomic prescribing systems are being developed by academic institutions. These typically employ a web-based portal which displays interactive, patient-specific, pharmacogenomic results in the form of a patient-tailored synopsis including prescribing recommendations and suggested alternative medications. Finally, the turnaround time for these tests needs to be short so that clinicians are able to receive actionable results in a time frame which doesn't delay the instigation of treatments in the acutely unwell patient.

Summary Points

- Adverse drug reactions to drugs commonly used in IBD result in significant morbidity and cost throughout the world
- Pretreatment genotyping is now affordable and could offer a personalised approach to tackling this issue, allowing clinicians to avoid drugs predicted to cause an adverse drug reaction, reduce the target dose or increase pharmacovigilance
- Barriers that have hindered the clinical translation of pharmacogenetic research are now being resolved through international collaboration and technological advances
- Broadly speaking there are two types of ADRs: Type A is more common and shows strong dose relationship, and Type B is less predictable and often has an immunopathological mechanism, many of which are being uncovered through the use of modern genetic techniques

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Biomarkers for Remote Monitoring

22

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Abstract

Since the prevalence and incidence of inflammatory bowel disease (IBD) is increasing worldwide, the access to effective treatment options is crucial in handling the present and future global impact of the disease. With an increased access to high-speed Internet, smartphones, and tablets, digital technologies are rapidly emerging in the field of health care. In IBD, electronic health (eHealth) technologies are used as tools to facilitate and reduce the burden of IBD management as they are based upon elements of self-management. The majority of eHealth technologies have previously relied on patient-reported outcome measures. However, during the last decade point-of-care (POC) analyses of C-reactive protein (CRP) and fecal calprotectin (FC) have been introduced and integrated in the remote monitoring of infectious and inflammatory diseases, including IBD. Also, newer technologies incorporating ingestible biosensors in the measurement of adherence have emerged and are showing positive results in chronic diseases. This chapter will highlight the application of biomarkers (FC and CRP) in the remote monitoring of IBD and the potential use of ingestible biosensors to facilitate measurement of adherence.

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22.1 Background for Remote Monitoring

There is a substantial health-care burden associated with inflammatory bowel disease (IBD) which involves both direct (e.g., medication and hospitalization) and indirect costs (e.g., sick leave). IBD patients are likely to be off from work for approximately 3–6 weeks/year, and direct health-care costs in Europe alone are estimated to be 4.6–5.6 billion euros/year [1]. The incidence and prevalence of IBD are rapidly increasing globally with the highest incidence in Asia found in areas of the two most populous countries in the world, China and India [2]. Hence, an enormous impact on health-care systems worldwide is expected, and therefore the need for new initiatives to cope with the increased health-care burden is necessary [1, 3].

Remote monitoring via electronic health (eHealth) technologies has been used with success in several chronic conditions such as diabetes, respiratory diseases, IBD, and heart failure as well as in patients receiving anticoagulant thrombosis prophylaxis [4–8]. In IBD, there is increasing evidence that tight monitoring using eHealth/

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mobile health solutions has the potential to reduce time to remission, increase compliance with medical therapy, reduce number of outpatient visits and hospital admissions, and also involve and empower patients [3, 5, 9]. Unfortunately, many eHealth technologies exclusively rely on patient-reported outcomes (PROs). To our knowledge only Pia Munkholm and team, North Zealand University Hospital, Denmark, have incorporated a remote biomarker for IBD home monitoring in the Constant Care © web application (www.ibd.constant-care.dk) described in this chapter.

The Constant Care © web application has a holistic approach of educating and involving IBD patients in their disease and treatment. Integrated in this web application is a disease monitoring algorithm (Fig. 22.1) illustrated to both patients and health-care provider via a traffic light system (green = remission, yellow = mild to moderate activity, red = severe activity). It consists of a disease activity score (Simple Clinical Colitis Activity Index [10] or Harvey–Bradshaw index [11]) that the patients fill out at home and a fecal calprotectin (FC) point-of-care (POC) test that can be performed at home within 18 minutes (CalproSmart™ by Calpro AS, Norway). The disease activity scores and FC measures are added together in a weighted manner giving the total inflammation burden score (TIBS). Quality-of-life measurement tools are also integrated in the application, where patients are requested to fill out the short IBD questionnaire [12] (SIBDQ) and the newly developed IBD Disk [13]. The disease algorithm is used by the patients and physician to tightly monitor disease activity and direct individualized treatments exactly when needed. The Constant Care © web application has been further described elsewhere [14, 15], and the validation of the TIBS by endoscopic and histologic findings is currently under progress. The gastroenterology department of North Zealand University Hospital will during 2018 be the first department in the world to offer IBD patients disease monitoring by the Constant Care © web application as an alternative to standard care.

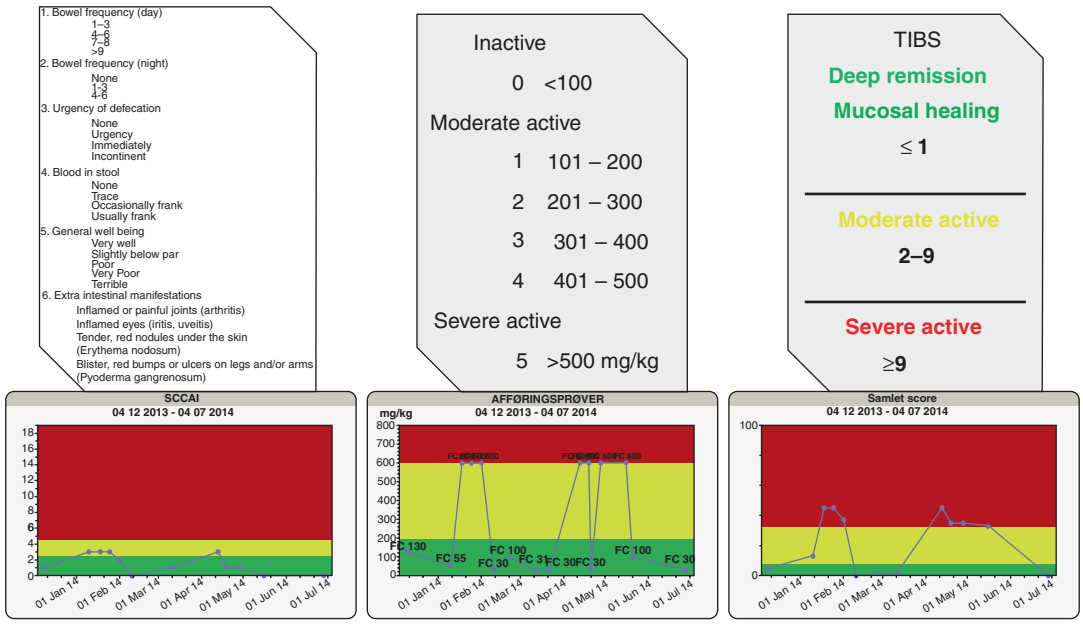

Fig. 22.1 Disease web algorithm in Constant Care © web application, consisting of a Simple Clinical Colitis Activity Index (SCCAI) for ulcerative colitis or Harvey–Bradshaw index for Crohn's disease and fecal calprotectin added together giving the total inflammatory burden score (TIBS). The patient-reported symptom score and point-of-care fecal calprotectin test can be performed at home by the patients in 5 and 18 minutes, respectively. (Reproduced from Burisch and Munkholm 2016 [15] with permission)

22.2 Integrating Biomarkers in Remote Monitoring

22.2.1 CRP

C-reactive protein (CRP) is an acute phase protein, first described in 1930 by William S. Tillet and Thomas Francis [16]. It has since become an important biomarker in the monitoring of inflammatory disorders and infections [17]. The half-life of CRP is approximately 19 hours, making it a better indicator of acute inflammation than most other acute phase reactants [17, 18]. Baseline concentrations of CRP in the plasma vary from 0.8 mg/l to 5 mg/l under normal noninflammatory conditions [19]. However, conditions affecting the functions of the liver, e.g., liver failure, or therapies affecting the acute phase stimulus might decrease CRP concentration in the serum, since it is mainly produced by hepatocytes when stimulated by cytokines, e.g., IL-6 [17]. In IBD patients, CRP levels ≥5 mg/l have a high specificity but a poor sensitivity for correlating with endoscopic activity [20]. Moreover, since the rectal venous plexus drains into both the portal vein and iliac vein, cytokines from inflammatory sites of the rectum partly bypasses the liver and only trigger CRP production in secondary pass. Thus, CRP is often normal in active proctitis [21]. To further complicate matters, it seems like both basal and increased CRP levels are strongly influenced by mutations in the CRP gene which make the interpretation complex [22].

Numerous studies have verified a strong association between elevated CRP levels and clinical relapse of IBD, with a relative risk ranging from 3 to 58 [23]. Differences in CRP response between UC and CD patients have previously been verified, with elevated levels more frequently described in CD compared to UC [24]. One explanation of this phenomenon might be the elevated expression of CRP producing adipocytes in the mesentery of CD patients [25]. Though approximately 20–25% of CD patients with flares do not express a CRP elevation due to genetic single-nucleotide polymorphisms (SNP) in the CRP gene [23], a systemic review found the sensitivity and specificity of CRP in detecting endoscopically verifiable remission to be 49% and 92%, respectively [20]. All things considered, one should take heed when interpreting solely elevated CRP level as a predictor of flare.

CRP also serves as a marker in the determination of drug effectiveness in IBD. The use of CRP as a marker of inflammation and treatment target has been used in a majority of CD trials but to a lesser extent in UC trials during the last decade [26]. In CD patients, an elevated CRP has been associated with a better response to antitumor necrosis factor (TNF) treatment, and rapid normalizations of CRP seem to correlate well with long-term response to treatment [27]. Moreover, in patients with a loss of response to anti-TNF treatment, CRP levels are frequently elevated. Therefore, CRP might not only serve as a biomarker of prediction of relapse but also serve as a biomarker of loss response in IBD patients, in particular CD [23]. In UC, CRP has mostly been investigated in patients with severe disease [27]. CRP levels in combination with stool frequency on the third day of treatment have been found to be a reliable predictor of failure of treatment with intravenous steroids in both adult and pediatric UC patients [28].

To apply and integrate the use of CRP in remote monitoring of IBD, home testing kits are key. Unfortunately, there are a limited number of CRP home tests on the market, and to our knowledge there is only one available in Europe. Prima® Home Test produces a CRP test that can be performed by the patients at home, generating a result within 5 minutes [29]. According to the manufacturer, the test has been validated against the Roche Cobas laboratory-based kit (cutoff from 8 μg/ml) with a sensitivity of 100% and a specificity of 93.3%.

To our knowledge, there is no eHealth tool that incorporates a CRP home test in the disease monitoring of IBD. Since CRP response is not a specific marker for gastrointestinal inflammation, an increase of CRP in IBD patients could be associated with other medical conditions such as infections or extraintestinal inflammations. Therefore, in order to achieve the most accurate picture of IBD activity, CRP should preferably be accompanied with another biomarker or a PRO in the remote monitoring of IBD.

22.2.2 Fecal Calprotectin

Fecal calprotectin (FC), first described by a Norwegian group in 1980, is a calcium-binding protein expressed in the cytosol of neutrophils and macrophages [30]. Elevations of FC are seen in multiple gastrointestinal conditions such as IBD, infections, and colon cancer due to the migration of neutrophils to the gastrointestinal tract [31, 32]. FC is considered to be a stable biomarker that can be detected in the stool for more than 1 week, if stored at room temperature [31]. Some of the symptoms of IBD, such as abdominal pain and diarrhea, are shared with irritable bowel syndrome (IBS). At times it is therefore difficult to distinguish between the two diseases based exclusively on symptoms. However, FC has in a variety of trials been verified as a useful diagnostic tool that discriminates IBS from IBD [33]. Previous studies have shown that IBS symptoms are common in IBD patients in biochemical remission [34]; therefore disease monitoring with FC could potentially facilitate and reduce overtreatment of IBS symptoms with IBD medication [32].

Several studies have investigated the optimal cutoff value for FC as a predictor of endoscopic activity in IBD patients. However, the cutoff values differ widely depending on the study population, the type of assay used, and the method of stool sample collection [20]. Nonetheless, FC is considered to be a better surrogate marker of endoscopic activity in symptomatic IBD patients than CRP, mainly due to the higher sensitivity of FC [20, 28]. In a meta-analysis, the pooled sensitivity and specificity of FC for detecting endoscopically active IBD was estimated to be 88% and 73%, respectively. However, when analyzed separately, UC had an higher specificity (79%) compared to CD (67%) [20].

An early study of FC as a predictor of relapse found that UC patients in clinical remission with FC values ≥150 μg/g were at 14 times greater risk of relapse, while CD patients just carried a twofold increased risk [35]. These results are in line with a more recent study reporting that relapse rates in IBD patients in clinical remission were significantly higher among those with FC ≥150 μg/g [36]. In addition, a meta-analysis reported a sensitivity of 78% and specificity of 73% of FC in predicting flares in patients with quiescent IBD [37]. A recently published systematic review found that patients with two consecutively elevated FC values were highly associated with disease flares, while repeated measures in the normal range suggested sustained remission [38].

Patient-reported outcomes, such as activity scores in combination with nonspecific serum markers and endoscopy, have previously served as measures for treatment response [33]. However, during the last decade, FC has become a frequently used marker of treatment response, possibly due to its nature of being a noninvasive but yet reliable marker of IBD activity. In a previous study of CD patients treated with antibiotics, 5-aminosalicylates, immunomodulators, or steroids, FC was significantly decreased from the baseline value in responders (defined by endoscopy); however, it remained abnormal in partial and non-responders [39]. Moreover, in a study of UC patients treated with anti-TNF, elevated FC levels were detected up to 3 months prior to relapse [36]. These results point to the utility of FC as a marker of treatment response in IBD patients. Furthermore, based on improvements in the natural history of rheumatologic diseases for which treatment escalation has been adjusted based on biomarkers rather than symptoms alone, it has been suggested that a similar approach using biomarkers ought to be adopted in IBD, a treatment approach which has been recently supported by the CALM study [40, 41].

FC is normally analyzed by the enzyme-linked immunosorbent assay (ELISA) method, first described in 1992; however, this can be time consuming since the analysis is only performed every other week in some laboratories [42]. Thus, the results might be of limited value in guiding clinical decision-making due to this delay. However, during the last decade, newer techniques with point-of-care (POC) tests have emerged, and to our knowledge two home FC tests have been launched and used with success: CalproSmart™ and IB*Doc*®. Both tests consist of a lateral flow-based calprotectin test accompanied by a mobile application that turns the camera into a reader of the test [38, 42]. Both CalproSmart™ and IB*Doc*® have

been validated by laboratory personnel, comparing their performance with laboratory-based stool extraction and calprotectin ELISA testing [42, 43]. More recently the CalproSmart™ was also validated by patients, resulting in an optimal cut of at 150 μg/g and a sensitivity and specificity of 82% and 85%, respectively [44]. The IB*Doc*® also showed promising results when validated by ELISA in IBD patients with a Spearman test of 0.85 [38]. Both tests have high usability, and patients have a positive attitude toward these home testing devices [45, 46].

22.2.3 Biosensors: Wearable Technology to Monitor Adherence

Wearable biosensors are some of the newest technologies in the field of health care [47]. To date, the most well-known wearable biosensors are fitness trackers, which monitor step counts and physical functions such as heart rate and sleep [48]. In terms of health care, biosensor techniques have been introduced as implants in muscles to monitor activity or to automatically sample blood and measure biomarkers [49]. They have also been be incorporated in pharmaceutical products to measure adherence to medicine [50]. In diabetes and cardiology, wearable biosensors have been used with mostly positive results in the monitoring of glucose levels and arrhythmias, respectively [51, 52].

To date there are a limited number of biosensors used in diseases of the gastrointestinal tract [49]. One device, the AbStats (GI Logic), is developed to measure intestinal rate, by a low-profile microphone that adheres to the abdominal wall in postsurgical patients. Previous research has suggested that this biosensor can predict which postsurgical patients will subsequently develop postoperative ileus, and ongoing research is investigating whether it can differentiate between meal sizes [48].

Good adherence to medical therapy is key in sustaining remissions and reducing the inflammatory burden of the gastrointestinal tract in IBD patients. Several approaches focusing on increasing medical compliance have been introduced, e.g., reduced pill burden, education programs, and setting alarms on mobile phones/watches [53]. During the last decade, newer technologies including innovative telemedicine devices have been created and have resulted in increased adherence in adult and pediatric IBD patients [5, 54]. However, evaluating adherence is a challenge in itself, and strategies for obtaining reliable measurement measurements are required. To date, several methods exist from self-reported measures to directly observe therapy and measurement of drugs or their metabolites in urine or blood. However, all methods come with different disadvantages and gold standards are still lacking [52]. In the era of mobile health technologies, novel approaches to measuring adherence have emerged [50, 55]. In a trial of high-risk cardiovascular patients, an ingestible, biodegradable sensor was investigated for the direct measurement of medication ingestion. The ingested sensor is able to be detected by an externally worn patch when it encounters the acidic environment of the stomach. Subsequently, the patch sends a Bluetooth signal to a software application on a tablet/smartphone [55]. A similar approach, with an ingestible sensor, wearable sensor, and software application, has been investigated in patients with schizophrenia [56]. Both studies reported positive results, and the methods were well tolerated by the patients, indicating that ingestible biosensors can be used as clinical tools to measure adherence and encourage better medical adherence. To our knowledge, no research regarding the possible effects of ingestible biosensors on adherence has been performed in the field of IBD. Since biosensors have shown great potential in other chronic diseases and with the expanding availability of technologies, positive results are to be expected in gastroenterology, as well. However, these new technologies are still far from being integrated into clinical practice, and future studies are needed to investigate their place in the remote monitoring of adherence [57].

22.3 Future Aspects of Remote Monitoring

Remote monitoring of diseases has during the last decade become possible due to the emerging access to high-speed Internet and smartphone devices [47]. Patients are, therefore, to a larger

extent now requesting digital technology options in order to manage their health. Until recently a majority of eHealth devices have focused on results of PROs in the remote monitoring of IBD. However, recent studies on eHealth technologies combining a PRO with a POC analysis of FC have showed promising results [58, 59]. These data suggest that combined use of PRO and patient-reported information (PRI) might possibly build a more complete and accurate picture of disease progression and activity [49]. Ingestible biosensors are the latest technologies in measuring adherence, with one system developed by Proteus Digital Health [50] already having achieved approval by the Food and Drug Administration [57]. While patients appear positive toward using eHealth devices to maintain their health, both health-care providers and systems seem to be the biggest hindrance for spreading remote monitoring, possibly due to the limited available evidence [60]. Therefore, in order to integrate eHealth devices into the daily practice of gastroenterologists, future studies will have to focus on whether telemedicine self-care approaches improve the disease course as well as the long-term direct and indirect health-care costs.

Summary Points

- Inflammatory bowel disease (IBD) is increasing worldwide, resulting in an accumulating demand for effective disease management options.
- Electronic health technologies have the potential to screen the inflammatory burden, thus reducing time to remission and number of outpatient visits, as well as increasing compliance with medical therapy and patients' empowerment.
- Point-of-care tests of fecal calprotectin (FC) have been introduced, validated, and used with success in the remote monitoring of IBD.
- Ingestible biosensors have shown promising results as measurements of medical adherence.

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Part V

Scientific Platforms for Biomarker Discovery

Exposome and Diet

23

Jakob Seidelin and Johan Burisch

Abstract

The underlying etiopathogenic factor for inflammatory bowel disease (IBD) remains unclear. It is generally accepted that IBD results from a complex relationship between genetic susceptibility, environmental factors, and intestinal microbiota, resulting in a self-perpetuating abnormal mucosal immune response. While several environmental factors have been associated with the onset of IBD, to date no specific environmental/microbial causative factors have been identified. Less is known about the influence of the exposome, the sum of all environmental exposures faced by a human being during his life, on the disease course in established IBD. In this chapter we summarize what is known about the influence of environmental exposures such as lifestyle factors, drugs, appendectomy, infections, diet, and external factors such as altitude and pollution. We demonstrate how the influence of the exposome on disease course remains poorly investigated and understood underlining the need for more clinical epidemiological and mechanistic research.

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23.1 Introduction

There is strong evidence that the exposome – meaning all of the environmental exposures faced by a human being from conception to death [1] – plays a substantial role in the pathogenesis of inflammatory bowel disease (IBD). For one, the rapid increase in the occurrence of both Crohn's disease (CD) and ulcerative colitis (UC) across the globe, and especially in regions where these diseases were previously rare [2], cannot be explained by genetics alone [3]. Furthermore, studies of migrants have shown that their risk of IBD can change significantly depending on whether their destination has a higher or lower incidence of IBD than their home region [4, 5]. Several observational studies have described the influence of various environmental factors on the risk of IBD, but less is known about their influence on established diseases and disease course. This chapter provides an overview of the environmental factors that have been associated with the natural history and prognosis of IBD (Fig. 23.1).

23.2 Lifestyle

23.2.1 Smoking

Smoking is the environmental factor whose influence on the disease course of IBD has been described in greatest detail. Most observational

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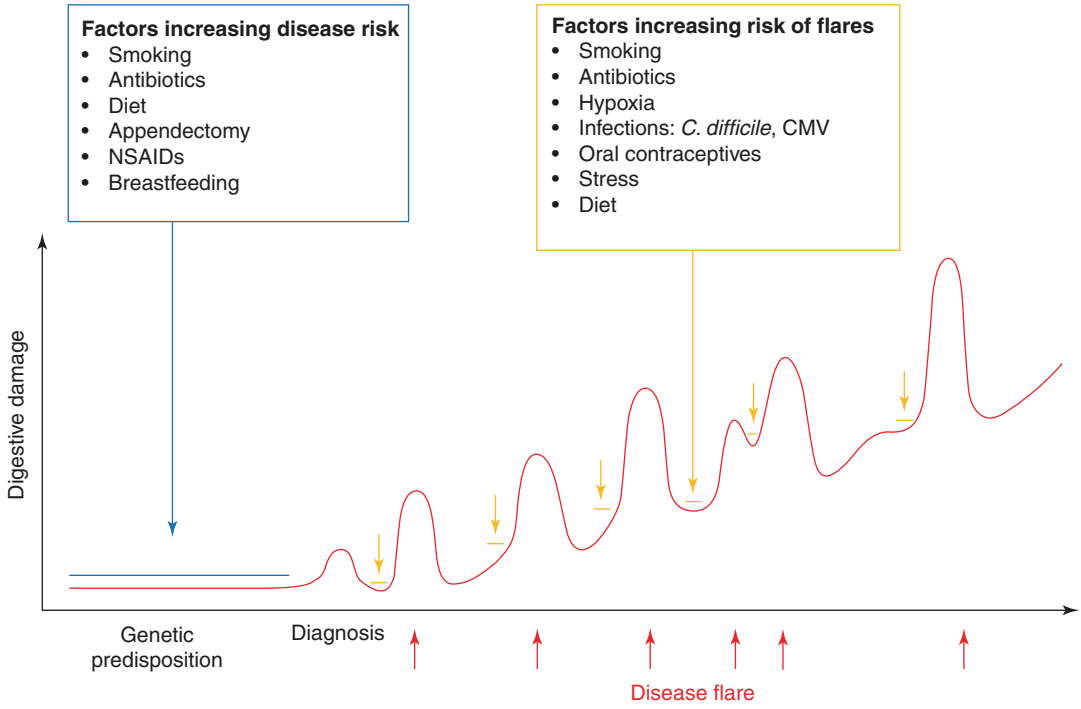

Fig. 23.1 The association of the exposome in inflammatory bowel disease

cohorts have found that smoking has a detrimental effect on the disease course of CD. A meta-analysis of 33 studies found that active smokers are twice as likely as non-smokers to experience relapse, relapse after surgery, require surgery in the first place, or need additional surgery [6]. Furthermore, there seems to be a dose-dependent effect of smoking on the risk of disease flare-ups [7]. However, many of these increases in risk are reversed when smoking is discontinued [8]. In UC, smokers do not seem to benefit from an improved disease course when compared to ex-smokers or non-smokers [9, 10], as confirmed by a recent meta-analysis of 16 studies [11]. Smoking does not seem to affect the efficacy of treatments, including tumor necrosis factor inhibitor drugs [12].

23.2.2 Stress

IBD patients experiencing symptoms of active disease report higher levels of stress than those who are asymptomatic [13]. Furthermore, several studies have observed that perceived stress can predict future relapses. For example, in one population-based cohort study, higher perceived levels of stress during a 3-month period predicted future exacerbation of symptoms among IBD patients [14]. In another prospective study of 62 UC patients, those in the highest tertile of perceived stress were 6 times more likely to suffer a relapse [15]. Recently, a Canadian study of 417 IBD patients demonstrated that higher perceived levels of stress predicted a worsening of symptom activity 3 months later in patients with CD, but not in patients with UC [16]; however, in this particular study, no association between perceived stress and intestinal inflammation could be found.

23.2.3 Physical Activity

Little is known about the impact of physical activity on the course of IBD, and most studies have been conducted among patients with CD. One small study found that a 3-month walk-

ing regimen improved quality of life, stress levels, and body mass index and aerobic capacity among patients with CD [17], while in another study a low-intensity, 30-minute walk three times per week across 3 months was found to improve quality of life and to decrease CD-related symptoms [18]. More recently, a prospective study found that patients who did more weekly exercise at baseline saw a 32% reduction in risk in CD, and 24% reduction in risk in UC, of experiencing active disease after 6 months [19].

23.3 Drugs

23.3.1 Nonsteroidal Anti-inflammatory Drugs

While laboratory data suggest several mechanisms for nonsteroidal anti-inflammatory drugs (NSAIDs) to induce relapse in IBD [20], observational studies have not provided consistent results. For example, a retrospective study found that of patients experiencing a flare-up, more had been taking a daily dose of NSAIDs the month before relapse than those who had not (OR 6.31 CI95% 1.16–34.38) [21]. Another case-control study found that NSAIDs were positively associated with relapse (OR 20.3 CI95% 2.6–159.7) [22]. However, other studies could not demonstrate any association between NSAIDs and relapses [23, 24]. One open-label trial investigated the effect of administrating paracetamol, aspirin, or a variety of NSAIDs to patients with quiescent IBD; while no patients taking aspirin, paracetamol, or nimesulide had a flare-up of their disease within the 4-week study period, 17–28% of those taking nonselective NSAIDs did experience a relapse, with symptoms appearing within days of first taking the drug [25].

23.3.2 Oral Contraceptives and Hormone Replacement Therapy

While the use of exogenous hormones in the form of oral contraceptives has been linked to the risk of CD [26], the precise association with disease course is less clear and the number of studies addressing the topic limited. One study found that during a follow-up period of 12 months, the use of oral contraceptives was not associated with an increased risk of relapse [27], while another study of women who underwent primary surgical resection for CD found no difference in the risk of surgical recurrence between users of contraceptives and nonusers [28]. On the other hand, one study nested in the placebo arm of a randomized controlled trial found that current and previous users of oral contraceptives had a significantly higher risk of relapse than did nonusers (HR 3.0 CI95%: 1.5–5.9) [29]. However a large, nationwide study from Sweden demonstrated that long-term (more than 3 years) use of oral contraceptives was associated with an increased risk of surgery (HR 1.68 CI95% 1.06–2.67) [30]. Another study investigating the use of oral contraceptives among UC patients found no statistically significant difference in relapse rates between users and nonusers (26.5 vs. 40%) [31].

Only one study has investigated the association of hormone replacement therapy and disease course. In this study, rates of flare-ups among premenopausal and postmenopausal patients with IBD did not differ; however hormone replacement therapy (among the postmenopausal women) was associated with a decreased risk of flare-ups (HR 0.18 CI95%: 0.04–0.72) [32].

23.3.3 Statins

Few studies have investigated whether certain drugs have a beneficial effect on IBD disease course. In a large administrative data set of almost 12,000 IBD patients, exposure to statins was associated with a reduced rate of treatment with steroids among patients with UC, including lower rates of the use of antitumor necrosis factor, fewer abdominal surgeries, and fewer hospitalizations among all IBD patients combined [33]. Furthermore, two small studies have investigated the effect of treating IBD with statins directly and concluded that statins can improve disease activity [34] as well as the markers of inflammation, such as C-reactive protein [35].

23.4 Appendectomy

While many studies have provided evidence of an association between appendectomy and disease risk, fewer studies have investigated whether appendectomy influences disease severity and its course – with conflicting results. Some have found that UC patients who underwent an appendectomy had a less severe disease course, including either fewer relapses, a decrease in immunosuppressant requirements, or a decrease in colectomy rates [36–38], while others did not observe any differences regarding colectomy rates or the need for immunosuppressants [39, 40].

Recently, a Swedish national cohort study of more than 60,000 UC patients found that an appendectomy occurring before a diagnosis of UC, if performed for appendicitis in patients younger than 20 years old, was associated with a lower risk of colectomy (HR, 0.44; CI95%, 0.27–0.72) and hospitalization (IRR: 0.75 CI95%: 0.69–0.82) [41]. Interestingly, appendectomy performed for appendicitis after a diagnosis of UC appeared to increase the risk of colectomy significantly (HR 1.56, 95% CI 1.20–2.03). Similar findings were made in a study from the National Institute of Diabetes and Digestive and Kidney Diseases Inflammatory Bowel Disease Genetics Consortium database [42], in that an appendectomy after a diagnosis of UC was significantly associated with colectomy (OR, 2.22; CI95%, 1.10–4.49). However, its authors also performed a meta-analysis that could not demonstrate any impact of appendectomy on colectomy risk, regardless of timing.

Several studies have found that among patients with CD, an appendectomy increases the risk of developing disease [43]; however, this finding has proven controversial and was most likely caused by confounding due to the similar clinical presentations of appendicitis and acute ileitis. Only limited data are available regarding the influence of appendectomy on disease course among patients with CD. Some studies have demonstrated an increased risk for intestinal resection in patients who had an appendectomy performed before their diagnosis of CD (e.g., IRR, 2.7; CI95%, 1.9–4.0) [44, 45], while one study found that appendectomy delayed diagnosis [38].

23.5 Tonsillectomy

While some studies have shown tonsillectomy to be a risk factor for CD, others have been unable to confirm this relationship [46]. Although a meta-analysis of 17 observational studies showed an adjusted OR of 1.37 (CI95%:1.16–1.62) for development of CD after tonsillectomy (with no association found with UC [46]), it seems reasonable to conclude that if an association does exist between tonsillectomy and CD, it is a weak one. Unfortunately, there are no data concerning the risk of flare-ups in patients with established IBD who are tonsillectomized.

23.6 Infections

23.6.1 Non-*Clostridium difficile* Pathogenic Bacteria

Infections with enteropathogenic *Salmonella* or *Campylobacter* spp. have been shown to increase the risk of a flare-up following a recent diagnosis of IBD [47], but the increased risk might be explained by simple detection bias because negative stool cultures also predispose one to IBD [48–50]. Accordingly, a large, single-center study at the Mayo Clinic in Rochester, MN, USA, showed a surprisingly low rate of non-*Clostridium difficile* infection (CDI) positive stool PCR or culture tests in IBD patients with flare-ups (0.9% in UC, 2.5% in CD) [51]. Patients with positive tests were less likely to need adjustments in their IBD-specific treatment, had a less severe disease course, and were more likely to remain in remission through the following year as compared to patients with a negative test result. Non-CDI enteropathogens thus have a limited impact on the disease course of IBD and are easily managed by antibiotics, when indicated.

The roles of other specific pathogens (e.g., *Mycobacterium avium*) in the development of IBD have been widely discussed but are far from established as causal factors for IBD [52].

23.6.2 *Clostridium difficile* Infection

In contrast to non-CDI gastrointestinal infections, CDI is well-documented as having a significant impact on IBD disease course: IBD predisposes one to CDI, resulting in a higher risk of CDI in patients admitted with CD (OR 2.9 CI95%: 2.1–4.1) and UC (OR 4.0 CI95%: 2.4–6.0), as compared to non-IBD patients [53]. Furthermore, a large epidemiological study in the USA using the National Hospital Discharge Survey found that IBD patients with CDI had more acute hospital admissions and longer stays than did non-IBD patients with CDI (6, rather than 4 days) and higher mortality rates (OR 4.5 CI95% 4.2–4.9) than admitted IBD patients without CDI [54]. Risk of colectomy is also increased in IBD patients with CDI as compared to non-IBD-CDI patients (6.4% vs. 0.3%) [55]. While glucocorticoids, antibiotics, and proton pump inhibitor use are established risk factors for CDI in the general population, data about these factors from the IBD population have been conflicting [56]. Conflicting data also exist for other forms of immunosuppressants and immunomodulators used to treat IBD, with some larger studies suggesting that immunomodulators increase the risk of CDI [57]. Systemic glucocorticoids might aggravate the CDI disease course in patients with IBD [58]. The increased risk and impact on disease course justify routine testing for CDI in IBD patients experiencing a flare-up.

23.6.3 Cytomegalovirus Infection

Cytomegalovirus (CMV) is common as a latent infection in the general population; however the prevalence of CMV colitis has not been determined; retrospective PCR-based analyses suggest that rates above 15% in the UC population are highly biased by the selection of participants [59, 60]. The use of glucocorticoids and antimetabolites is associated with an increased risk of CMV colitis [60]. On the other hand, CMV infection is associated with steroid refractory disease, suggesting that CMV colitis is able to drive the inflammation in IBD patients rather than being passively reactivated during inflammation and treatment-induced immune system suppression [59, 61]. Patients with CMV infection and IBD have a higher risk of severe and extensive colitis and colectomy (e.g., 33% vs. 13% of children with acute severe colitis with and without CMV infection, respectively) [59]. Treatment with infliximab or cyclosporine, along with the antiviral treatment, does not seem to affect the risk of colectomy in patients with CMV colitis [62].

23.7 Diet

23.7.1 Carbohydrates, Proteins, and Fatty Acids

The increasing prevalence of IBD and its association with a western lifestyle suggest that diet plays a role in the pathogenesis of IBD [63]. A western diet typically is high in processed proteins and fat and an unbalanced intake of polyunsaturated fatty acids (PUFAs), combined with a low intake of vegetables and fruits. A high-fiber intake, in the form of fruits and vegetables, protects against CD (HR 0.59 CI95 0.39–0.90), whereas the data regarding the association of fiber intake and risk of UC have been contradictory [64]. Accordingly, a high-fiber diet has been associated with fewer exacerbations of CD (OR 0.58 (0.43–0.81)) [65]. A larger prospective study has suggested that flare-ups in UC are associated with a high intake of myristic acid (coconut oil, palm oil, and dairy products) when compared to a diet low in myristic acid (OR 3.01 (1.17–7.74)) [66], while the beneficial effect of n-3 PUFAs, found in earlier intervention studies, could not be confirmed [67]. High intake of other specific components of a western diet (such as processed meats and protein) was not associated with flare-ups in this study, as opposed to the positive association found in an earlier study [68].

23.7.2 Emulsifiers/Additives

Although there are several theoretical and experimental links between IBD and food additives such as aluminum silicate, titanium oxide, and emulsifiers, no direct evidence exists for their role in IBD development or disease course [69]. The diet typical of countries with a high prevalence of IBD contains high levels of emulsifiers such as lecithin, and several studies have shown that this compound and other emulsifiers like it increase intestinal permeability, possibly through their action as detergents [70]. Other studies on mice suggest that exposure to emulsifiers induces colitis and metabolic syndrome-like features through changes to the microbiota [71].

23.7.3 Vitamin D

Patients with IBD tend to have lower vitamin D levels than the general population, and this has been associated with the inflammatory activity that they experience [72]. Furthermore, genetic polymorphisms of the vitamin D receptor have been associated with IBD [73]. A prospective study has shown that low vitamin D levels are associated with an increased risk of flare-ups in UC patients, even among those with no histological evidence of inflammation, i.e., patients in deep remission [74]. In a randomized clinical trial, vitamin D supplementation of CD patients showed a trend toward lowering the risk of flare-ups [75]. Vitamin D levels are positively correlated with patients' response to biologics [76]. There is also a beneficial role of vitamin D supplements in helping to avoid osteoporosis [77]. In light of these benefits, prophylactic vitamin D and calcium supplements are recommended, at least during the winter months in both northern and southern hemispheres, and measurements of vitamin D levels should be taken in order to guide supplementation. Conflicting views exist about the optimal dose of vitamin D for patients with IBD; it should most likely be higher than general recommendations, with studies suggesting that levels above 75 ng/ml are sufficient [78].

23.7.4 Alcohol

In a single study of patients with UC, alcohol was found to be associated with flare-ups, but these results were not able to be replicated in a later study examining alcohol as a risk factor in IBD [68].

23.8 Microbiome

Whereas *C. difficile* and CMV are the only specific enteropathogens of clinical importance associated with IBD flare-ups, it remains the case that the overall microbiota of the gut seems consistently to be less diversified in patients with IBD than in healthy subjects, and this dysbiosis tends to worsen during flare-ups [79]. It is an open question as to whether the dysbiosis is secondary to the intestinal inflammation or if it reflects the presence of a specific pathobiont, i.e., a composition of microbes capable of causing IBD [80]. IBD seems to be associated with the use of antibiotics in early life [81], and antibiotics may have a place in the treatment of IBD [82], all of which might suggest that microbiota changes lead to intestinal inflammation and IBD in susceptible persons. Although trials on fecal microbiota transplant for the treatment have been contradictory, they nonetheless indicate that normalization of the microbiota could improve intestinal homeostasis and reduce inflammation [83, 84]. A prospective study on pediatric IBD patients suggests that changes in the microbiota are paralleled by flare-ups but are also substantially influenced by the use of antibiotics and dietary changes [85]. Similarly, in pediatric patients the grade of dysbiosis appears to correlate with disease activity and could predict the response to immunomodulatory or biologic treatments [86]. However, data are lacking on other outcome mea-

sures in IBD, including the risk of surgery and the recurrence of disease after surgery.

23.9 Geography

23.9.1 High Altitude

High altitudes (above 2000 m on land or aboard commercial flights) reduce the partial pressure of oxygen, resulting in lower availability of oxygen to internal organs. Humans not acclimatized to high altitudes are liable to experience hypoxia-induced organ inflammation, with increased circulating inflammatory mediators like C-reactive protein and IL-6 [79]. In one Swiss study, high altitude was shown to predispose IBD patients to a significantly greater risk of flare-ups for a 4-week period after their exposure to a high altitude, with 40.4% of them suffering a flare-up compared to 15.7% of patients not exposed [87].

23.9.2 Travel

Travelling per se has been studied as a risk factor in IBD. One larger study from Israel showed a 1.4-fold risk of illness during trips among IBD patients as compared to healthy controls. The increased risk was almost entirely in the form of a worsening of their IBD and was correlated with frequent flare-ups and IBD-related hospitalizations prior to the trip [87], whereas IBD-specific treatments did not affect the risk. As such, the increased risk does not seem to be related to factors associated with travelling itself, such as GI infections, but rather to the disease characteristics of the individual patient; travelling in and of itself appears to present only a low risk to IBD patients.

23.9.3 Pollution

Although IBD is more frequent in urban settings, only a few studies have focused on how air pollution contributes to the risk of IBD flare-ups. One single study showed a correlation between the pollutant emission density and the risk of IBD-related hospital admissions. The increased risk was seen in both UC and CD, and no specific pollutant could be identified as more important than any of the others [88]. No reports exist that directly link water supply pollution to IBD flare-ups, but a single study has related specific bacterial compositions of tap water to the risk of IBD [89]. Similarly, the iron content of tap water has been associated with increased risk of developing IBD [90].

23.9.4 Seasonal Variation

Both CD and UC patients have more flare-ups during winter, and this seasonality seems to be more pronounced for younger than for older IBD patients than for older [91]. Multiple reasons most likely exist for this pattern, including seasonal variation in vitamin D levels, prevalence of GI infections, and composition of diet. Lower vitamin D levels in the winter should be counteracted through supplements in order to decrease the risk of flare-ups among those with IBD.

23.10 Future Directions

In contrast to genetic risk factors, the exposome can be modulated, and thereby its influence on disease course is mitigated. In light of this, it is crucial to improve our understanding of how different environmental factors affect the course of disease in order to give patients the best advice on how to prevent environmentally triggered flare-ups, as well as how to improve clinical outcomes and health-related quality of life in the long term. To date, most studies investigating the exposome and IBD have been limited by factors such as a small sample size, retrospective design, and limited ascertainment of exposures and clinical factors influencing disease course. Furthermore, and a topic not able to be addressed in this chapter, the mechanisms by which environmental factors influence the disease course of

IBD are poorly understood; hence, more clinical epidemiological and mechanistic research is needed.

Summary Points

- The influence of the exposome on the disease course of inflammatory bowel disease remains poorly understood.
- While some factors such as smoking and *C. difficile* infections have been found to significantly influence disease course, epidemiological findings regarding other factors such as oral contraceptives, appendectomy, diet, and NSAIDs are inconsistent.
- In order to give patients the best advice on how to prevent environmentally triggered flare-ups of their disease, more clinical epidemiological and mechanistic research is needed.

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The Microbiome in IBD

24

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Abstract

In individuals with IBD, the microbiome occupies an integral juncture between their genetics and disease profile and may participate to the manifestations of the disease and the severity of its course. This chapter will highlight major microbiome research studies showing the pathobionts and symbionts in the human microbiome associated with IBD. It will also include a brief outline of various bacterial, viral and fungal sequencing techniques, including 16S rRNA and hiSeQ, along with a post-processing interpretation of the data to arrive at potential biomarkers of IBD. It will also cover the major microbial signatures found in association with IBD, including postoperative recurrence of Crohn's and the occurrence of pouchitis.

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24.1 Introduction

Microorganisms present in the gastrointestinal tract (the microbiome) interact with each other and with host cells in both health and disease (including IBD). Their death, survival and development are controlled by environmental factors such as diet, antibiotics and inflammation. Hence, the presence or absence of certain microorganisms may be used as a bioindicator of ecological selective pressures, including those resulting from disease. Molecular methods have revolutionised our ability to describe changes in the microbiota. Microorganisms (which include bacteria, archaea, yeast, fungi and viruses/bacteriophages) have many functions. They secrete bioactive molecules, transform exogenous or endogenous molecules and are sensed by host cell receptors, such as NOD-like and Toll-like receptors. There is thus great value in the pursuit of

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disease markers in (or from) the microbiome and as therapeutic targets in IBD, which we review in the present chapter. To date, ecological description tools are not used in clinical practice but are only available to researchers. Individual data on qualitative and quantitative measurements of microbiota ("microbial profiles") are not sufficiently standardised to guarantee their accuracy and reliability, although this may happen soon.

24.2 Overview of the Gut Microbiome in IBD: Description and Potential Role in Pathogenesis

Many tools can be used to describe the microbiota, its genetic potential and its metabolic activity (respectively, the metagenome, transcriptome, proteome and metabolome). Most studies of the bacterial composition of the gut microbiome have used a 16S rRNA gene-based approach. This allows characterisation of the microbiome at the family or genus level and includes the quantitative polymerase chain reaction (qPCR) and restriction fragment length polymorphism (RFLP) or pyrosequencing. A higher resolution can be reached using shotgun sequencing (brands such as Illumina, Ion Proton, PacBio, etc.). Microbial diversity can be assessed within a day for numerous samples in parallel for costs that have steadily diminished, currently reaching approximatively 50€ for 16S rDNA and 500€ for shotgun sequencing. In the context of research, it is commonly applied to assess the microbiome in cohort studies and clinical trials.

The microbiota differs between niches in the gastrointestinal tract. This is because segments vary in their ecological conditions as measured by pH, gastrointestinal transit time, the availability of nutrients and the presence of bile acids and substrates for adhesion. The microbial composition of faeces varies from chime microbiota in the right colon [27] to mucosa-adherent microbiota present in the ileum and entire colon of both healthy people and those with IBD [15, 19, 22]. Micro-biomarkers of IBD have been found in both faecal and mucosal biopsy samples.

The role of the microbiota in IBD pathogenesis has been approached through interventional studies and descriptive studies in human and animal models. Interventional studies have tested the influence of candidate microorganisms on cells, tissues or animals. They have also studied the consequences of altering the microbiota in patients or animals by administering antibiotics, prebiotics, fibres or probiotics and faecal transplantation. The main features that suggest the microbiota's role in the pathogenesis of IBD are summarised in Table 24.1. The main dysbiotic characteristics repeatedly observed in IBD cases are shown in Table 24.2.

Pathogens or pathobionts are rarely observed in IBD, and treatments that attempt to eradicate or limit them have little effect on IBD symptoms and lesions. Some microbes are significantly less represented in IBD, especially those from the *Firmicutes* phylum [15, 36, 46]. The dominant *Firmicutes* from the *Clostridium* cluster IV, *F. prausnitzii*, belongs to the core microbiota in the vast majority

Table 24.1 Main arguments for the role of the microbiota in IBD

	Animal models	Humans
Arguments	Researchers have not been able to induce experimental IBD in germ-free animals Some microbiota are more colitogenic than others [28]	IBD lesions predominate where bacteria are most abundant (at the end of the ileum and the colon) Genetic polymorphisms associated with IBD risk factors include genes involved in bacterial recognition and/or autophagy [17, 20, 35, 39] The microbiota of IBD patients differs from that of healthy participants (dysbiosis) Metronidazole and ciprofloxacin are effective treatments for pouchitis The probiotic mixture VSL #3 is effective in preventing the recurrence of pouchitis The transfer of faecal microbiota is an effective treatment for UC

Table 24.2 Main dysbiotic features repeatedly observed in IBD

Rate of occurrence	Dysbiotic features
Very often	Unstable composition of the dominant microbiota over time Decreased microbial richness Restricted biodiversity, especially among *Firmicutes* Decreased proportions of *Faecalibacterium prausnitzii*, *Roseburia* spp., *Butyricicoccus pullicaecorum* or *Akkermansia* spp.
Often	Increase in *Enterobacteriaceae* including adherent invasive *Escherichia coli* (AIEC) associated with the ileal mucosa in ileal CD Increase in H_2S producers [31] Increase in fusobacteria in patients with UC
Sometimes	Acquisition of *Clostridium difficile* Presence of *Mycobacterium avium paratuberculosis* during CD

of healthy humans. Research has shown that it is lower in individuals with UC and CD [45]. However, this finding was more significant in CD, especially ileal CD. The concentration of *F. prausnitzii* often mirrors that of *Enterobacteriaceae* [23], and the ratio of *E. coli* to *F. prausnitzii* is a proposed method for assessing dysbiosis in CD [3, 45]. The microorganism *F. prausnitzii* can lower the production of pro-inflammatory cytokines IL-12 and IFN-γ and can increase IL-10 secretion in peripheral blood mononuclear cells. It can also block the NF-κB pathway in intestinal cell lines. Its in vivo anti-inflammatory effects and that of its culture supernatant have been demonstrated in mice with TNBS-induced colitis [43].

Levels of butyrate-producing bacteria are often lower in people with IBD compared with healthy controls. These groups encompass *Roseburia* [24, 38], *Eubacterium* [38] and *F. prausnitzii* [24]. Butyrate has several anti-inflammatory effects on the intestine [7, 9, 41, 47, 50]. Levels of *Akkermansia muciniphila*, a member of the phylum *Verrucomicrobia*, may be low in individuals with UC. Experimental studies have shown that it reinforces the epithelial barrier at tight junctions and has very little pro-inflammatory effect on epithelial cells.

Several studies have shown differences in the microbiota (either faecal or mucosa-associated) between UC, ileal CD and colonic CD. In addition to different genetic risk factors and phenotypes, this strongly suggests that these are three different diseases [15, 21, 24, 32]. For instance, several groups have reported that levels of *F. prausnitzii* were lower and levels of *Enterobacteriaceae* were higher in ileal CD than in colonic CD [32], regardless of the biopsy site.

Ecological alterations in IBD affect archaea, bacteriophages and fungi in addition to bacteria [14]. Sokol and colleagues studied the bacterial and fungal compositions of samples of faeces from 235 participants (IBD patients and healthy controls) using 16S and ITS2 sequencing. They observed that the fungal microbiota was skewed in IBD, with an increased *Basidiomycota/Ascomycota* ratio, a decrease in *Saccharomyces cerevisiae* and an increase in *Candida albicans* [44].

The microbiota and host cooperate to transform endogenous and exogenous substrates; hence candidate metabolites and the metabolome are sources of potential micro-biomarkers. For example, the faecal concentrations of conjugated bile acids (BA) were found to be significantly higher in active colonic IBD, whereas that of secondary BA were significantly lower. Additionally, in IBD, decreased concentrations of isomerised forms of BA, including isolithocholic acid (iso-LCA), isodeoxycholic acid (iso-DCA) and ursodeoxycholic acid (UDCA), are observed. The ratio of iso-LCA to LCA in faeces has been shown to have a strong correlation with dysbiosis, IBD activity and IBD diagnosis [4].

24.3 Microbial Signatures and Other Microbial Biomarkers in Clinical Situations of IBD

The presence or absence of a single microorganism or metabolite is not specific enough to be a signature of IBD, but some combinations of microbial variables described below have interesting biomarker characteristics.

24.3.1 Associations with Crohn's Disease

Gevers et al. conducted a pioneering and impressive work on a large series of children with previously untreated IBD. They studied the faecal- and mucosa-associated microbiota and inferred a taxon–taxon interaction network [15]. After observing that some groups tended to exhibit co-occurrence while others exhibited co-exclusion, the authors proposed a microbial dysbiosis index (MD-index). They chose to calculate this arbitrarily as the log of the total abundance of organisms increased in CD over the total abundance of organisms decreased in CD. They took samples at different sites to evaluate how well the MD-index classified the CD state of participants using a receiver-operating characteristic (ROC) analysis. The best performances were obtained for ileal mucosa biopsy samples (AUC = 0.85) and rectal biopsy samples (AUC = 0.78), while faecal samples did not perform that well (AUC = 0.66). In another paediatric series with prospective longitudinal microbial follow-up, the MD-index was significantly correlated with severity of IBD, but not with treatment response [42].

Further evidence of the association between specific microorganisms and CD was from studies by V. Pascal and colleagues. They analysed faecal samples from a large cohort of IBD and non-IBD participants using 16S rRNA sequencing with the aim of developing an algorithm to discriminate between those who had CD and those who did not [36]. Eventually, the algorithm retained samples that did not contain "*Faecalibacterium* or *Peptostreptococcaceae* and *Anaerostipes* and *Christensenellaceae*" or those that contained "*Fusobacterium* and *Escherichia* but not *Collinsella* and *Methanobrevibacter*". The algorithm was tested on several data banks and obtained an average of 77.7% true positives for CD detection and an average of 7.3% and 12.8% false positives for healthy controls and UC patients, respectively. However, when applied to a French cohort, its accuracy was only 64% when discriminating between CD and UC (60% sensitivity and 68% specificity) and 77% when discriminating between CD and healthy controls (60% sensitivity and 94.8% specificity). There is therefore clear hope that consensual micro-biomarkers will soon be found, although the critical step of standardising methods is not yet complete [10].

24.3.2 Association with Early Recurrence of Ileal Crohn's Lesions After Surgery

In CD, lesions may recur early after surgery (i.e. less than 1 year) in a large proportion of patients, and several authors have wondered if microbial characteristics at the time of surgery (especially in the surgical specimen) could help predict this outcome. Only a small series of patients have been studied.

A study of 21 participants by H. Sokol and colleagues found that a higher level of *F. prausnitzii* in the ileal mucosa of surgical specimens was associated with a lower risk of early postoperative recurrence [43]. In a study of six participants, at the time of surgery, the microbiota of CD patients who remained in remission had more richness and was more similar to controls than that of patients with subsequent recurrence [13]. In a series of studies on 12 participants, De Cruz and colleagues showed that patients with recurrent disease harboured more *Enterococcus* and *Veillonella* spp., while those maintaining remission had higher levels of *Bacteroides*, butyrate-producing *Firmicutes* and *Prevotella* and *Parabacteroides* spp. [12]. Mondot and colleagues studied whether the microbiota composition of faeces collected from 20 participants just before surgery could help predict recurrence [30]. They found that four specific molecular species had biomarker potential. The presence of *Coprococcus catus* and a relative of Clostridiales bacterium (*Butyricicoccus* genus) were significantly associated with the absence of recurrence. The presence of *Proteus mirabilis* and a relative of *Eubacterium rangiferina* were associated with future postoperative recurrence. Wright and colleagues studied the differences in taxa observed in the surgical specimens of 34 participants. Patients who had early endoscopic recurrence had higher levels of members of the *Firmicutes*

phylum, the *Bacteroides* genus and the *Bacteroidaceae* and *Pasteurellaceae* families than those without recurrence [52].

24.3.3 Associations with IBD Activity and Severity

Some authors have investigated the correlation of faecal microbial profiles as biomarkers to distinguish between CD patients in remission and those with active disease. For example, Tedjo D.I. and colleagues conducted a longitudinal study in which they collected faecal samples from CD patients in remission and during active disease. A random forest analysis highlighted 50 OTUs (or bacterial taxa) as able to discriminate between remission and active disease, with a sensitivity of 0.79 and a specificity of 0.73. As expected, *F. prausnitzii* was associated with remission [48]. Varela and colleagues used quantitative real-time PCR to determine the total faecal bacteria counts of *F. prausnitzii* in 116 UC patients in remission, 29 first-degree relatives and 31 healthy controls [49]. They found lower counts of *F. prausnitzii* in UC patients and their unaffected relatives compared to healthy controls (faecal counts of 1.4×10^8 copies/g and 1.7×10^8 copies/g vs 6.5×10^8 copies/g). Patients who had experienced a disease flare less than 12 months before the study had lower counts of *F. prausnitzii* compared to patients with longer remission. Faecal counts of *F. prausnitzii* $< 10^8$ copies/g increased the probability of having a relapse within 12 months fourfold ($p < 0.001$).

24.3.4 Prediction of Pouchitis

Machiels and colleagues collected faecal samples from 21 patients with UC before colectomy and ileal pouch anal anastomosis (IPAA) and at regular intervals for the following 12 months. They observed that the presence of *R. gnavus*, *B. vulgatus* and *C. perfringens* and the absence of *Blautia* and *Roseburia* in faecal samples before surgery were associated with a higher risk of developing pouchitis [25]. In line with this, N. Maharshak and colleagues used 16S rRNA gene pyrosequencing to analyse the faecal microbiota of 20 patients with a normal pouch after IPAA. They compared samples collected before the development of pouchitis in some patients ("pre-pouchitis group", $n = 7$) with those collected from patients who did not develop pouchitis ($n = 13$). Genera *Ruminococcus*, *Lachnospira* and *Coprococcus* were significantly lower in pre-pouchitis patients than in the other group [26].

24.3.5 Microbial Markers of Primary Sclerosing Cholangitis (PSC)

Bajer and colleagues reported that PSC was associated with specific gut microbes independently of concomitant IBD. They analysed the faecal composition of 31 healthy controls, 32 UC patients without PSC and 43 patients who had PSC with concomitant IBD ($n = 2$) and without ($n = 11$). They found that *Rothia*, *Enterococcus*, *Streptococcus*, *Veillonella* and three other genera were markedly overrepresented in PSC regardless of concomitant IBD. They tracked *Rothia*, *Veillonella* and *Streptococcus* to the species level, and this allowed them to identify *Rothia mucilaginosa*, *Streptococcus infantis*, *S. alactolyticus* and *S. equi* along with *Veillonella parvula* and *V. dispar*. The microbiome in PSC was also characterised by decreased abundance of *Adlercreutzia equolifaciens* and *Prevotella copri*. A decrease in the genus *Phascolarctobacterium* was linked to the presence of colonic IBD. In patients with UC, *A. muciniphila*, *Butyricicoccus pullicaecorum* and *Clostridium colinum* were decreased along with the genus *Roseburia* [2].

24.3.6 Theragnostics

S. Rajca and colleagues studied the composition of gut microbiota in patients from a prospective cohort trial designed to identify predictive factors of clinical relapse after discontinuation of infliximab in CD. They collected faecal samples from 33 patients with CD at baseline, at 2 months, at 6 months and at the end of the follow-up period. Of these, 19

relapsed and 14 did not. Low percentages of *F. prausnitzii* and *Bacteroides* were associated with a high risk of relapse independently of a high concentration of serum C-reactive protein (CRP) [37]. Ananthakrishnan and colleagues conducted a prospective study with CD and UC patients starting vedolizumab therapy. Community α-diversity was significantly higher, and *Roseburia inulinivorans* and a species of *Burkholderiales* were more abundant at baseline in CD patients who later entered remission with Vedolizumab treatment [1].

24.4 Therapeutic Strategies to Manipulate the Gut Microbiota in IBD

Researchers have documented four ways of influencing the microbiota in IBD [5], which include the use of antibiotics, prebiotics and fibres, probiotics and faecal microbiota transplantation (FMT). The current use of these approaches is very limited (Table 24.3), but randomised controlled trials (RCTs) are increasing, and some of these have yielded positive results. During these treatments, the endogenous microbiota is influenced by ecological selection pressure. After this has occurred, the microbiota may either keep some of its new characteristics or move back to its dysbiotic composition (resilience). These findings are summarised below.

24.4.1 Antibiotics

Antibiotics may promote dysbiotic features including *Clostridium difficile* infection and the promotion of antibiotic resistance. The use of antibiotics early in life has been reported as a significant risk factor of IBD in the western world and a protective factor in Asia [33]. The (few) indications for antibiotics in IBD are shown in Table 24.3.

24.4.2 Prebiotics and Fibres

As *F. prausnitzii* is reduced in patients ingesting low amounts of fibre, it is quite possible that low

Table 24.3 Indications of treatments targeting the microbiota in IBD (according to ECCO guidelines) [16, 18]

IBD type	Indications
Crohn's disease	Antibiotics are considered appropriate for septic complications, perineal disease or symptoms due to small bowel bacterial overgrowth Anti-mycobacterial treatment cannot be recommended based on the results of controlled trials A double-blind clinical trial recently tested rifaximin in patients with moderately active CD. A dose of 800 mg of rifaximin appeared to be more effective than a placebo in achieving remission, but dosages of 400 and 1200 mg did not. No confirmation of this data is available Ciprofloxacin has been shown to significantly increase the efficacy of adalimumab in the healing of perianal fistulas. Other data has confirmed its utility in perianal disease Trials testing probiotics and prebiotics have been negative
Ulcerative colitis	Antibiotics are recommended only when an infection is suspected (i.e. in a short-term first attack, after recent admission to hospital or after travel to an amoebiasis-endemic area) or immediately before surgery There is some evidence for the therapeutic benefit of probiotics when added to standard therapy to induce remission, particularly VSL #3 Results of RCTs evaluating faecal microbiota transfer are encouraging. These support the use of FMT to induce remission in active UC Data on the use of antibiotics to maintain remission was considered to be insufficient by the ECCO consensus (2016) A total of three RCTs comparing *E. coli* Nissle 1917 to 5-ASA suggested that this probiotic was not inferior to 5-ASA for the maintenance of remission in UC No evidence has been reported that any other probiotic is effective for maintaining remission
Pouchitis	The majority of patients respond to metronidazole or ciprofloxacin, but the optimal modality of these treatments is not clearly defined In chronic pouchitis, a treatment combining these antibiotics is effective The probiotic mixture VSL #3 is effective in maintaining antibiotic-induced remission and in preventing pouchitis

Table 24.4 Methods and efficacy of FMT in active UC (results of four RCTs)

	Rossen et al. [40]	Moayeddi et al. [29]	Paramsothy et al. [34]	Costello et al. [11]
N° of patients (verum/placebo)	23/25	38/37	41/40	38/35
Placebo	Autologous faeces	Water	Coloured water	Autologous faeces
Treatment duration (weeks)	12	6	8	8
N° of FMT	2	6	40	3
Route of administration	Nasoduodenal	Enemas	Coloscopy then enemas	Coloscopy then enemas
Donor(s)	Single	Single	Pool of 3 to 7	Pool of 3 to 4
Remission verum vs placebo	30%/20% $p = 0.51$	24%/5% $p = 0.03$	27%/8% $p = 0.02$	32%/9% $p < 0.01$
Response verum vs placebo	48%/52% $p = 0.58$	39%/24% $p = 0.16$	54%/23% $p < 0.01$	55%/20% $p < 0.01$

fibre diets (which are often recommended to patients suffering from IBD) participate in dysbiosis. A prebiotic is a substrate (usually a sugar but some polyphenols are also of interest) that is undigested in the small intestine that increases the populations of microorganisms supposedly beneficial in the colon [51]. The most studied have been fructans (fructo-oligosaccharides (FOS) and inulin). The few therapeutic trials of these agents in IBD have shown poor tolerance and no efficacy [5, 6, 51]. Such drawbacks were expected considering that these substances are fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs). This should not discourage researchers, and studies for other products and/or doses are ongoing.

24.4.3 Probiotics and Faecal Microbiota Transplantation

In RCTs on probiotics, researchers have shown that VSL #3 is effective in preventing pouchitis and that *E. coli* Nissle 1917 is effective in preventing relapse of UC. Studies testing probiotics in patients with CD have been negative [5]. Receipt of FMT from a healthy donor or from a pool of donors is effective in patients with recurrent *Clostridium difficile* infection [8]. The results of controlled trials in participants with active UC are encouraging, as shown in Table 24.4 [11]. However, there are many issues for researchers to resolve before patients, clinicians and society can validate or refute this treatment. Currently, there are only few examples of the use of this technique in IBD, but this could soon change. Studies targeting pathogens or pathobionts such as *Mycobacterium avium paratuberculosis* or AIEC should be performed in patients carrying these microorganisms (and probably not on those who do not carry them).

24.5 Unmet Needs and Future Directions

Microorganisms living in the inflammatory environment seem to be good bioindicators that integrate the ecological disturbances of IBD. Researchers should not consider the candidate markers presented here in relation to causality in IBD. Instead, they should consider only their marker characteristics. We have shown here that several candidates could be "micro-biomarkers" of IBD, but proper validation requires large-scale studies with longitudinal assessment of candidate markers and the consideration of potential confounders. As the microbiota is influenced by diet, age, ethnicity, etc., it is possible that micro-biomarkers could vary between countries. This should be anticipated, properly assessed and should not discourage research and development. We must also guarantee that the medical value of these markers will not be affected by drugs, antibiotics and bowel preparation. It is likely that

markers with a suspected link to disease causes or mechanisms will also be used as surrogate markers for clinical trials. For example, trying to improve *F. prausnitzii* concentration, restore richness and diversity or robustness makes sense for intestinal ecologists. To improve their sensitivity/specificity, future predictive models of IBD could encompass a combination of both microbiomarkers and bio-clinical parameters.

Summary Points

- Alterations of the microbiota (dysbiosis) occur in IBD, and some features differ significantly between healthy participants, patients with ileal CD, patients with colonic CD, patients with UC and patients with primary sclerosing cholangitis (PSC).
- These may serve as bioindicators (as they integrate ecologic pressure due to disease burden) and may be used to develop diagnostic algorithms.
- Confounding factors, including ethnicity, diet and medical treatments must be studied.
- The Dysbiosis Microbiota Index for identifying ileal CD from rectal biopsies (even when the rectum is normal) and the Pascal Index for CD are promising.
- Large-scale international studies with proper controls and phenotyping of specific situations are required.

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Metabolic Profiling in IBD

25

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Abstract

Metabolites are small molecules derived from biochemical processes in metabolism, and their profiling enables the analysis of physiological functions. Metabolic profiling through cross-sectional studies has moved forward to longitudinal cohort studies and metabolome-wide association studies (MWAS) which have helped unveil numerous discoveries in amino acid, fatty acid and energy metabolism pathways and their link in inflammatory bowel disease (IBD). This chapter will introduce metabolic profiling approaches and discuss the role that the metabolites play in the link between the gut microbiome and the host with regard to IBD. We will discuss the various biomarkers, which have been uncovered by metabonomics currently through separation of IBD phenotypes and the future for this area in relation to biomarkers for pathogenesis of IBD and personalizing medical therapy.

25.1 Introduction

There is an increasing use of "omics" technologies in gastrointestinal disease research to identify novel diagnostic targets and gain insight into the underlying molecular mechanisms of IBD pathogenesis. Metabonomics or metabolite profiling overcomes the limitations imposed by other "omic" approaches such as genomics, transcriptomics or proteomics by integrating information about gene regulation, post-translational modification and pathway interaction. Therefore, metabolites can act as an immense readout of the cell phenotype [1, 2]. In addition, this synthesis of diverse upstream "omics" signals in metabolites makes them useful for detecting subtle changes in metabolic pathways before the phenotypic changes occur [2]. Metabolomics is defined as "the unbiased identification and quantitative measurement of metabolites in a biological system" [3], whereas metabonomics is defined as "the quantitative measurement of the time-related multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification", addressing the dynamic changes [4]. Both terms are used and often interchangeable [1, 5].

Metabolites reflect host physiological and pathological states providing a picture of the host phenotype or metabotype [6]. Bodily fluids encompassing serum or plasma, urine and faeces and tissues provide adequate matrices for metabolite profiling. Depending on the nature of the chosen matrix, metabolites of different origins, such as the host and/or the microbiota, can be identified and quantified. For example, faecal water extracts provide a pool of microbiota-derived and dietary metabolites, whereas the

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host metabolites prevail in the serum and tissue samples. The urinary samples contain host, dietary and microbial metabolites, the latter of which are either absorbed through the intestinal and the colonic mucosa and excreted directly or undergo further modification by host metabolic processes, such as sulphation, glucuronidation and conjugation with glycine, glutamine, etc. The metabolites that are derived from the interactions between the host and the microbiota are known as host-microbial co-metabolites [1], e.g. hippurate (a glycine conjugate of benzoic acid).

25.2 Metabolic Profiling Methods and Workflow

Many analytical platforms are available for metabolic profiling, such as gas chromatography-mass spectrometry (GC-MS), high-/ultra-performance liquid chromatography coupled with MS (H/UPLC-MS), nuclear magnetic resonance (NMR) spectroscopy [1, 2], Fourier-transform infrared spectroscopy (FT-IR) and capillary electrophoresis (CE) coupled to MS. Moreover, mass spectrometry imaging (MSI) is a powerful tool that can be used to visualize the spatial distribution of preselected metabolites in the tissue samples [2]. Among these analytical platforms, proton NMR spectroscopy and UPLC-MS are the most widely used instrumentation in metabolic profiling. NMR spectroscopy exploits the spin property of nuclei, where the nuclei can absorb and re-emit electromagnetic radiation in a magnetic field [3, 7]. The NMR spectra provide rich information on the molecular structures, and it is both qualitative and quantitative [1, 4]. NMR spectroscopy is extremely robust with high reproducibility, and hence it is of great value in studying large sets of samples from longitudinal or epidemiological studies, which require a high instrumental power. However, NMR has a relatively low sensitivity and may not detect the compounds with very low concentrations, i.e. at the sub-micromolar level. In contrast, UPLC-MS is more sensitive and provides retention time and mass-to-charge ratios (m/z) of metabolites [3, 7]. The UPLC system separates a complex sample into multiple simpler fractions, and each fraction is in turn analysed by mass spectrometry in positive or negative mode depending on the positive/negative charges of the molecules. Another advantage of UPLC-MS-based metabolic profiling is that the UPLC methods can be modified to target a specific set of molecules, e.g. bile acids, lipids and amino acids [1]. The untargeted or global metabolic profiling methods offer the advantage of de novo metabolite identification and characterization for exploring the metabolic disturbances induced by both intrinsic and extrinsic factors. In contrast, the targeted analyses, which are based on a defined group of metabolites and often require prior knowledge of the metabolites of interest, can be applied to investigate specific pathways[1].

The typical workflow of metabolic profiling consists of (1) experimental design, (2) sample collection and processing, (3) sample preparation and analysis, (4) data treatment and modelling, (5) metabolite identification and (6) result interpretation and validation [8–11] (Fig. 25.1). Throughout the workflow, it is essential to follow the standard operating protocols to ensure the quality of both samples and the data. For example, consistent sampling protocols should be used at multiple centres, and samples should be randomized prior to the analysis. Quality control samples, which are usually formed by pooling a small volume from each of the analytical samples, should be analysed together with the analytical samples to check the stability of the instrument over the entire data acquisition time. Appropriate data preprocessing methods need to be applied to the metabolic datasets obtained from various analytical platforms before statistical analyses. Typically, these preprocessing methods include calibration, phasing, baseline correction, peak picking and

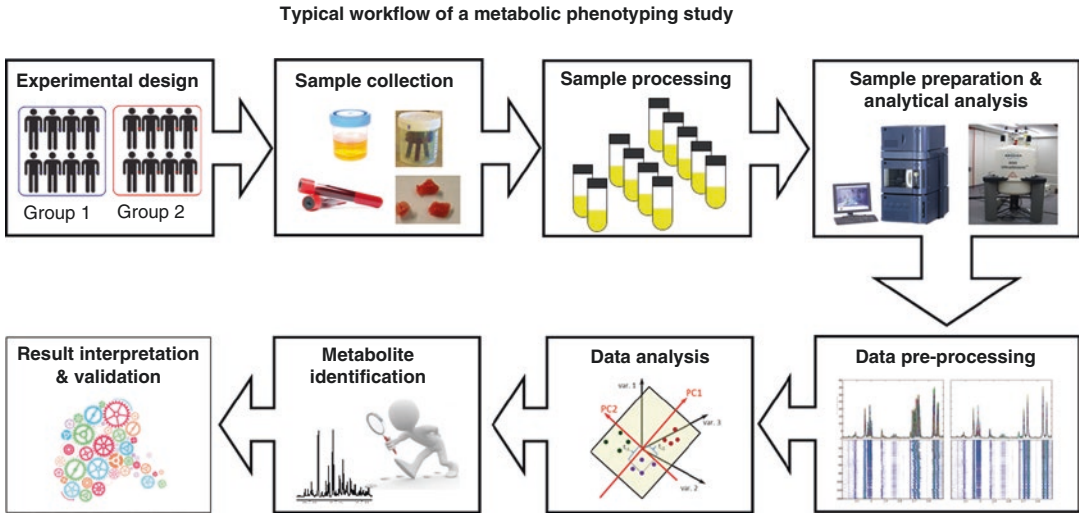

Fig. 25.1 Schematic illustration of a typical workflow for metabolite analysis and identification. Each metabolite study comprises the following steps: experimental design, sample collection and storage, sample processing, sample preparation and data acquisition, data preprocessing, statistical analysis, metabolite identification and result biological interpretation and validation

alignment, normalization and scaling [12, 13]. Multivariate statistical analysis methods, such as unsupervised principal component analysis (PCA) and supervised orthogonal signal corrected-projection to latent structure-discriminant analysis (OPLS-DA), can subsequently be applied to model the data [14, 15]. The key discriminant features (e.g. chemical shift in NMR spectra, retention time-mass to charge ratio in UPLC-MS data) can be identified as metabolites for biological interpretation. Metabolite identification is a challenging step in untargeted metabolic profiling and often requires additional experiments to elucidate the molecular structures. The techniques often used in metabolite identification, in combination with various databases, include two-dimensional (2-D) NMR spectroscopy, statistical correlation spectroscopy (STOCSY), MS/MS or molecular fragment patterns, hyphenated HPLC-NMR-MS systems and spike-in authentic compounds [8, 16–18]. Two-dimensional NMR spectroscopy, such as ^{1}H-^{1}H correlation spectroscopy, ^{1}H-^{1}H total correlation spectroscopy, ^{1}H-^{13}C heteronuclear single-quantum correlation and ^{1}H-^{13}C heteronuclear multiple bond correlation spectroscopy, show the connectivity of atoms in the molecules and provide structural information of the molecules of interest. STOCSY is a statistical approach and calculated based on a large set of one-dimensional NMR spectra. It provides correlation information between the spectral peaks derived from both the same molecule and the compounds involved in the same pathway. The hyphenated HPLC-NMR-MS technique allows us to combine the information from both NMR spectroscopy and MS. Tandem MS/MS is often used to obtain the structural information by breaking the parent ion into multiple fragments, providing information on the substructure of a molecule. These identified metabolites can not only provide mechanistic understanding of the biological processes but also could be used as biomarkers for disease diagnosis and prognosis, assessment of therapeutic treatment and patient stratification.

25.3 Application of Metabolic Phenotyping in IBD

The first metabolite profiling study that was able to discriminate IBD patients from healthy individuals took place in 2007 and was based on ^{1}H NMR analysis of faecal water extracts [19]. Since then, numerous studies have been performed (Table 25.1) and have consistently shown that the metabolic phenotype of IBD patients differs from a healthy state [19–21]. Interestingly, different subtypes of IBD such as Crohn's disease (CD) and ulcerative colitis (UC) can also be distinguished [19, 21]. Bacterial metabolites including short chain fatty acids (SCFAs), methylamine and trimethylamine were depleted in the faecal water extracts from IBD

Table 25.1 Summary of selected metabolic profiling studies in IBD

References	Analytical platform	Biological fluid	Disease	Observations
Marchesi et al. [19]	^{1}H NMR	Faecal extract	CD, UC, HC	Depletion of SCFAs (butyrate, acetate), methylamine and trimethylamine in CD and UC. Higher amounts of amino acids in CD and UC
Jansson et al. [20]	Ion cyclotron resonance – Fourier transform (ICR-FT)/MS	Faecal extract	CD, HC (twins)	Discrimination based on affected area of the gut (ileum or colon). Differences in metabolites within the pathways of amino acid metabolism (in particular tyrosine, tryptophan and phenylalanine), bile acids and fatty acid synthesis including arachidonic acid
Le Gall et al. [21]	^{1}H NMR	Faecal extract	UC, HC	Discrimination of UC from HC. Elevated levels of taurine and cadaverine in UC. Non-significant differences in SCFAs and amino acid pathways
Williams et al. [38]	^{1}H NMR	Urine	CD, UC, HC	Differential clustering of CD, UC and healthy controls. Urinary metabolites related to the gut microbiota metabolism such as hippurate, formate and p-cresol sulphate were altered. Hippurate levels were significantly reduced in IBD cases. Formate levels were higher, whereas p-cresol sulphate was decreased in CD compared with UC patients or healthy individuals. Other discriminatory metabolites for CD include high levels of guanidinoacetate, glycine, methylhistidine and glycolate and reduction in citrate. UC shows upregulation in glycine, guanidinoacetate, methylhistidine and citrate and downregulation in NNN-trimethyllysine
Schicho et al. [35]	^{1}H NMR	Urine, serum and plasma	CD, UC, HC	Differentiation of UC and CD patients from healthy controls; however differences between UC and CD are less pronounced. Altered metabolite levels in serum and plasma: methanol, monosaccharides (mannose and glycose), amino acids, creatine, urea, citrate, acetate, succinate, choline and betaine. Decreased concentration of citrate, succinate, betaine, hippurate and methanol in urine in both UC and CD. Increased urinary levels of mannitol, allantoin, tryptophan in UC and saccharides such as lactose, galactose, maltose and xylose in CD
Williams et al. [40]	^{1}H NMR	Serum	CD, UC, HC	Discrimination between CD and UC in terms of lipid metabolism: reduced LDL cholesterol, unsaturated lipids and choline, increased N-acetyl glycoprotein and differing amino acids. CD and UC metabolic profiles were different from HC: reduced LDL and HDL cholesterol, low choline, increased very low-density lipoprotein (VLDL), N-acetyl glycoprotein and lactate. Combined CD and UC cohorts differentiated IBD from health: lower levels of LDL and HDL cholesterol, unsaturated lipid, choline, isoleucine and alanine and increased N-acetyl glycoprotein and lactate

Table 25.1 (continued)

References	Analytical platform	Biological fluid	Disease	Observations
Dawiskiba et al. [39]	^{1}H NMR	Serum and urine	CD, UC, HC	Differentiation of active IBD from remission state and healthy control individuals. Distinguishing metabolites between active and quiescent IBD include N-acetylated compounds and phenylalanine (increased in serum), LDL and VLDL (decreased in serum) as well as glycine (increased in urine) and acetoacetate (decreased in urine). Metabolites characterizing IBD from healthy state include leucine, isoleucine, 3-hydroxybutyric acid, N-acetylated compounds, acetoacetate, glycine, phenylalanine and lactate (increased in serum), creatine, dimethyl sulfone, histidine, choline and its derivatives (decreased in serum), as well as citrate, hippurate, trigonelline, taurine, succinate and 2-hydroxyisobutyrate (decreased in urine). No separation between CD and UC. Discrimination of IBD patients in remission from HC only in urine metabolite profiles
Ooi et al. [44]	GC/MS	Mucosal biopsy and serum	UC, CD, HC	Differences in the levels of amino acids and TCA cycle-related molecules between UC, CD and healthy controls. Also, metabolic differences between UC and CD

Abbreviations: *NMR* nuclear magnetic resonance, *UC* ulcerative colitis, *CD* Crohn's disease, *HC* healthy control, *MS* mass spectrometry, *GC/MS* gas chromatography MS

patients compared with healthy controls, whereas amounts of faecal amino acids were elevated suggesting a possible malabsorption in the intestine due to mucosal injury resulting from inflammation [19]. Methylamine and trimethylamine are products of bacterial degradation of dietary compounds including choline and carnitine, while SCFAs derive from bacterial fermentation of indigestible dietary fibre [22]. The fact that the differential metabolites are bacterial products indicates an imbalance in the gut microbial composition. Furthermore, the depletion of SCFAs was correlated with reduced abundance of *Clostridium coccoides* and *Clostridium leptum* in IBD patients, which are implicated in SCFAs production [19]. A growing series of evidence has shown a reduction in the levels of SCFAs in IBD, highlighting their importance [21, 23]. SCFAs, which are 1–6 carbons in length, comprise mainly acetate, butyrate and propionate and have a role in the regulation of host metabolism and immune system homeostasis [24]. In particular, butyrate is the main energy source of colonocytes and has a role in the maintenance of intestinal epithelial barrier integrity [22, 25]. Additionally, butyrate has been brought into the frontline of IBD research since it exerts an anti-inflammatory role suppressing inflammation through a range of mechanisms [24, 26–28]. Treatment of UC patients with butyrate enemas ameliorated intestinal inflammation, [29] and addition of butyrate in intestinal biopsy specimens from CD patients reduced the expression of pro-inflammatory cytokines [30]. A reduction in the levels of SCFAs, particularly butyrate, is associated with an altered microbial composition and presumably a decrease in butyrate-producing bacteria such as *Clostridia* [19, 26, 27, 31].

Metabolite profiling can discriminate IBD patients depending on the affected area of the gut (ileum or colon) [20]. A study on identical twins, including healthy individuals and twin pairs discordant or concordant for CD, differentiated CD from healthy controls and further stratified CD cases to affected tissue, i.e. ileum or colon [20]. Differences in faecal metabolites within the pathways of amino acid metabolism (in particular tyrosine, tryptophan and phenylalanine), bile acids and fatty acids synthesis were identified. Higher concentrations of tyrosine and tryptophan, bile acids, and saturated and unsaturated fatty acids were indicative of ileal CD phenotype [20]. The levels of bile acids, a family of cholesterol-derived molecules that are produced in the liver with a role in fat breakdown to release dietary lip-

ids and lipid-soluble vitamins, [24] are elevated in IBD [20]. Bile acids are concentrated in the gallbladder and in response to diet-dependent signals are emptied in the small intestine to perform their detergent-like role [24, 32]. After that, they are reabsorbed and directed back to the liver awaiting a new signal [33]. Inflammation and increased intestinal permeability, which are hallmarks of IBD, hinder their reabsorption leading to increased levels [20]. Metabolism of bile acids is dependent on deconjugation (removal of glycine and taurine residues) and dehydroxylation processes, which are directed by gut microbiota [24, 32]. IBD patients have high levels of conjugated bile acids and sulphated bile acids, but low proportions of secondary bile acids in the gut lumen compared with healthy individuals [34]. Of note, secondary bile acids (such as deoxycholic, lithocholic and muricholic acids) are derived from the transformation of primary bile acids (cholic and chenodeoxycholic acids) [24]. Therefore, these modifications in the luminal bile acid pool are directed by defective deconjugation, transformation and desulphation activities of the altered IBD microbiota composition. Given that secondary bile acids such as deoxycholic acid and lithocholic acid exert anti-inflammatory effects on the intestinal mucosa, alterations in bile acid metabolism enhance inflammatory pathways leading to a feedforward loop perpetuating inflammation [34].

The aforementioned studies have been primarily conducted on faecal samples. Other biofluids and tissues that have been used for metabolite profiling in IBD comprise urine, serum, plasma and colonic mucosa biopsies. Metabolic profiling studies based on these matrices were also able to discriminate IBD patients from healthy controls [35–45] as well as the different subtypes of IBD [40]. These studies have further revealed alterations in amino acid, lipid and energy metabolism pathways as evidenced by the decreased levels of amino acids (such as glutamine), lipoproteins (mainly low-density lipoprotein) and tricarboxylic acid (TCA) cycle intermediates (such as succinate and citrate). These metabolic changes indicate enhanced energy requirements and rapid utilization of metabolites that feed energy-producing pathways during intestinal inflammation [35, 46]. A common finding especially in urine metabolic profiling studies is the lower levels of hippurate in IBD, suggesting its potential use as a biomarker. Hippurate or N-benzoylglycine is a metabolite generated by bacterial fermentation of dietary aromatic compounds (such as polyphenols, purines or aromatic amino acids), and hence its low levels may reflect altered gut microbiota composition, given that hippurate levels positively correlate with *Clostridia* levels in the gut [47].

While the vast majority of metabolite profiling studies has focused on IBD in adults, there is a scarcity of data on paediatric IBD. Understanding the metabolic dysregulation in IBD children is particularly important, since IBD children suffer from growth failure and delayed puberty in addition to the known pathological features of the disease [48, 49]. A study on newly diagnosed paediatric IBD reported differences in faecal metabolite profiles between IBD patients and healthy individuals and categorized IBD cases in CD and UC [50]. Metabolite pathways involved in amino acid metabolism, sphingolipid metabolism, urea cycle and bile acid biosynthesis were perturbed in paediatric IBD [50]. A recent study following IBD paediatric patients over a year reported differences in amino acids with an emphasis on glycine metabolism, bile acids, urea cycle, metabolites of energy metabolism, signalling molecules (such as dopamine and gamma-aminobutyric acid (GABA)) and gut microbial metabolites [51]. In particular, urinary levels of pyroglutamic acid, glutamic acid, glycine, cysteine, as well as fumarate, isocitrate, 2-hydroxyglutaric acid, methylsuccinic acid, methionine and tyrosine were elevated in paediatric IBD patients during the course of the study, whereas hippurate appeared in lower concentrations [51]. Metabolite phenotyping provides insight in differential metabolic requirements in IBD children, which could lead to better disease management.

Metabolite profiling discriminates active disease from those in remission with IBD [23, 39, 52, 53]. Discriminating metabolites include

N-acetylated compounds, phenylalanine, glycine lipoproteins and acetoacetate. The levels of the medium chain fatty acid, hexanoate, were negatively correlated with disease status in CD, whereas the benzenoid compound styrene, which is produced by protein fermentation, was positively correlated with the status of UC [23]. Identification of metabolites whose variation is associated with certain stages of disease activity holds promise for the discovery of IBD biomarkers in the future.

25.4 Application of Metabolic Phenotyping in Interventional Studies in IBD

Different interventional studies intend to treat IBD symptoms and complications, and metabolic profiling sheds light on the underlying pathways. Iron deficiency anaemia is a common comorbidity in IBD patients, who require iron supplementation. The routes of iron intake (intravenous or oral) have an impact on the gut microbiome and metabonome landscape. Bile acids, steroids and cholesterol derivatives were higher in IBD patients receiving intravenous iron treatment, whereas phosphatidylglycerol, palmitate and its derivates were increased in patients taking oral iron supplements [54]. As oral iron therapy, which is the standard option, is associated with side effects and a possible inflammation exacerbation, knowledge of the metabolites involved can lead to better understanding of the limitations of the oral route treatment. A subset of IBD patients do not respond to current therapies, sparking an interest in the identification of natural compounds with anti-inflammatory properties. Dietary intervention using fine powdered eggshell membrane resulted in amelioration of mucosal inflammation in vivo in a murine mode of dextran sodium sulphate-induced colitis [55]. Gene expression of inflammatory mediators was reduced, whereas expression of genes involved in tissue regeneration and repair was increased. Supplementation with eggshell membrane also regulated energy metabolism as evidenced by the elevated TCA cycle and glycolysis metabolites [55] restoring an efficient energy supply. TCA cycle and glycolysis metabolites are dampened in inflammatory environments due to enhanced energy requirements. These findings suggest that dietary supplementation may have a functional effect on the host eventually alleviating mucosal inflammation and leading to better clinical management of IBD.

25.5 Future Directions

Studies pertaining to metabolic profiling in the context of IBD provide valuable insights into the metabolic dysregulation and the underlying mechanisms of this chronic inflammatory condition, allowing the design of better therapeutic strategies and more tailored drugs. Identification of metabolites associated with IBD may provide diagnostic biomarkers for early disease diagnosis and monitoring tools for disease surveillance. However, the associations found between metabolites and IBD do not necessarily indicate a causal relationship, as changes in those metabolites could be secondary to IBD development. Metabolite profiling studies on clinical IBD [19, 21, 35, 38, 40, 44] and experimental animal models [5, 46, 56–58] have focused on progression of disease after IBD had been established, confounding the role of metabolites in driving IBD progression. One study focusing on stages before the development of inflammation in colitis-prone animals reported stability of the metabolic network prior to disease manifestation [59]. Prospective longitudinal studies are warranted to reveal any causal relationship between metabolites and IBD pathogenesis. Integration of metabolomics with other "omics" techniques such as metagenomics enables a more comprehensive study of IBD pathophysiology. Also, validation studies in a large scale are deemed necessary to translate identified IBD metabolites in clinical use with high accuracy.

Summary Points

- Metabolites are low molecular compounds present in body fluids and tissues that describe the host physiology.
- There are many analytical platforms available for metabolite analysis including chromatographic separation coupled with MS and NMR.
- Metabolite profiling studies in IBD are able to discriminate IBD patients from healthy individuals, different IBD subtypes (i.e. ileal or colonic CD) and remission from active state.
- Although a biomarker of IBD has yet to be found, bacterial metabolites such as SCFAs, amino acids, bile acids and lipid- and energy-related metabolites are altered in IBD compared with a healthy state.
- Future studies on metabolite profiling in IBD will contribute to a better understanding of IBD pathogenesis and its complications improving current treatment modalities, and disease prognosis and early diagnosis.

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Innate and Adaptive Immunology

26

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Abstract

Crohn's disease and ulcerative colitis typically follow a relapsing-remitting pattern and with heterogeneous clinical outcomes between patients. As a result, there is an unmet need to develop biomarkers predictive of both clinical outcomes and response to therapeutics, particularly given the rapid expansion of therapeutic options.

Paralleling the rise in treatment options has been an increased understanding of immune dysregulation in inflammatory bowel disease, creating an array of potential biomarkers. However, most biomarker studies have focused on diagnostic aspects and the monitoring of disease activity. Studies of prognostic and predictive biomarkers may permit patient stratification, bringing "personalised medicine" a step closer.

In this chapter, we highlight some of the novel approaches taken to quantitatively and qualitatively assess the immune response in inflammatory bowel disease and review potential biomarkers which have been identified from both studies in model systems and from clinical trials. In particular, we discuss the manner in which novel techniques have helped to advance the field.

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26.1 Role of the Immune System and Immune Biomarkers

Crohn's disease (CD) and ulcerative colitis (UC) occur due to immune activation triggered by a complex interplay of genetic risk, microbial dysbiosis, and other external stimuli affecting the host immune system. The mechanistic interactions of these elements in the pathogenesis of inflammatory bowel disease (IBD) are beyond the scope of this review having been thoroughly reviewed elsewhere [1–3].

Current biomarkers in IBD are typically used to help diagnose the condition or to measure and monitor disease activity. An attractive alternative use of biomarkers would be to predict disease course or response to treatment. Such an approach would permit a more nuanced assessment of the risk/benefit ratio for any patient when considering treatment escalation, as well as a more informed selection of drug class. It is this use of biomarkers for prediction that we will focus on in this chapter. In particular, we consider how our developing knowledge of immunology may impact on biomarker discovery and development.

Biomarker discovery has conventionally been driven by hypothesis-based approaches, using observations relating to specific pathways to form testable hypotheses as to how evidence of activation of these pathways may inform prediction models. Such approaches, whilst being intellectu-

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ally satisfying, can inevitably only proceed at the rate of advances in our understanding of the complexities of cellular function and signalling during the host immune response. A further consideration of such approaches is that whilst the use of laboratory resources is kept manageable, a hidden cost may come from the use of precious clinical material to assess only a limited set of markers that form the basis of the hypothesis under test.

An alternative, complementary model comes from hypothesis-free approaches, employing technologies such as genomics, transcriptomics, proteomics and metabolomics, sometimes collectively referred to by the catch-all jargon of "multi-omics platforms". Critics may characterise hypothesis-free studies using these platforms as "fishing expeditions". This may well be a fair criticism of some less well-conceived studies but overlooks the clear impact of these approaches in driving our understanding of IBD genetics [4] or in assisting investigators in other fields such as oncology [5]. Most notably in the oncology field is the development of a biomarker assay to detect recurrence of breast cancer [6], which has been assessed in a randomised, clinical trial setting and shown utility to personalise therapy for a subset of patients [7]. Such multi-omics approaches will maximise the information that may be gained from any clinical material but come with a potentially much greater financial cost depending on the range of assays employed. Nevertheless, there have been recent changes in this field including huge reductions in the cost of, for example, next-generation DNA sequencing methodologies. These decreasing costs combined with increased transparency in data publication and sharing, as well as significant advances in development of computational models, have permitted integration of novel data sets with existing curated repositories of relevant data to improve target identification and validation [8].

Regardless of the approach taken, undertaking the design and analysis of these studies informed by advances in understanding of immune function in IBD will maximise their potential and drive the development of immunological biomarkers.

26.2 Biomarkers for Prognostication

Neutrophils play a key role in intestinal inflammation through multiple inflammatory mediators [2]. Amongst these, lactoferrin and calprotectin represent validated biomarkers of disease activity [9], as well as showing a more limited predictive value for disease relapse. A prospective study of 53 patients from 17 centres with UC demonstrated a faecal calprotectin <50 μg/g, 2 weeks following induction therapy with infliximab, was able to predict endoscopic remission seen at week 10 in 80% of patients [10]. A further prospective study of another 87 patients with UC demonstrated faecal calprotectin >300 μg/g correlated with higher likelihood of relapse, despite having no concurrent clinical symptoms to suggest a flare of their disease [11]. Indeed, two consecutively raised calprotectin levels >300ug/g within a 1-month interval were identified as the best predictor of a flare of UC, with 100% specificity, albeit with only 61.5% sensitivity.

Subsequently, faecal calprotectin was also examined in 135 patients with CD following resection in the POCER trial, compared with CRP and other clinical indices [12]. This trial included patients from 17 hospitals in Australia and 1 hospital in New Zealand, with 104 undergoing an ileocaecal resection. Faecal calprotectin samples were taken at 6, 12 and 18 months following resection. Calprotectin concentrations >100 μg/g were associated with endoscopic recurrence (Rutgeerts score of i2 or more) with a negative predictive value of 91%, suggesting that a calprotectin value that is not raised could help to reduce the need for colonoscopic surveillance in the post-operative setting. The CALM study has since demonstrated that faecal calprotectin is perhaps most effective in combination with serum CRP and clinical parameters, as a treatment target associated with mucosal healing in patients with CD at 1 year [13].

Changes in intestinal permeability have long been implicated in the pathogenesis of IBD [14]. Confocal microscopy and the adjunct process of fluorescent staining permit dynamic imaging of intestinal barrier function [15], and such functional imaging techniques have applied in a prospective pilot study of 47 patients with UC and 11 patients with CD [16], where detection of increased epithelial cell shedding and development of local barrier defects predicted disease relapse at 12 months with a specificity of 91.2% (95% CI, 75.2–97.7%). However, clinical utility in this study was compromised by a sensitivity of only 62.5% (95% CI, 40.8–80.4%). A similar imaging technique was applied in a study of 49 patients with CD, aiming to identify subgroups of patients based on clinical outcome [17]. 63% of patients showed evidence of abnormal barrier function, highlighted by the endomicroscopic detection of focal cryptitis and crypt architectural abnormalities, which were predictive of the need for subsequent medical treatment escalation within 12 months (positive likelihood ratio (LR) = 3.27, $p = 0.025$), in comparison to an elevated CRP (positive LR = 2.05, $p = 0.020$). Importantly, in this study, there was no prognostic utility identified for clinical disease activity, using the Crohn's Disease Activity Index (CDAI), nor for endoscopic activity, using the Crohn's Disease Endoscopic Index of Severity (CDEIS), highlighting the potential advantages of microscopic technology over macroscopic endoscopic views.

Further advances in biomarker development using a hypothesis-free approach have come from the analysis of transcriptomic signatures from $CD8^+$ T-cell subsets obtained from patients with newly diagnosed IBD. In expression microarray analyses, two distinct subgroups of patients were identified, with prospective follow-up for these patients over a 2-year period, showing significant differences in disease course with respect to the need for treatment escalation and the frequency of flares [18]. High-risk patients from this cohort demonstrated an earlier time to surgery and greater number of operations (positive LR = 4.87, 95% CI, 1.64–14) [18]. Indeed this pattern has held out for a further follow-up period of 4 years (*James Lee, personal communication, January 2018*). Crucially, prognostication based on expression profiling was superior to prognostication based on previously validated clinical variables of poor prognosis.

Taking promising biomarkers from the stages of initial cohort studies into clinical practice requires overcoming a large number of barriers including the replication of test results in wider cohorts and demonstration of their clinical utility. In addition, a common problem encountered by biomarkers discovered using contemporary research techniques is the need to adapt these to suit the resources of a wider range of clinical environments.

In this regard, further discussion of the prognostic $CD8^+$ T-cell signature described above is informative. This signature was originally based upon the use of microarrays applied to cell populations generated using cell sorting. Both microarray (or similar transcriptomic) methodology and cell sorting are universally available in contemporary immunology laboratories but neither sits within standard hospital laboratories, immediately limiting the test utility, even if validated. Therefore, the transcriptional biomarker was recapitulated in a further training cohort, using whole, unseparated blood [19]. The microarray signature was then used to form a real-time, quantitative PCR (RT-qPCR) assay before validation of this assay performance in another cohort of patients with a new diagnosis of CD [19]. Importantly, the initial discovery phase using cell separation material was necessary, since changes in the signal/noise ratio meant that hypothesis-free application of microarray technology to whole blood samples alone, without prior knowledge from the cell separation studies, was not informative (*James Lee, personal communication, January 2018)*. In order to prove clinical utility, this validated biomarker panel is being utilised in the PROFILE trial (ISRCTN 11808228), in which newly diagnosed patients with CD will receive either "accelerated step-up" conventional management or "top-down" early biologic treatment, following biomarker risk stratification [20].

Another current, large-scale study using newly diagnosed patients is the IBD Character study,

which aims to use a multi-omic approach to identification of novel biomarkers of disease course and response to treatment [21]. These studies all face common logistical challenges in the need to assemble large consortia and then coordinate efforts in recruiting patients, as well as obtaining and processing samples. However, such approaches will help further our understanding of the immunobiology of disease course and the heterogeneous outcomes currently seen in IBD.

26.3 Biomarkers for Predicting Response to Treatment

A variety of techniques have been employed in the development of biomarkers to predict response, including both hypothesis-driven and hypothesis-free approaches.

26.3.1 Hypothesis-Driven Approaches to Treatment Response

TNF-α represents an important therapeutic target in IBD associated with improved clinical and endoscopic outcomes [22, 23]. However, up to 40% of patients fail to show an initial response to anti-TNF therapy [24]. A pharmacogenomic study of 287 patients with CD identified variation in three single nucleotide polymorphisms (SNPs) in genes involved in apoptosis (Fas ligand-843 C/T, Fas -670G/A, caspase9 93 C/T), as predictive of clinical response to a first infusion of infliximab [25]. These three SNPs were identified from a panel of 21 coding SNPs, which had been preselected based on their association with programmed cell death [25]. Using this same cohort, prediction of treatment response was demonstrated based upon the presence of a hypofunctional genotype; 1 point could be scored for each of the three SNPs involved, allowing stratification of patients into one of three groups [26]. These groups had a 50% likelihood (low apoptotic index, score $\leq$ 1), 73.8% likelihood (intermediate apoptotic index, score 2) and 100% likelihood (high apoptotic index, score 3) of clinical response to their first infusion of infliximab.

Another novel study used fluorescent antibodies to TNF coupled with in vivo imaging during colonoscopy to test the hypothesis that levels of mucosal TNF may correlate to treatment response [27]. Endoscopic examination with fluorescent antibody application was performed in 25 patients with active CD, as measured by a CDAI $\geq$150, prior to starting adalimumab therapy. Labelled adalimumab was then applied to the most inflamed areas using a spray catheter, and patients divided into two groups based upon numbers of membrane-bound TNF (mTNF) mucosal immune cells identified per confocal image. Importantly, these two groups were indistinguishable based on inflammatory activity, both endoscopically and histologically, as well as by CRP levels. Following subsequent treatment with adalimumab, the group with a higher number of mTNF cells demonstrated clinical response to adalimumab in 92% at 12 weeks, compared to just 15% response in the group with low numbers of mTNF cells. The sensitivity and specificity for predicting therapeutic response were 92% and 85%, respectively, with an optimal cut-off value of $\geq$20 cells to differentiate these two groups (area under receiver operating characteristic = 0.933, 95% CI 0.917–0.942). Whilst this study was limited to 25 patients, it does offer significant potential as a biomarker to determine therapeutic response to anti-TNF treatment.

This same technology has also been applied to the anti-integrin vedolizumab, which binds to the α4β7 integrin and inhibits interaction with mucosal addressin cell adhesion molecule-1 (MAdCAM-1). The α4β7-MAdCAM-1 interaction is associated with movement of lymphocytes from mucosal vasculature to gut-associated lymphoid tissues, and inhibition of this process has been associated with decreased inflammation [28]. Fluorescent molecular imaging was performed to detect the α4β7 integrin in a small group of five patients, who were refractory to anti-TNF and due to start vedolizumab induction therapy [29]. Labelling α4β7 with fluorescein isothiocyanate (FITC) allowed identification of

two patients with α4β7 expressing mucosal cells, whereas in the remaining three patients, no $\alpha4\beta7^{+}$ cells were observed. Clinical response was reported in the two patients with identifiable α4β7 mucosal cells, whereas no response demonstrated for cells not expressing this integrin receptor. Application of this technology is still in the preliminary stages and, to date, has all been performed ex vivo, where biopsy samples required further examination and analysis in a laboratory setting. Clearly, this needs to be translated to an in vivo application and explored in larger patient cohorts to determine clinical utility as a point of care endoscopic biomarker. However, such a strategy combining immunology and endoscopy offers a feasible and attractive biomarker for clinical gastroenterologists.

Etrolizumab differs from vedolizumab by selectively binding the β7 subunit of both α4β7 and αEβ7 integrin heterodimers. The αEβ7-E-cadherin interaction is subsequently involved in retaining these lymphocytes to the intra-epithelial compartment. The EUCALYPTUS trial highlighted the changing landscape of trial design and incorporated biomarker development into a phase 2 trial of etrolizumab in 119 patients with moderate to severe UC [30]. Clinical remission was assessed at week 10 with two different doses of etrolizumab, which both independently showed significant differences compared to placebo, despite overall effect sizes being small ($n = 39$ in each etrolizumab dosage arm). However, these differences were more marked when patients were stratified according to levels of αE gene expression (*ITGAE*) using RT-qPCR, from colonic biopsies taken at baseline. Clinical remission at week 10 was achieved in 6 out of 16 patients (38%) in the αE^{high} group (greater than or equal to median gene expression) compared with 2 out of 16 patients (13%) in the αE^{low} group (less than median gene expression). Sub-analysis of patients who were anti-TNF naïve again suggested greater response in the high expression group, with six out of nine patients (67%) in the αE^{high} group in clinical remission at week 10, compared to one out of six patients (17%) in the αE^{low} group. The number of participants in this trial were small, and it is possible that baseline αE levels may simply be a marker for decreased epithelial integrity seen with higher severity of inflammation. However, given the significant promise shown, αE integrin is currently being assessed in a phase 3 development programme, as a biomarker for response.

Interleukin-23 (IL-23) is a pro-inflammatory cytokine with two subunits: p40, which is shared with IL-12, and p19, which is unique to IL-23. Monoclonal antibody treatment to inhibit the p40 subunit, ustekinumab, has efficacy in treating CD [31], and p19 antibodies are currently in development. In a trial of the anti-IL23p19 monoclonal antibody MEDI2070/brazikumab, 119 patients with CD, who had failed treatment with anti-TNF, were recruited to a phase 2a, placebo-controlled trial [32]. This study showed significant benefits of treatment compared to placebo in terms of clinical response and remission. Importantly, given the known role for IL-22 as an upstream regulator of IL-23 signalling, baseline IL-22 concentrations ≥15.6 pg/ml were associated with an increased likelihood of clinical response (70% vs. 30%) and clinical remission (45% vs. 10%) for those on MEDI2070 at week 8. These findings did not reach statistical significance (reflecting the small numbers of participants involved), although the results are in keeping with the EMBARK trial, which identified that serum IL-22 was able to reflect disease activity in a subset of 66 patients with CD [33]. EMBARK was originally set up as a discovery trial for biomarkers of disease activity in 107 UC patients and 157 CD patients; however, a subset of 66 CD patients had concurrent ileocolonoscopy and CT enterography data which were used as dependent variables to demonstrate association with serum IL-22 [33]. These phase 2 observations provide promise for further biomarker development and informing future trial design. If analysis of cytokine levels can be taken forward to routine clinical assay development, this may offer a potential method for stratification using IL23p19 antibody.

26.3.2 Hypothesis-Free Approaches to Treatment Response

Gene expression array studies of mucosal biopsies from IBD patients have reported panels predictive of nonresponse to infliximab in both UC and CD. Forty-five patients with UC, naïve to anti-TNF therapy, were recruited across two cohorts and had biopsy samples obtained from flexible sigmoidoscopies prior to starting infliximab therapy [34]. At 4 weeks in the first cohort and at 8 weeks in the second cohort, patients were classified as either responders or nonresponders based upon endoscopic responses. In total, 74 gene probes from 53 different genes were consistently expressed at lower levels in responders as compared to nonresponders. Increased expression of the top 5 genes (*TNFRSF11B, STC1, PTGS2, IL-13Ralpha2, IL-11*) across both cohorts predicted nonresponse to infliximab with an accuracy of 89%. All five of the proteins encoded by these genes are characterised by their role in the host immune response and inflammatory process, most notably IL-13 receptor alpha 2 due to downstream effects on prostaglandin metabolism [35]. In an extension of this work, a similar approach was applied to 37 patients with active CD, consisting of 19 patients with colonic disease and 18 patients with predominantly ileal disease (although 9 of these patients also had colonic involvement) [36]. Biopsies were taken before and after induction treatment with infliximab. In this study, a panel of the top 5 genes (*TNFAIP6, S100A8, IL-11, G0S2, S100A9*) was able to predict response of colonic CD to infliximab with an accuracy of 100%. Again, all these genes encode proteins associated with pro-inflammatory responses. Notably, the previously identified panel of genes for response in UC showed significant overlap with colonic CD, with all of the top five genes for UC, also being associated with response, albeit to a lesser extent. However, no predictive panel of genes could be identified for patients with ileal CD suggesting that, despite their genetic and immunobiological overlap, there remain important differences between ileal and colonic CD.

Subsequently, bioinformatic modelling from this molecular phenotyping approach has been suggested as a possible strategy to identify response to anti-TNF therapy [37]. In a further refinement of whole-tissue transcriptomic analysis, in recent years, the ability to generate transcriptomic analysis of specific cell populations sorted from small amounts of biopsy samples has been developed [38]. This offers a novel approach to understanding immune function as well as to assess the cell-specific impact of therapies and is now being applied to studies of therapeutic interventions, such as the use of IL-36 antibody in UC (EudraCT number 2017-000100-20) [39].

An elegant study examined oncostatin M (OSM), which is part of the IL-6 family [40]. Higher levels of OSM were found to be expressed in mucosal biopsies of patients with IBD (162 with CD and 74 with UC). Using hierarchical clustering of expressed cytokines and chemokines, a module of these mediators was associated with high OSM. Patients with high mucosal expression of OSM were found to have reduced mucosal healing (10–15% vs. 69–85%) and higher levels of primary nonresponse on treatment with anti-TNF. This would be in keeping with previous work highlighting that primary nonresponse to treatment often reflects a high inflammatory burden [41]. Further study of OSM has two potential clinical benefits, both as a biomarker to identify likely nonresponders to anti-TNF at an early stage and as a potential target for novel therapeutic agents.

Analysis of mucosal biopsy transcriptomics to predict response has been applied not just to anti-TNF therapies but also to anti-integrin medications. Colonic biopsies were analysed for transcriptional signatures from 41 UC patients from both the GEMINI I or GEMINI long-term study [42]. Biopsies were taken from these patients prior to vedolizumab induction and then at three further time points throughout the year. Analysis of gene probe sets from these biopsies of vedolizumab responders demonstrated 462 downregulated probes (notable genes including *LPHN2*, *FGF7*, *GNG11*, *EMCN*) and 131 upregulated probes (notable genes including *MIR192*,

SLC3A1, *FABP6*) at week 52 compared to baseline. These probe sets further highlighted the critical role of the immune system and represented genes involved in immune cell trafficking, cellular movement and the inflammatory response.

26.4 Conclusions and Future Directions

Biomarker studies have uncovered a range of potential markers for use in disease prognosis or prediction of treatment response. All of these studies face major barriers in terms of validation and clinical utilisation. In particular, there has been a commendable drive by the pharmaceutical industry to build analyses into the design of late-phase clinical trials to permit the identification and validation of drug-specific biomarkers of response. To date, with the exception of some of the initial findings discussed in this article, much of this data remains unpublished. It is difficult to conclude whether such approaches will ultimately yield results applicable in a clinical context, despite the large sample sizes assembled as part of these commercial drug development programmes. To take the much smaller vedolizumab and infliximab transcriptomic response experiments described above, it is interesting to note that there was significant overlap of gene probe sets for vedolizumab and infliximab responders, with the majority of genes predicting response shared by both drugs. This suggests that models of treatment-specific prognostication, at least using blood or whole-tissue transcriptomes alone, may yet remain elusive, whilst more general models may permit the differentiation of disease that is more or less likely to respond to treatment.

It is sobering to reflect that all of the biomarkers described in this article have emerged from analyses of studies examining relatively restricted elements of the complex interplay between genetics, immune function and microbial dynamics that underlie IBD pathogenesis. Integrative approaches using some or all of these elements are still some way off generating validated targets or biomarkers of clinical utility. However with development of novel computational approaches to manipulation and integration of large data sets, investment in large biobanks and decreased technology costs, the promise of significant future advances is strong.

Summary Points

- The immune system provides a rich target for biomarker discovery in predicting both outcomes and treatment response in IBD.
- The process of biomarker discover can be driven by prior knowledge of biology such as the observations that neutrophilic biomarkers, such as faecal calprotectin, correlate with early or ongoing intestinal inflammation in the POCER and CALM trials.
- Combining endoscopic techniques with advances in immunology offer hope for prognostic biomarkers using confocal endomicroscopy and for treatment predictive biomarkers using fluorescent antibody application to intestinal mucosa.
- A complementary approach to biomarker discovery is to use a range of modern "-omics" approaches to achieve an unbiased, global readout of immune function and then use the resulting data set to test for correlates with the outcome under study. In this way, analysis of transcriptomic signatures from individual cell types, whole blood, and intestinal biopsies have yielded insights for development of both prognostic and predictive biomarkers.
- Integrative approaches using a range of different technologies and data sets along with careful validation are required to develop clinically useful biomarkers.

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27 Genetic and Genomic Markers for Prognostication

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Abstract

Genetic and genomic studies have provided key insights into the biology responsible for inflammatory bowel disease (IBD) susceptibility, but the biology of disease outcome remains relatively unexplored. Like most autoimmune and inflammatory diseases, IBD has a highly variable course, with the potential to have a devastating impact on patients' lives. As a result, being able to reliably predict prognosis in IBD remains a major ambition of clinicians and patients alike.

In most fields of medicine, the goal of delivering personalised medicine has become increasingly important. For this to become a reality, however, it will first be necessary to better understand what determines disease prognosis. A major step towards this goal may lie in the emerging evidence that the biology that drives prognosis in IBD is distinct from the biology that underpins disease susceptibility. Indeed, it is hoped that by better understanding the mechanisms that determine disease progression, it might ultimately be possible to develop clinically useful biomarkers, which could be translated back to the clinic to improve patient care.

In this chapter we will review the efforts that have already been made using genetic and genomic tools to develop prognostic and predictive biomarkers in IBD. We will discuss the important requirements for such biomarkers, both in terms of their development and validation, and the evidence that will be required in order for them to be translated back into clinical practice.

27.1 Introduction

In the last 15 years, research in genetics and genomics has evolved from labour-intensive studies of small numbers of genes (in DNA and RNA, respectively) to relatively straightforward and affordable studies that can be performed on a genome-wide scale. These advances have made genetic and genomic studies incredibly powerful, not least because it is now possible to discover genes and pathways that play a key role in disease biology but which were never previously sus-

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pected. The potential for such insights to provide clinically useful biomarkers has unsurprisingly spawned a new field of "Genomic Medicine", which, while still in its infancy, has already begun to fulfil its potential in certain areas.

To date, the major beneficiary of Genomic Medicine has undoubtedly been oncology. Indeed, one of the first applications of personalised medicine was based on the observation that patients with breast cancer expressing the human epidermal receptor 2 showed improved survival following treatment with a monoclonal antibody that targeted the receptor, trastuzumab (Herceptin) [1]. Subsequent attempts to identify clinically meaningful subgroups of patients using -omic approaches have also been successful. For example, transcriptional profiling of tumours has facilitated the development of gene expression biomarkers that can predict important features of cancer behaviour, including response to therapy, risk of metastasis and risk of recurrence [2, 3].

In other fields, however, progress has been much more limited – not least because in non-oncological diseases, it is often difficult to determine which tissue to study. Moreover, in inflammatory and autoimmune diseases, most research efforts have focused upon disease susceptibility rather than on disease prognosis. However, it is notable that in IBD, these efforts have been very successful, with 244 susceptibility loci having been identified to date [4–6]. This success, together with a small number of studies that have recently provided insights into the biology of prognosis in IBD [7–10], has generated optimism that genetic and genomic prognostication should be possible in the future.

27.2 Personalised Medicine in IBD

It is well known that the clinical course of IBD can vary dramatically between patients [11]. Given this spectrum of prognosis, it is not surprising that a "one-size-fits-all" approach to treatment is generally considered inappropriate (Fig. 27.1). For example, while a reactive or "step-up" treatment strategy (in which treatment is only escalated in response to ongoing active disease) might be appropriate for patients with mild disease, this would inevitably expose patients with more aggressive disease to avoidable disease-related complications while potentially ineffective treatments are trialled [12]. Similarly, while there is good evidence that patients with aggressive disease would be best managed by early use of potent therapies [13, 14], the indiscriminate use of such treatment in all patients would expose those with milder disease to the risks and side-effects of unnecessary treatment (Fig. 27.1). Moreover, with the growing number of available treatments, it will soon be important to not only determine which patients require more potent therapy but also to assess which is the most appropriate for them – in terms of minimising side-effects and maximising efficacy.

Against this backdrop – which is shared across many diseases – personalised, or precision, medicine has become an attractive proposition [15]. Indeed, the simple notion of giving the "right treatment to the right patient at the right time" has recently garnered considerable political and financial support, with a $215 million precision medicine initiative being set up in the USA in 2015 and similar initiatives being established elsewhere, including the UK [16, 17]. Importantly, the need for such initiatives also highlights the fact that although there are many reports of clinical and/or biochemical measures associating with disease course, these are typically insufficient to guide therapy and/or have never been tested in appropriately powered, prospective studies [18]. Accordingly, there is now a clear need for clinically useful biomarkers, that are not only associated with a particular phenotype of disease or treatment response but are sufficient to base treatment decisions on and which can accordingly lead to both patient-centred and health economic benefits [15, 19].

27.3 Genetics

Since the advent of genome-wide association studies (GWAS), genetic research in IBD has been successful. However, these efforts have principally focused on understanding the genetic contribution to disease susceptibility [4–6, 20–22]. Nevertheless, in many diseases – including IBD – there is evidence that a genetic contribution to

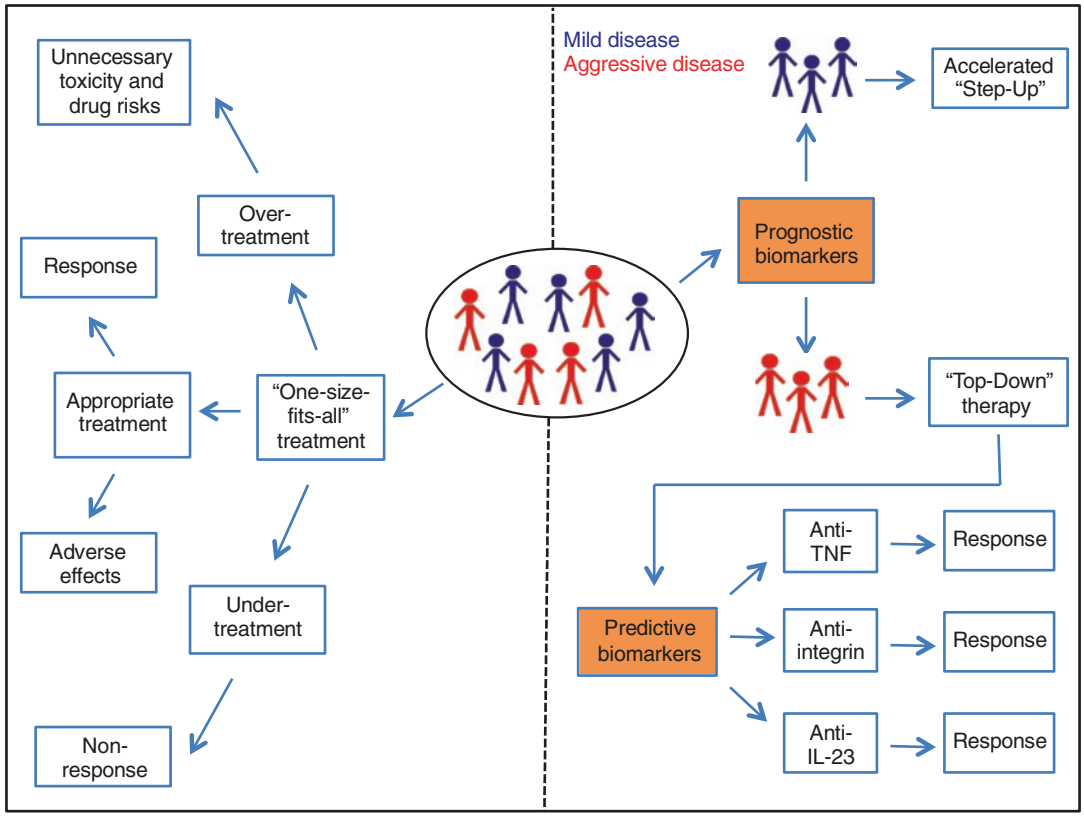

Fig. 27.1 Future use of biomarkers to personalise treatment in Crohn's disease. A comparison of the current treatment approach in IBD (left) with the aspirational goal of delivering personalised medicine by incorporating prognostic and predictive biomarkers (right). On the left, where all patients are treated with a "one-size-fits-all" approach – irrespective of whether that is "step-up" or "top-down" – there will be some patients for whom the treatment is inappropriate, because of either under-treatment and exposure to avoidable disease-related complications or overtreatment and exposure to the risks and side-effects of unnecessary immunosuppression. Moreover, even if the potency of treatment is appropriate, there will be risks of side-effects related to the therapy. This is contrasted with a personalised approach (right) where prognostic biomarkers are used to identify patients with mild disease who can be safely treated using conventional "accelerated step-up" therapy, and those with aggressive, poor prognosis disease who require a more potent, "top-down" approach. In those patients, additional predictive biomarkers would be used to select the most appropriate treatment based on maximising the efficacy and minimising the probability of side-effects

prognosis may also be present, largely thanks to similar disease patterns often being observed within multiply affected families [23–25].

This observation initially led to a series of candidate gene studies to investigate how genetic variation might contribute to disease course. Notably, the majority of these studies focused on known susceptibility variants – most likely because these represented a convenient and manageable list of SNPs, rather than because of any a priori hypothesis that shared effects on susceptibility and prognosis being particularly likely. However, it is important to realise that if susceptibility variants are also the principal determinants of prognosis, then this would imply that the development and subsequent course of IBD are driven by shared biological pathways. In support of this hypothesis, early studies identified apparent links between susceptibility SNPs and clinical outcomes, including associations between *SMAD3* variants and need for recurrent surgery in Crohn's disease [26] and *IRGM* variants and fistulating behaviour [27]. Unfortunately, much like candidate gene studies of disease susceptibility in the pre-GWAS era, many of these associations have not been able to be replicated

subsequently [28]. One association that has been frequently reported is between *NOD2* variants and need for surgery [29, 30], but this has recently been shown to be due to the association between *NOD2* variants and ileal disease – for which surgery is more commonly used – rather than any true effect on disease course. Indeed, if disease location is taken into account, no effect on disease course is detectable [28].

Following the general failure to identify robust associations between susceptibility variants and disease course, there has been increasing interest in whether non-susceptibility variants might contribute to prognosis in IBD. This possibility was supported by the results of a large sub-phenotype study, which demonstrated that although associations were detectable between susceptibility variants and Crohn's disease location, there was little or no association between these variants and disease behaviour [28]. Accordingly, this would imply that any genetic contribution to prognosis in IBD must arise from non-susceptibility variants. In order to discover this contribution, however, a genome-wide approach would be necessary. Such an approach has been used before to discover genetic contributions to sub-phenotypes in IBD. For example, in 2010 a "within-cases" GWAS was performed in UC, comparing 324 patients with medically refractory acute severe UC with 537 patients with non-medically refractory disease [31]. This identified a signal within the MHC that surpassed a genome-wide significance threshold and led the authors to develop a genetic risk score using a combination of 46 SNPs, which associated with risk of colectomy in the same cohort. This tool clearly requires external validation, but nonetheless highlights the potential for genetic studies to yield tools that might have prognostic utility.

In Crohn's disease, a "within-cases" GWAS of prognosis has also recently been performed in which subgroups of patients at opposite ends of the prognostic spectrum were identified and compared [8]. In this analysis, good prognosis Crohn's disease was defined as disease that did not require surgery or immunomodulator therapy with a minimum of 4 years' follow-up, while poor prognosis Crohn's disease was defined as disease that had required two or more immunomodulators, biologics or surgical operations (or any combination of these). By combining data from two cohorts of good and poor prognosis cases, four distinct loci were identified that surpassed a genome-wide significance threshold (*FOXO3*, *XACT*, *IGFBP1* and the MHC region). Interestingly, none of these SNPs had previously been associated with susceptibility to Crohn's disease, and a genetic risk score comprising 170 susceptibility SNPs did not associate with prognosis – thus supporting the notion that disease susceptibility and prognosis have distinct genetic architectures.

There is little doubt that studies such as this will provide important clues as to the biology that determines prognosis in IBD. For example, the *FOXO3* association has been shown to regulate a novel TGFβ-dependent pathway that controls inflammatory cytokine production in monocytes [32], while the multigene haplotype that is tagged by the MHC association (ancestral MHC 8.1) is known to be associated with defects in T cell activation [33]. However, whether these variants will also have prognostic utility is currently unknown. This is because the odds ratios observed at these associations indicate that none of these SNPs are either necessary or sufficient to determine outcome in isolation. This is true of GWAS results in general and means that simply genotyping these four SNPs is unlikely to provide a robust prognostic test. However, there are methods that can develop genetic classifiers from an extended list of associated variants [34, 35] which could make genetic prediction possible, although there is clearly still much work to do in this area.

One area of genetic research where the odds ratios of associated SNPs are sufficient to facilitate predictive testing is in pharmacogenetics. For example, it is already common practice to assess TPMT genotype and/or activity before commencing thiopurines due to the high risk of myelosuppression in people who carry inactivating mutations in the gene. Similarly, a non-synonymous SNP in *NUDT15* has also been shown to associate with myelosuppression with an odds ratio of 35 [36]. Genetic associations with thiopurine-induced pancreatitis and 5-ASA-induced nephrotoxicity have also been reported [37, 38]. Screening for such variants is

likely to eventually represent an important component of personalised medicine in IBD, not just in terms of assessing which patients require more potent therapy (prognostic biomarkers) but in terms of assessing which treatments are most appropriate for individual patients (predictive biomarkers) (Fig. 27.1).

Currently there remains a need for larger studies to better characterise the role of genetics in prognosis and to assess whether clinically useful prognostic tools can be built from stratified analyses of patients. However, it is clear that a genetic contribution to prognosis does exist and that this is principally driven by variants that are distinct from known susceptibility loci – a finding that also has important implications for future drug development [39].

27.4 Genomics

While genetic studies measure the relative frequency of polymorphisms within DNA and can be performed similarly well, irrespective of the tissue that the sample was extracted from, genomic studies measure changes in RNA – the nucleic acid that is produced when genes are expressed. RNA levels are highly tissue- and context-specific and thus are much more difficult to assay meaningfully, as there are many potential confounders to consider. Nonetheless, RNA is ultimately what determines cellular identity and behaviour and has been shown to represent a valuable source of biological information, which can have prognostic utility. For example, many of the established biomarkers in oncology are based on measuring the expression (or RNA) from key genes [2, 3].

Much like advances in DNA analysis, technological developments have made genome-wide measurement of RNA a realistic and affordable option in research settings. However, unlike DNA-based approaches, there are several additional considerations that must be made when analysing RNA. For example, because gene expression is tissue-specific, assaying RNA from a heterogeneous tissue – such as whole blood or a mucosal biopsy – will typically produce results that are simply reflective of the relative proportions of the constituent cell types [40]. This can create misleading results if the composition differs between health and disease, with "disease-specific" transcriptional signatures often simply reflecting numerical differences in the cell types present in the starting material. For this reason, variables such as the tissue examined, disease duration, disease activity and concomitant treatments must be considered in genomic studies in order to control for potentially confounding effects. One of the simplest ways to control for duration of disease and treatment effects is to use samples from newly diagnosed, treatment-naïve patients, although a trade-off may be required between the logistical challenges of obtaining such samples and the potential benefit of such a collection. In addition, unlike DNA, gene expression has been shown to change once a tissue is removed from the body meaning that it is important to have the local facilities and expertise to work on freshly collected samples [40]. Indeed, this multitude of potential confounders may explain why most candidate biomarkers do not progress beyond initial reporting [41].

Despite the additional challenges that come along with genomic research, there are several examples of well-performed studies that have provided important and novel insights into the pathogenesis of IBD. For example, in a prospective inception cohort of 913 children and adolescents with newly diagnosed Crohn's disease (the RISK cohort), a gene signature was identified in ileal biopsies that was associated with future stricturing complications and which was enriched for extracellular matrix genes [9]. Moreover, the same data has also been used to develop a 29-gene transcriptional risk score, by integrating GWAS and expression quantitative trait data, which showed a modest association with progression to penetrating or stricturing disease [10]. However, it is worth restating that gene expression is dynamic and susceptible to changes in response to treatment and disease activity, which means that the utility of such biomarkers may be limited to patients presenting with active disease at diagnosis, where confounders such as drug therapy, previous surgery or a protracted disease course are not present.

RNA samples from intestinal biopsies have also been shown to be useful in developing predictive biomarkers (that could help assign the

right treatment to the right patient). For example, an analysis of cytokine expression in colonic biopsies from IBD patients identified upregulation of oncostatin M (*OSM*) and its associated inflammatory mediators as being predictive of non-response to anti-TNF therapy [42]. Similarly, pretreatment expression of αE integrin subunit in colonic biopsies has been shown to positively correlate with response to etrolizumab, a monoclonal antibody that targets the β7 subunit of the α4β7 and αEβ7 heterodimers [43].

However, one practical downside to studies using intestinal biopsies is that biomarkers based on the results are likely to require endoscopic procedures in order for the biomarker to be assessed. For this reason, other studies have investigated whether it might be possible to develop clinically useful biomarkers from blood, which is much easier to sample in clinical practice. An early example of such an approach was performed in a cohort of paediatric acute severe UC patients by comparing whole blood gene expression on day 3 of intravenous steroids between responders and nonresponders. This identified 41 differentially expressed genes, of which the 10 most strongly associated genes were able to classify the same patients with a sensitivity and specificity of 80% [44]. However, an important criterion for biomarker development is that when differentially expressed genes are identified by comparing predefined subsets of patients, then the performance of these genes as a classifier must be tested on an independent validation cohort and not the same cohort that was used for the initial discovery. Failure to do this is likely to lead to "overfitting" where a classifier is too tightly modelled on the training data, and consequently incorporates noise in addition to any real signal, and thus does not perform well on new samples.

Another blood-based prognostic biomarker, which has since been optimised for use in clinical practice and is currently undergoing late-phase clinical testing, was identified by performing gene expression analysis on leucocyte subsets from patients with active, untreated IBD [7]. This approach overcomes the variable composition limitation that is inherent in analysing heterogeneous tissues, by first purifying leucocytes into individual cell types [40]. Interestingly, this study identified a gene expression signature in CD8 T cells associated with prognosis in both Crohn's disease and ulcerative colitis and which was analogous to a prognostic CD8 T cell signature that was previously described in systemic lupus erythematosus and ANCA-associated vasculitis [45]. In all four diseases, the gene signature divided patients into two distinct subgroups, which were all clinically indistinguishable at presentation but which had very different courses of disease thereafter [7, 45]. Subsequent exploration of the biology responsible for these transcriptional differences demonstrated that patients in the good prognosis subgroup were enriched for a signature of T cell exhaustion – a phenomenon where antigen-experienced T cells lose their ability to respond to their target antigen [46]. This would accordingly be consistent with a disease course characterised by fewer flare-ups, less end-organ damage and a lower requirement for treatment. This biomarker has since been devolved into a multigene qPCR assay that can be performed on unseparated whole blood, and a biomarker-stratified trial is currently underway to determine whether this is able to effectively stratify patients such that treatment can be personalised (*P*redicting *O*utcomes *f*or Crohn's D*i*sease Using a Mo*l*ecular Biomark*e*r (PROFILE) trial) [47]. This represents the first biomarker-stratified trial in any inflammatory disease to date and could represent a significant step towards personalised therapy.

27.5 Conclusions

Understandably, the initial focus of genetic and genomic studies in IBD was on disease susceptibility, but it has become increasingly clear that there would be much to gain from applying these approaches to understanding prognosis – as has been shown in oncology. Indeed, without an understanding of what determines prognosis in IBD, and ultimately an ability to reliably predict the future disease course at an individual patient level, it seems unlikely that the goal of personalised medicine will be realised. Such efforts will need to be coupled to the development of predictive biomarkers so that the most appropriate option can be selected for patients requiring more

potent therapy from the ever-growing armamentarium of treatments.

It is clear that tools to predict prognosis using genetic or genomic data are not yet ready for routine clinical use, but there is considerable early promise on several fronts that gives plenty of room for optimism. We would predict that advances in technology, coupled with an increasing awareness of the importance of personalised medicine and large-scale financial support, will ultimately converge on a situation akin to that in oncology, where genetic and genomic biomarkers are used in routine practice. Importantly, there are likely to be other benefits of the work needed to arrive at that point, including the identification of novel targets for future treatments and a better understanding of disease pathophysiology.

27.6 Future Directions

With increased financial support and ongoing technological advances, it is clear that interest in developing prognostic and predictive biomarkers will only continue to grow. For this reason, it will be important that we hold any potential biomarker to a robust set of standards (Table 27.1). We should not, for example, assume that associations with a clinical phenotype automatically mean that a biomarker would have sufficient predictive performance on which to base treatment decisions. This must be specifically tested in order to ensure that any resulting tools are genuinely capable of improving patient care. Likewise, wherever possible we should expect prospective, well-validated studies as the norm and ensure that retrospective associations are scrutinised to prove causation, not just correlation.

Holding prognostic biomarkers to such standards has borne substantial progress in other fields, notably oncology, and coupled with national and international efforts to better understand the determinants of prognosis in IBD should bring similar advances. We look forward to a time when prognostic and predictive biomarkers can be routinely used to ensure that all patients can receive the right therapy at the right time and so truly receive a personalised approach to their treatment.

Table 27.1 Checklist for prognostic biomarkers

Have they been shown to be genuinely prognostic? An important distinction here is between retrospective associations where an apparent biomarker may in fact be the result of a particular disease course, not its cause. There is a need for prospectively performed, well-validated studies that can establish a prognostic link
Have they been externally validated in an independent cohort? This is particularly important when the biomarker being investigated was discovered in a training cohort through a comparison of predefined patient subgroups
Can the biomarker guide effective treatment stratification? This requires formal testing and cannot simply be presumed because an association is detectable
Can the biomarker be performed in routine clinical practice? Is the technique feasible and affordable, and is the material required easily obtainable?
Is the biomarker clinically useful? Is it detectable in all patients or simply a subgroup of patients? Does it produce clear, comprehensible results?
Is the biomarker acceptable to patients? Will they be willing to provide the necessary samples and have treatment decisions based on the result?
Is the biomarker cost-effective? Have health economic studies been performed to model the financial impact of using the biomarker on health-care systems?

Summary Points

- Genetic contributions to prognosis in inflammatory bowel disease have been discovered by performing "within-cases" analyses from existing cohorts of patients from genome-wide association studies.
- The genetic contribution to prognosis appears to be due to genes distinct to those associated with disease susceptibility, suggesting the biology of prognosis is also likely to be distinct from that of disease susceptibility.
- Adequate prognostic biomarker associations need to be established from well-performed, prospective investigation and not simply derived from retrospectively observed associations.
- Following discovery of a potential prognostic biomarker, external validation is required in an external cohort in order to prevent the phenomenon of "overfitting" of data.

- Ultimately, such prognostic biomarkers should then be used in a clinical trial setting in order to demonstrate clinical utility, cost-effectiveness and the ability to personalise therapy for patients with inflammatory bowel disease.

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28 Risk Alleles for Drug Targets: Genomic Markers of Drug Response

Ryan Ungaro and Judy Cho

Abstract

The armamentarium for the treatment of inflammatory bowel disease (IBD) is growing with the introduction of new targeted therapies. Developing precision medicine approaches using our knowledge of IBD genetics is of great interest and importance. To date, research on genomic markers of drug response has largely focused on anti-tumor necrosis factor alpha (TNF) agents. Currently, no genetic markers have been validated for use in clinical practice to guide treatment selection. However, several studies have highlighted promising blood- and tissue-based markers of drug response. In this chapter, we provide an overview of the current evidence for genomic markers of response to biologics and discuss potential future directions for predictive biomarker discovery.

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28.1 Introduction

Extensive research has elucidated the genetic underpinnings of susceptibility to inflammatory bowel disease (IBD) with nearly 200 susceptibility loci identified to date [1–3]. The associated genetic polymorphisms have highlighted key immune and molecular pathways and processes that are important in IBD pathogenesis. The central role of innate immunity in Crohn's disease (CD) was supported by the discovery of *NOD2*, the key role of autophagy was pointed to by *ATG16L1*, and the association of *IL23R* with IBD underlined the importance of IL-23 immune pathways [4]. Moreover, there are many connections between gene loci identified in genome-wide association studies (GWAS) and IBD therapeutics (Table 28.1). For example, the *IL23R* risk allele has been demonstrated to lead to a loss of function of the IL-23 receptor with subsequent decreased signaling in the IL-23 pathway with the recently approved ustekinumab inhibiting IL-23 [5, 6]. Anti-tumor necrosis factor alpha (TNF) agents have long been the mainstay treatment in IBD, and various TNF-associated candidate genes have been identified in genetic susceptibility studies (TNFRSF18, TNFRSF14, TNFRSF9, TNFAIP3) [2]. Last, anti-integrin therapy vedolizumab is now used in clinical practice, and a recent GWAS study implicated multiple integrin genes as being activated in IBD [7].

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Table 28.1 Associations between genetics and IBD therapies

Treatment	Mechanism of action	Genetic correlate
Anti-tumor necrosis factor alpha (TNF) biologics (infliximab, adalimumab, certolizumab, golimumab)	Blockade of TNF signaling and apoptosis of TNF-expressing cells	TNFAIP3- and TNFRrelated genes associated with risk of IBD
Anti-interleukin 12 and 23 biologics (ustekinumab, anti-p19 therapies in development)	Blockade of IL12 and IL23 signaling (p19 blockade specific to IL23)	Loss-of-function allele in IL23 receptor that is protective against IBD
Janus kinase (JAK) inhibitors (tofacitinib, JAK-specific inhibitors in development)	Blockade of transcription of multiple pro-inflammatory cytokines	Multiple IBD-associated genes related to inflammatory cytokines
Anti-integrin biologics (vedolizumab, etrolizumab in development)	Blocks α4β7 expressing leukocyte trafficking from the periphery to the intestine	Integrin genes associated with increased risk of IBD

For many years, anti-TNF medications were the only biologics available for IBD. However, new drugs with novel targets have recently become available or are in the pharmaceutical pipeline. Vedolizumab, an anti-integrin, was approved in 2014, providing a new class of biologic therapy for IBD. Most recently, the anti-IL12/23 biologic ustekinumab was approved for treatment of CD. Medications that inhibit the p19 subunit of IL-23, Janus kinase (JAK) inhibitors, and other anti-integrin therapies will become commercially available in the coming years. Clinicians will have to choose which drug to use and which molecular pathway to target. Since anti-TNF agents have been around longest, gastroenterologists may preferentially use anti-TNF due to familiarity. However, this will not be the right strategy for every patient. Identifying genetic and genomic biomarkers of therapeutic response offers significant promise to help usher in an era of precision medicine in IBD, allowing clinicians to select the best treatment for the individual patient. There is great interest in identifying markers of response or non-response to anti-TNF in particular as then alternative new (and expensive) biologic agents may be better positioned in the armamentarium. Decreasing costs and higher-throughput genomic, transcriptomic, and proteomic platforms make incorporation of such technologies into IBD clinical practice increasingly feasible. This chapter aims to give an overview of the current research on genomic predictors of drug response in IBD with a focus on markers of response to anti-TNF agents.

28.2 Blood Genomic Markers of Anti-TNF Drug Response

Blood-based genomic markers of anti-TNF response would offer the greatest ease and practicality to incorporate into clinical practice. Various studies have been conducted looking at the impact of specific gene polymorphisms or sets of related genes on anti-TNF response. A few studies have found associations between TNF and TNF receptor-related genes and response to anti-TNF therapy given the direct relationship with mechanism of action. A study of 75 CD patients utilizing data from the original infliximab placebo-controlled trials found that patients with the TNF haplotype 11-4-1-3-3 had a lower response rate based on change in the Crohn's Disease Activity Index (CDAI), although this did not reach statistical significance [8]. The authors then looked at genes in the same region as TNF and focused on *Nco*I, TNFc, aa13L, and aa26 polymorphisms for lymphotoxin-α (LTA). None of the patients ($n = 6$) who were homozygous for the *Nco*I-TNFc-aa13L-aa26 1-1-1-1 haplotype responded to anti-TNF therapy. These patients were also more likely to be positive for perinuclear anti-neutrophil cytoplasmic antibodies

(pANCA) which was also associated with lower rates of infliximab response. Another study investigated the impact of polymorphisms in TNF receptors 1 and 2 (TNFR1/2) on infliximab response in CD patients using both a prospective German cohort ($n = 90$) for discovery and a randomized trial (ACCENT I, $n = 444$) for validation [9]. This group tested polymorphisms in the TNF, TNFR1, and TNFR2 genes. In their discovery cohort, the 196 Arg allele in exon 6 of TNFR2 and a polymorphism in exon 2 of TNFR2 were associated with non-response to infliximab. The authors found an 83.3% non-response rate in 196 Arg homozygotes compared to 36.9% in heterozygotes and wild-type patients ($p = 0.036$) as well as an 85.7% non-response rate in homozygote exon 2 patients compared to 36.1% in heterozygotes and wild types ($p = 0.01$). However, when the authors attempted to replicate these findings in the second cohort, no association was found. A similar study was conducted in Leuven, Belgium, investigating 166 infliximab-treated CD patients [10]. In contrast to the German group, the authors found that patients with one or two specific TNFR1 alleles (A36G genotype) were less likely to have a response to infliximab (OR 0.47, 95% CI 0.23–0.95, $p = 0.03$) defined as a decrease or normalization in C-reactive protein (CRP). No association was seen with TNFR2 polymorphisms. These results, while intriguing, demonstrate the inconsistent findings related to genetics of TNF-related genes.

Other specific genes that are related to IBD susceptibility or potential anti-TNF mechanism have been investigated as markers of anti-TNF response. Studies have had conflicting results when examining the association of one of the strongest IBD susceptibility genes, nucleotide-binding oligomerization domain containing 2 (NOD2), and anti-TNF response. One study in a prospective cohort of 245 CD patients studied the impact of the 3 main NOD2 variants (R702W, G908R, and 1007 fs) on clinical response at 4 weeks and 10 weeks of infliximab therapy for inflammatory and fistulizing disease, respectively [11]. Although the authors did observe differences in mucosal TNF production in a subset of patients with biopsies (lower in NOD2 mutation carriers), they found no significant association between NOD2 and clinical response to infliximab. In contrast, another study of 50 CD patients treated with infliximab or adalimumab found an association between NOD2 and anti-TNF response [12]. Patients who had wild-type NOD2 status appeared to have a higher response rate to anti-TNF induction therapy defined by decrease in clinical activity assessed through medical record review. The IBD5 susceptibility locus (chromosome 5q31) has been associated with clinical response to infliximab in a Spanish cohort [13]. In a small study of CD and ulcerative colitis (UC) patients, there was an association with IBD5 homozygosity and non-response to infliximab in CD (RR = 3.88, 95% CI 1.18–12.0) but not in UC patients. The autophagy gene ATG16L1 has been associated with susceptibility to CD in GWAS [14]. A prospective cohort of adalimumab-treated CD patients found that the ATG16L1 SNP rs10210302 was associated with treatment response defined as a decrease or normalization of CRP at 12 weeks. Eighty-five percent of patients with the CT or TT genotype responded to adalimumab compared to 37.5% of patients with CC genotype (OR 9.44, 95% CI 2.49–35.83) [15].

Proposed mechanisms of action of anti-TNF include effects on TNF-expressing cells following binding of drug to transmembrane TNF including apoptosis induction or antibody-dependent cell-mediated lysis [16, 17]. A French group studied the association of polymorphisms in the receptor for Fc portion of IgG III (FcγRIII), which is important in cell-mediated cytotoxicity, with anti-TNF response [18]. FcγRIII had previously been associated with response to another antibody therapy, rituximab, with the purported mechanism of improved binding of the Fc portion of rituximab to target cells. The *FCGR3A*-158 polymorphism was assessed in a group of 200 CD patients treated with infliximab. There was no association found between genotype and clinical response; however, 100% of patients with the homozygous V/V *FCGR3A* genotype had response to infliximab when defined by a decrease

or normalization in CRP after 4 weeks of treatment (RR = 1.43, 95% CI 1.27–1.61). This association held in multivariable analysis. However, the association of *FCGR3A* with treatment response was unable to be replicated when later tested in a clinical trial cohort [19]. Genes in apoptotic pathways have also been investigated given that this process may be important for anti-TNF efficacy. A study of 287 anti-TNF-treated CD patients found an association between specific apoptosis genes and short-term clinical response [20]. Patients with the TT genotype of the Fas ligand-843 gene had lower response rates (OR 0.11, 95% CI 0.08–0.56), and those with the caspase-9 93 TT genotype were more likely to respond (OR 1.50, 95% CI 1.34–1.68). Interestingly, concomitant immunomodulator therapy negated the impact of genotype.

Although not directly related to the possible mechanisms of anti-TNF, various other studies have looked at the relationship between pro-inflammatory cytokines, receptors, and proteins with anti-TNF response. For example, NFκB-related cytokines have been of interest as they are critical in mediating the inflammatory response in IBD. A Danish study of 738 anti-TNF-treated IBD patients found that functional polymorphisms in genes within the NFκB pathway have been associated with clinical response to anti-TNF within 22 weeks of initiating therapy [21]. Nineteen different gene polymorphisms were associated with response. Most were associated with anti-TNF response in both CD and UC, while one polymorphism was associated with CD only – rs1816702 in Toll-like receptor 2 (TLR2) – and three polymorphisms, TLR2 (rs4696480), CD14 (rs25669190), and IL1RN (rs4251961), were associated with UC only. Examples of other pro-inflammatory protein genes that have been investigated as potential markers of anti-TNF response include IL-1B, IL-11, IL-12, IL-13, IL-17F, and IL-18 [22–24]. Given multiple studies of different samples sizes investigating an array of potential genes, a systematic review and meta-analysis was performed [25]. When compiling data across studies, polymorphisms in TLR2, TLR4, TLR9, TNFRSF1A, IFNG, IL6, and IL1B were significantly associated with clinical response to anti-TNF. Only the FCGR3A gene had data supporting an association with objective biologic response (CRP). The various genes associated with anti-TNF response cannot be recommended to be used clinically given the current nature of the evidence which has heterogeneous populations, differing definitions of response, and disparate results lacking prospective validation.

28.3 Composite Genomic Scores for Anti-TNF Drug Response

Given the complex pathogenesis of and genetic susceptibility to IBD, studies have investigated the performance of combining multiple genes to predict anti-TNF response. One group developed an apoptotic pharmacogenetic index (API) that created a score based on the number of polymorphisms a patient had in single nucleotide polymorphisms (SNPs) in apoptotic genes associated with anti-TNF response (Fas ligand-843C/T, Fas-670 G/A, and caspase-9 93 C/T) [26]. In a cohort of CD patients (208 with inflammatory and 83 fistulizing phenotype), clinical response at 4 and 10 weeks (for inflammatory and fistulizing patients, respectively) significantly increased as the API score increased ($p = 0.005$). When combining the API with specific clinical factors (age, disease location, and concomitant immunomodulator use), the overall predictive performance of the model increased significantly. Another study in pediatric IBD patients investigated a composite of predictors of primary non-response, defined as a failure to improve clinical disease activity (assessed by Harvey-Bradshaw Index or partial Mayo score) at weeks 10–14 of infliximab treatment [27]. Previously reported IBD susceptibility genes as well as genes associated with anti-TNF response in GWAS analysis within this cohort of 94 patients were tested to develop a predictive model. A final model combining polymorphisms in four genes (BRWD1, TACR1, FAM19A4, PHACTR3) with pANCA and a UC diagnosis had an area under the curve (AUC) of 0.98 for anti-TNF non-response.

A different, but similar, approach was taken in two studies of adult CD patients [28, 29]. Both of

these studies created a composite genetic burden score to assess its association with anti-TNF response. In a retrospective cohort of 201 anti-TNF-treated CD patients, a genetic risk score (GRS) was created by summing the odds ratios for each of 140 of the CD and shared IBD (between both CD and UC) risk loci to create a continuous variable. In univariable analysis, the GRS was not associated with clinical primary non-response at week 14. A base model incorporating clinical variables only (age, body mass index, and prior surgery) was significantly associated with anti-TNF primary non-response (AUC 0.80, 95% CI 0.67–0.93). The accuracy of the predictive model did not improve after adding the GRS (AUC 0.78, 95% CI 0.65–0.91). The second study utilized a large prospective registry linked to genotyping data of patients from a tertiary care center [28]. Patient's response to anti-TNF (infliximab, adalimumab, or certolizumab) was assessed through medical record review. Patients were classified as either having primary non-response (no response by week 12) or durable response (continued response to anti-TNF therapy for at least 24 months). All patients had genotyping done on an immunochip that included nearly 200,000 polymorphisms in genes associated with immune function or autoimmunity. The investigators examined which of the immunochip gene SNPs and the 163 IBD risk alleles were associated with primary non-response or durable response. Two separate GRS were then calculated, one for primary non-response (15 SNPs) and one for durable response (16 SNPs), by summing the immunochip and IBD susceptibility SNPs that were associated with each endpoint. Patients with primary non-response and durable response each had significantly higher response-specific GRS. A model that included the GRS with clinical variables had better performance than a model with clinical variables alone (AUC 0.93 vs. 0.70, $p < 0.001$) at predicting primary non-response. The GRS for primary non-response and for durable response were not correlated suggesting distinct mechanisms depending on the type of response outcome. The approach of using composite scores that utilize multiple genes of interest as well as clinical factors is a potentially promising approach to predicting response. However, the ideal combination of genes is unclear, and findings need replication in external cohorts.

28.4 Tissue Genomic Markers of Anti-TNF Drug Response

While genetic markers from peripheral blood have offered some potential predictors of drug response, studying the site of inflammation in intestinal tissue may more directly reflect the nature of IBD inflammatory pathways. Two studies from the Leuven group have investigated the ability of pre-treatment mucosal gene expression to predict response to infliximab. In the first study, microarray data from colon biopsies from 46 UC patients treated with infliximab were analyzed [30]. Response to therapy was defined as a composite of endoscopic (Mayo score 0 or 1) healing and histologic remission (Geboes score) at week 4. Out of 212 differentially expressed probe sets, the top 5 differentially expressed genes included osteoprotegerin (TNFRSF11B), stanniocalcin-1 (STC1), prostaglandin-endoperoxide synthase 2 (PTGS2), interleukin 13 receptor alpha 2 (IL13Ralpha2), and interleukin 11 (IL11). This five gene signature predicted response to anti-TNF with 95% sensitivity and 85% specificity. In a second similar study of a group of 19 patients with Crohn's colitis and 18 patients with ileal CD, microarray data from mucosal biopsies was analyzed for association with infliximab response [31]. Response was also rigorously defined as composite of complete endoscopic healing and a decrease in histologic activity score at weeks 4–6 of treatment. The authors were unable to identify a signature associated with response in ileal CD patients. However, the authors found that a panel of five genes was able to predict anti-TNF response with 100% accuracy in Crohn's colitis. The five genes included in this panel were TNF-[alpha]-induced protein 6 (TNFAIP6), S100 calcium-binding protein A8 (S100A8), IL11, G0/

G1switch 2 (G0S2), and S100 calcium-binding protein A9 (S100A9). Another group performed real-time PCR on pre-infliximab treatment colon biopsies in 74 UC patients [32]. The authors found that clinical remission (assess by the ulcerative colitis disease activity index) after induction with infliximab was more likely in patients with higher baseline expression of IL-17A (OR 5.4, $p = 0.013$) and IFN-γ (OR 5.5, p-0.011). One of the most promising potential biomarkers of anti-TNF response is the cytokine oncostatin M (OSM) and its receptor (OSMR). OSM and OSMR expression is increased in intestinal stromal cell in the inflamed mucosa of IBD patients [33]. Microarray analysis of RNA from pre-treatment colon biopsies from UC patients was performed in three observational cohorts and three clinical trials (total $n = 227$) with response defined as endoscopic or clinical remission depending on the individual study's definition [33]. The authors found that high baseline OSM gene module expression was significantly associated with anti-TNF non-response (relative risk = 5, 95% CI 1.4–17.9) with an AUC of 0.99. These results require further validation but come from one of the largest and most carefully phenotyped studies of drug response to date. Last, the Risk Stratification and Identification of Immunogenetic and Microbial Markers of Rapid Disease Progression in Children with Crohn's Disease (RISK) study investigated markers of disease complications over time in an inception cohort of pediatric CD [34]. Based on RNA sequencing of biopsies from ileal mucosa at the time of diagnosis from patients without complications, the authors found distinct gene signatures that were associated with either an increased risk of penetrating (fistulizing) complications or stricturing complications. Of note, high ileal expression of extracellular matrix (ECM) production genes was associated with later development of strictures, and the risk of complications was not decreased by treatment with anti-TNF agents. Patients with high ECM expression may therefore represent a distinct group in which CD pathogenesis is distinct and non-responsive to anti-TNF.

28.5 Markers of Response to Other Biologic Therapies

Most research on predictors of drug response to date has focused on anti-TNF therapies. There is currently little to no evidence on genomic or molecular predictors of response to other biologic medications. However, a few recent studies have suggested some potential predictive biomarkers to biologics with other mechanisms of action. A recent prospective observational study of IBD patients treated with the anti-integrin vedolizumab identified an association between stool microbial taxonomic composition and function and response [35]. Eighty-five patients (42 CD and 43 UC) were included. Baseline alpha diversity was significantly higher in CD patients who achieved clinical remission at week 14. Baseline abundance of two specific bacterial species, *Roseburia inulinivorans* and *Burkholderiales*, was higher in responders. In addition, 13 metabolic pathways including branched chain amino acid synthesis were significantly enriched in CD patients achieving week 14 remission. No significant association was found between gut microbiota and clinical remission in vedolizumab-treated UC patients. Another anti-integrin that is in development, etrolizumab, had a potential predictor of therapeutic response identified during its clinical trial [36]. Etrolizumab is a subcutaneous monoclonal antibody that blocks the β7 subunit of the heterodimeric integrins α4β7 and αEβ7. In a phase II trial in UC patients, etrolizumab response rates were markedly higher in patients with high αE gene expression levels in their baseline colonic biopsy by both immunohistochemistry and quantitative PCR (clinical remission in 50 or 67% of αE high compared to 7 or 25% in αE low among all patients and anti-TNF naive, respectively). Last, an interesting phase IIa study of a novel biologic that targets the p19 subunit of IL23 (MEDI2070) demonstrated efficacy in CD patients and also identified a potential blood biomarker of response [37]. Clinical response and remission rates were significantly higher among patients with high baseline serum IL22 levels compared to those with lower levels.

Predictors of response to anti-tumor necrosis factor alpha therapy	Ulcerative colitis	Crohn's disease
Blood-based gene markers		
TNF haplotype 11-4-1-3-3		
TNF receptors 1 and 2		
TNFRSF1A		
TNFAIP3		
NOD2		
IBD5		
ATG16L1		
Fc portion of IgG III (FcγRIII) -FCGR3A gene		
Fas ligand-843		
Caspase-9		
Toll like receptor 2		
Toll like receptor 4		
Toll like receptor 9		
LY96		
MAP3K14		
IL1RN		
CD14		
IL6		
Composite blood gene marker scores		
Apoptotic gene pharmacogenetic index		
Genetic risk score incorporating IBD associated risk loci only		
Genetic risk score with IBD associated risk loci and immune related disease polymorphisms		
Tissue-based gene markers		
Composite of TNFRSF11B, STC1, PTGS2, IL13Ralpha2, and IL11		
Composite of TNFAIP6, S100A8, IL11, G0S2, S100A9		
IL-17A		
IFN-γ		
OSM and OSMR		
Extracellular matrix production genes		

Positive predictor
Negative predictor
Conflicting data
No association / Unknown

Fig. 28.1 Overview of current genomic markers for anti-TNF response

28.6 Future Directions

In this chapter, we have provided an overview of current genomic markers of drug response, summarized in Fig. 28.1. While much research has been done on genomic predictors of drug response, no markers are currently ready for use clinically. The major obstacles to adoption in clinical practice include replication in larger validation cohorts, effect sizes that will be meaningful clinically (with understanding of sensitivity, specificity, and likelihood ratios of candidate markers), and reproducible high-throughput assays with quick turnaround times. Although some genetic markers show promise, research that focuses on gene expression or protein markers may ultimately be more useful. Moving from GWAS to transcriptomic assays, for example, may be more likely to reflect the actual nature of inflammatory pathways that are driving an individual patient's IBD. Blood biomarkers of response would be preferred over tissue-based assays due to ease and convenience of obtaining and processing samples. However, tissue-based assays may prove more informative since coming from site of inflammation. Future research should compare performance of blood and tissue biomarkers within the same patient cohorts. A major need in developing genomic markers of drug response is the incorporation of blood and tissue bio-samples into clinical trials of novel therapeutics. Clinical trial patients are best characterized with objective endpoints to assess response (endoscopy). In order to make best use of these cohorts, study designs should allow for exploratory analyses aimed at developing biomarkers of response. Ultimately, the best prediction tools will likely involve a panel of blood and/or tissue markers that also take into account clinical features associated with drug response. With current advances in genomic and molecular assays, we are in an era in which the development of predictive biomarkers of drug response and IBD precision medicine is now possible.

Summary Points

- IBD susceptibility genes have highlighted immune and molecular pathways that are critical in disease pathogenesis as well as current and potential drug targets.
- Genes associated with risk of IBD have demonstrated modest predictive capacity for anti-TNF response. Genetic markers that are related to mechanisms of anti-TNF efficacy, such as genes related to leukocyte apoptosis, also have shown promise as predictive biomarkers.
- Composite scores that incorporate multiple IBD risk alleles appear to perform better as predictors of drug response than individual genes.
- Tissue-based markers of anti-TNF drug response may ultimately be the most predictive. OSM, OSMR, and extracellular matrix production genes are some of the more currently intriguing biomarkers of non-response.
- Some research on non-anti-TNF biologics has investigated markers of response including blood cytokine levels, tissue immunohistochemistry, and microbiome composition.

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Part VI

Conclusion

29 Big Data Meets Real World! The Use of Clinical Informatics in Biomarker Research

Siddharth Singh

Abstract

Electronic health records (EHRs) are being increasingly utilized and form a unique source of extensive data gathered during routine clinical care. Through use of codified and free text concepts identified using clinical informatics tools such as natural language processing, disease phenotyping can be performed with a high degree of accuracy. At the same time, technologies such as genome sequencing, gene expression profiling, proteomic and metabolomic analyses, and patient-reported health information are generating large amounts of data from various populations, cell types, and disorders (big data). However, to make these data useful for promoting biomarker discovery, precision medicine, and clinical practice, it is imperative to harmonize and integrate these diverse data sources. In this article, we introduce important building blocks for personalized treatment, such as common data models, text mining and natural language processing, privacy-preserved record linkage, machine learning for predictive modeling, and health information exchange.

29.1 Introduction

Adoption of electronic health records (EHRs) has continued to increase, spurred by federal incentives and mandates. These electronic systems collect vast amounts of clinical data either as structured elements (vital parameters, laboratory data, etc.) or unstructured clinical notes and facilitate increasingly effective clinical decision support (CDS), defined by HealthIT.gov as systems or processes that "[provide] clinicians, staff, patients or other individuals with knowledge and person-specific information, intelligently filtered or presented at appropriate times, to enhance health and health care." These data, currently used primarily for clinical care and administrative purposes, hold tremendous potential for advancing biomarker discovery and providing precision medicine at point of care.

In parallel with the EHR revolution, there have been tremendous advancements in computational biology techniques with proliferation of standardized genetic platforms and sequencing technologies, explosion of multi-omics approaches, along with streamlined analytic

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pipelines, facilitating pooling of research data across populations. However, such efforts have relied on carefully curated cohorts with research teams manually identifying patients from clinical care by review of individual charts to identify eligible individuals, which requires significant personnel support and is resource intensive. Moving forward, utilizing the EHR to curate large disease-based cohorts in a short amount of time with modest resources, carefully performing automated detailed disease phenotyping utilizing text mining and natural language processing, and then integrating these diverse "big data" sources through privacy-preserved linkage, can promote effective and efficient discovery research, rapid translation, and integration and adoption at point of care. In this chapter, we discuss important concepts of clinical informatics required to facilitate such advancement. Figure 29.1 summarizes the approach to precision medicine using EHRs.

29.2 Common Data Models

An intrinsic limitation to any big data approach is the issue of data quality in terms of volume, variety, velocity, and veracity [1–3]. Hence, to make EHR data usable across formats and institutions, it is critical to develop a common data model with use of standard terminology. Each type of data has an associated terminology that enables the vocabulary to be operationalized within the context of the EHR. These terminology systems have unique data formatting, coding, domain coverages, and hierarchical relationships between a specific instantiation, such as amoxicillin capsule 250 mg, and a concept, such as penicillin. Table 29.1 shows the common EHR data sources relevant to clinical decision support. Precision medicine is developing a new vocabulary related to genetic conditions, which has yet to be standardized in the EHR. Genetic test results should

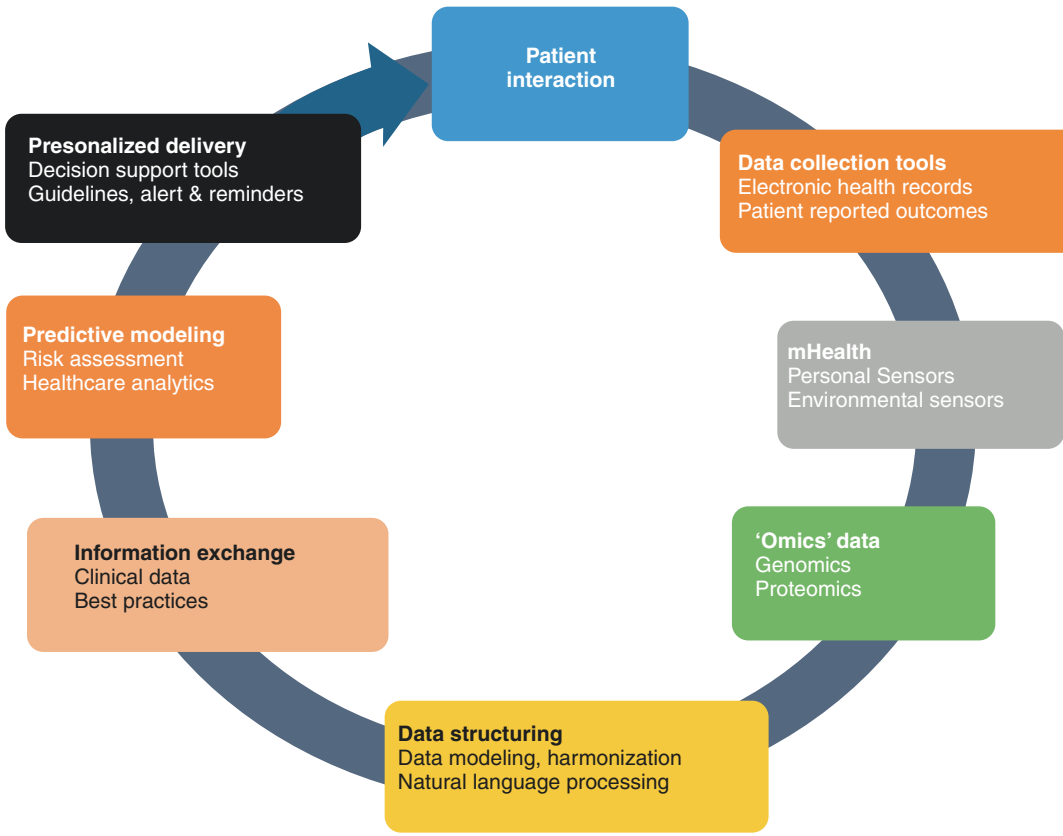

Fig. 29.1 Key tools for precision medicine using electronic health records

Table 29.1 Data sources for EHR relevant to drive clinical decision support

Type of information	Standardization	Opportunities	Challenges
Laboratory	LOINC, HGVS, HL7 FHIR value sets	Clinical laboratory tests have a mature standardization capabilities via LOINC LOINC and HL7 genomic groups have started developing standards for genetic tests that enable standardized discrete coding of some genetic test information	Not all clinical lab tests are encoded with LOINC (still in process in many institutions) Discussions on including genetic text in EHR in a structured way have only recently commenced Significant volumes of tests are performed at external laboratories with processes and results that lack standardization Laboratory orders are frequently matched in the computer to component results Genetic test results are not systematically incorporated into EMR in a searchable way. For example, they are non-discretely stored in the EMR as a scanned PDF document or image at the UCSD medical center
Medication	RxNorm, NDC	Clinical drug names have been standardized using these codes Dictionaries provide the opportunity to include manufacturer, dosing, and route information	Categorization is not clean as medications may have multiple indications both on and off label that skew groupings Combination drugs may not neatly fit into clinical groupings Deriving relevance related to effect over time, dosing intensity, or adherence is problematic
Diagnosis	ICD 9, ICD 10, SNOMED-CT	Most institutions adopt ICD system to support both active problem lists and encounter diagnoses Diagnosis names are interrelated, meaning that terms encoded with other one terminology such as SNOMED-CT can be converted to ICD through cross mapping established between the two systems	Coding is frequently completed by a clinician with time constraints that may not search through the extensive terms for the true best fit (undercoding, miscoding) ICD9 and 10 contain level of detail that may deviate from clinical relevance ICD9 is historic and ICD10 current (codes expire and newly develop) Not all codes are billable (irrelevant) Some diagnoses are not encoded (missing) SNOMED concepts are frequently not parsed into terms that support clinically specific workflows IMO updates can impact term groupings and insert clinically mismatched concepts
Radiology	RadLex, SNOMED-CT DICOM	Standards to capture the key findings and metadata about the radiologic studies exist	Radiology test-related metadata may not be formatted in a structured way using a standard like DICOM Radiology reports are in an unstructured narrative text format. Processing the text to tease out the key findings and mapping them to the standardized codes require additional efforts/resources that involves natural language processing (NLP)
Pathology	SNOMED-CT HL7 (anatomic pathology)	Standards to capture the key findings and metadata about the pathology test exist NAACCR is interested in adopting standard for cancer pathology reporting	Pathology reports are in an unstructured narrative text format or PDF. Processing the text to tease out the key findings and mapping them to the standardized codes require additional efforts/resources (NLP) Pathology frequently utilizes standardized nomenclature but does not record data in structured format

(continued)

Table 29.1 (continued)

Type of information	Standardization	Opportunities	Challenges
Clinical evidence and outcomes	OMOP CDM and all terminology systems listed above	EHR data stored in a clinical data warehouse serves a powerful knowledge resource OMOP CDM is recognized as a de facto standard and adopted by many institutions	There are types of data that are not sufficiently represented by the OMOP CDM such as patient-reported outcomes OMOP has not been universally adopted across organizations
Procedures	Terms to represent clinical procedures	Standardized terms that define common clinical procedures and their associated charges	Process for approving new procedural codes is onerous; as a result the library may incompletely represent activity detail Many procedural codes are fairly generic and do not incorporate the level of details that impact outcomes

follow relevant data standards, such as LOINC, HL7 Genomics, HGVS, etc., that contain information about test findings and potential risk; yet, this is a challenge since these standards are not adopted by all laboratories. The rapid evolution of tests makes this challenging for the field of genetics, posing challenges for discrete data retrieval of this information in the EHR. Precision medicine also relies on other types of data that were not traditionally recorded in EHRs, such as patient-reported outcomes (PROs), which are still early in standardization, and the reporting is highly variable according to race, ethnicity, and literacy.

29.3 Text Mining and Natural Language Processing

While several elements handled through common data models are based on structured or codified elements, free text or narratives still dominate in terms of clinically relevant information contained in EHRs. While free narrative is effective and convenient for medical record keeping, its unprocessed form is difficult to search, summarize, or analyze for secondary purposes such as research or quality improvement. Natural language processing (NLP) is any computer-based algorithm that handles, augments, and transforms natural language so that it can be represented for computation. Because a computer cannot comprehend meaning from a block of text, a series of operations must be defined to transform the data into usable information, which is the essence of NLP. In elegant use of this combination of codified data and NLP to develop an EMR-based cohort, Ananthakrishnan and colleagues created a cohort of 11,000 patients with IBD within two hospitals in Boston [3–5]. From among all patients with at least 1 billing code for Crohn's disease or ulcerative colitis, a chart review revealed a positive predictive value of only 60% with frequent misclassification. Extraction of codified data ascertaining disease complications as well as narrative free text data comprising number of mentions of individual disease names ("Crohn's disease") or disease-related terms in clinical notes ("abdominal pain," "diarrhea"), radiology reports ("ileal wall thickening"), endoscopy ("ileitis" "aphthous ulcer"), and pathology ("crypt abscess") allowed for development of a classification algorithm using machine learning that was able to achieve a positive predictive value of 97%. The addition of free text data to codified information not only improved the accuracy of identifying cases but also increased the number of patients who could be classified as having a disease. In addition, this approach also allowed identification of phenotypes of disease, such as primarily sclerosing cholangitis, which is limited by lack of specific diagnostic codes or high frequency of use of codes for competing diagnoses (e.g., cholelithiasis), determining status of disease activity in relapsing and remitting disorders, or identifying

response to treatment. Natural language processing software is increasingly sophisticated to be able to distinguish positive findings ("has diarrhea") from negative ones ("does not have diarrhea"), assign specific contexts for occurrence of phrases ("abdominal pain" from "joint pain"), separate personal from family history ("family history of colon cancer"), and search within specific components of the note (such as indication for procedures). Despite the inherent variability in structure and content of EHR data and differences in quality of provider documentation across institutions, disease-defining algorithms created at one institution are portable to other institutions using distinct EHRs and retain their accuracy, which is key for multi-institutional consortia, such as the Electronic Medical Records and Genomics (eMERGE) Network. With advances in the field of NLP, detailed phenotyping is feasible, allowing performance of large-scale, integrated genome-wide and phenome-wide studies to promote biomarker discovery and precision medicine.

29.4 Privacy-Preserved Record Linkage

In a research network, information from the same individual may be partitioned among several sites such as healthcare providers, sequencing facilities, insurance companies, research institutions, etc. There are mainly two types of patient data partitioning across institutions: (1) horizontal partitioning, where different institutions hold information on the same, and (2) vertical partitioning, where different institutions hold information on different attributes. The former one consists of records with the same features, for an overlapping or non-overlapping set of individuals. Feature values are the same in the case of true overlap, or they can differ when patients switch healthcare systems or receive complementary care in different health systems (e.g., patients cared for primarily at the Veterans Health Administration system but receiving specialty care in another system). In vertical partitioning there is information about different features for the same individual at different sites. In both situations, patient record linkage is an essential step to combine data in cross-institutional studies. For example, if the truly duplicated records across different institutions cannot be sufficiently removed, the estimation could become biased in the study with horizontally partitioned data. For the case of vertically partitioned data, the genome data of a particular group hosted in a sequencing facility can be significantly enriched by linking the data to EHRs. In addition to linking patient records across research networks, existing clinical data research networks can link their data to publicly available databases of vital statistics (such as the National Death Index), pharmaceutical databases, etc. allowing comprehensive and simultaneous capture of multiple exposures, health status, interventions, and outcomes. Existing record linkage methods can be categorized into two approaches: deterministic and probabilistic [6]. If there exists explicit identifiers (e.g., name, social security number, etc.) among different datasets, deterministic record linkage methods are used. Probabilistic linkage methods are more complex, as they assign different weights for different discriminative linkage variables to compute an overall score that indicates how likely it is that a record pair comes from the same patient. Furthermore, due to concerns of invasion of privacy, institutions and patients alike may be hesitant to share personal health information outside the health system. Hence, robust privacy-preserving record linkage tools are clearly needed before this rich environment is ripe for research use. Figure 29.2 depicts an example of a record linkage system for vertically partitioned data between a hospital and a biobank where DNA data are available.

29.4.1 Health Information Exchange

One of the limitations of EHR-based research is that data are contained in silos in health systems which do not interact adequately with each other. While patients move in and out of health systems, their data does not move and gets lost in translation. Not only does this disrupt clinical care, but

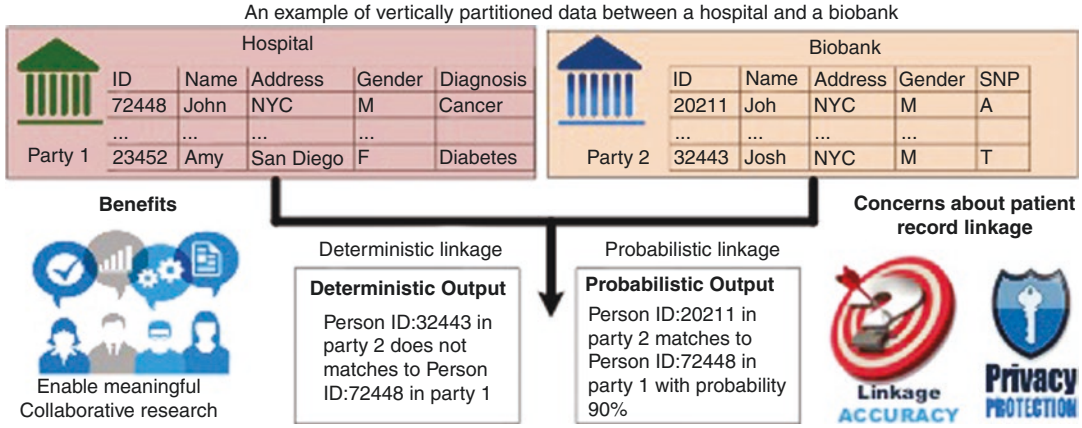

Fig. 29.2 Privacy-preserved record linkage approaches

it also impedes phenome-wide association studies and biomarker discovery due to misclassification of clinical data. However, through approaches of health information exchange (HIE), a special case of privacy-preserved record linkage, this barrier may be overcome [1]. The Health Information Technology for Economics and Clinical Health (HITECH) Act of 2009 was proposed to promote interoperable health information. HIE initiatives aim at realizing timely and appropriate level of access to the patient level of health information stored in the EHR by healthcare providers through a secure means to exchanging health data among healthcare organizations. Having complete information about disease progression and treatment data at the point of care helps healthcare providers make better treatment decisions and achieve better patient outcomes. Utilizing information collected from different healthcare systems is an important step toward this goal.

HIE covers three types of data exchange:

1. *Directed exchange* that occurs between healthcare providers to complete the planned healthcare services such as sending and receiving laboratory test orders and results, exchanging patient referral documents, etc.
2. *Query-based exchange* that occurs when a healthcare provider delivers unplanned services and requires accessing necessary health information about the patient, for example, when an emergency room physician needs to access patient's disease history, current medications, allergies, etc.
3. *Consumer-mediated exchange* that lets patients control their health information. In this model, patients grant access to their health information to healthcare providers.

However, establishing a sustainable HIE is not a trivial task; there are a number of technical and nontechnical barriers that need to be addressed first. For example, lack of business incentives, specifically concerns on losing patients to other hospitals by making their health data available anywhere, has long been recognized as a factor that makes some healthcare systems hesitant to embrace HIEs. Patients and providers sometimes opt out from HIEs due to privacy concerns. Other recognized challenges are poor data standardization, inefficient processes of sorting through overloaded unselective information of a patient, and difficulties in understanding the shared data in the absence of context when detailed clinical notes are withheld due to privacy concerns.

29.5 Statistical Approaches Including Machine Learning

With the vast amount of data being generated from diverse sources, novel and powerful analytic approaches are needed. Figure 29.3 summarizes

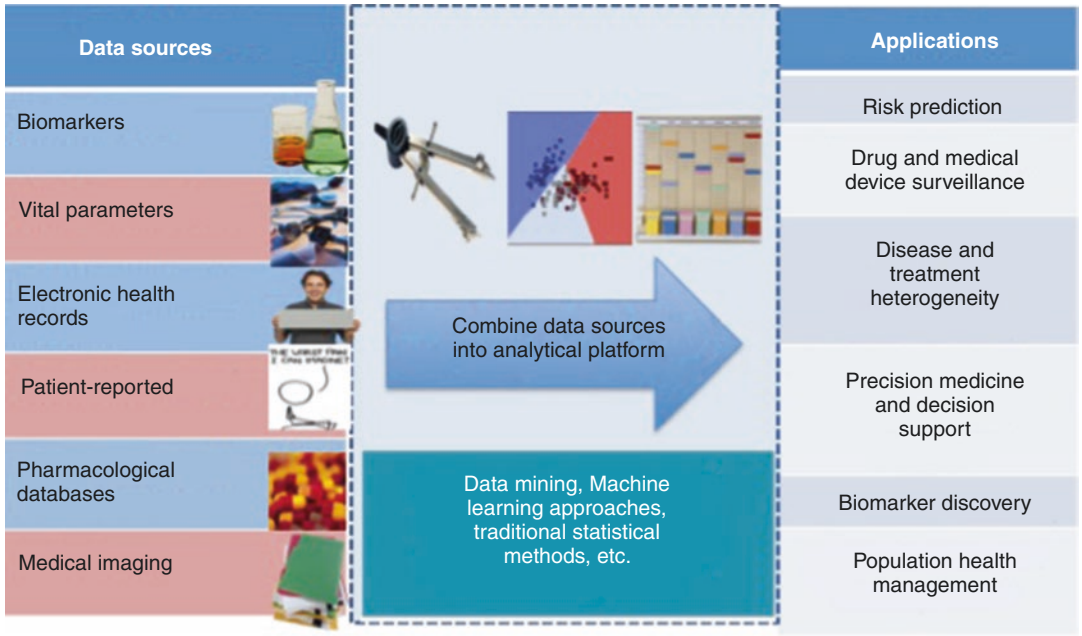

Fig. 29.3 Analytic approaches for big data

different approaches to analysis. Machine-learning methods consist of computational algorithms to relate all or some of a set of predictor variables to an outcome [7]. To estimate the model, they search, either stochastically (randomly) or deterministically, for the best fit. This searching process differs across the different algorithms. However, through this search, each algorithm attempts to balance two competing interests: bias and variance. In the machine-learning context, bias is the extent to which the fitted predictions correspond to the true values—i.e., how accurately does the model predict the "true" risk of death in the population? Variance is the sensitivity of the predictions to perturbations in the input data, i.e., how does sampling variability impact the predictions? Even though it is not possible to separately quantify a model's bias and variance, these two values are summarized together by loss functions. Many machine-learning methods can be grouped into different families based on their underlying structure. The two largest families are those that amend the traditional regression model (such as regularized methods, including common ridge regression and LASSO), tree-based methods (such as classification and regression trees), and others including artificial neural networks, nearest neighbors, support vector machines, etc.

In summary, marrying EHR-based clinical research approaches with advancements in computational biology is immensely promising for biomarker discovery and promoting precision medicine. One can readily envision this approach being applicable across a wide swath of diseases relevant to gastroenterology, including colorectal polyps, gastrointestinal cancers, celiac disease, eosinophilic esophagitis, microscopic colitis, Barrett's esophagus, and liver disease. All of these diseases have in common varying, and often poor, accuracies of existing administrative coding-based diagnoses but can be readily identified in the EHRs using data (e.g., serology, pathology, and endoscopy) that are a routine part of clinical care and that can be mined using clinical informatics tools. Linkage of such disease registries to biobanked genotyped samples, ensuring appropriate data protection and de-identification, can be enormously valuable to advance scientific discovery. This, however, is contingent on standardization of reporting methods and attributes and the ability to receive structured data from outside sources.

Summary Points

- To make EHR data usable across formats and institutions, it is critical to develop a common data model with use of standard terminology.
- Besides structured data, abstraction of free text narratives from electronic medical records, through text mining and natural language processing, is vital to detailed phenotyping.
- Due to the structure of the healthcare system, patient data is often horizontally and vertically partitioned across multiple institutions and organizations. Privacy-preserving record linkage is vital to making this data utilizable for biomarker discovery and scientific advancement.
- With this vast amount of data being generated from diverse sources, novel and powerful analytic approaches such as machine-learning methods are needed.

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30 Bringing It Altogether: A Systems Biology Approach to Biomarkers in Inflammatory Bowel Disease

Claudio Fiocchi

30.1 Introduction

The challenges posed by complex diseases such as chronic inflammatory, autoimmune, metabolic, and neoplastic disorders are many, ranging from correct clinical diagnosis to proper classification, precise and effective therapy, long-term monitoring, and prediction of ultimate outcome. This is certainly the case for the two main forms of inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC), despite the considerable progress witnessed in the last couple of decades. Facing the above challenges, it is not surprising that physicians and patients alike yearn for objective and highly specific tests that can provide clear answers to what is the correct diagnosis, what is the best treatment, and how the patient will fare in the long run. The quest for simple answers to complicated questions is intrinsic to human nature, but in biology and medicine, expectations are seldom matched by reality. Nevertheless, the medical community is still searching for ideal biomarkers [1], traditionally defined as "cellular, biochemical or molecular alterations that are measurable in human tissues, cells, or fluids" [2] and more recently including "biological characteristics measured as indicators of normal and pathogenic processes, or pharmacological responses to a therapeutic intervention" [3]. These are broad and ambitious definitions that reflect the desire of having tests that can inform about all aspects of a disease like IBD, as denoted in the preceding chapters of this book.

The search for biomarkers of IBD is being continuously pursued, and justifiably so, but it is becoming increasingly clear that we are still facing a huge number of obstacles. At the same time, it is also being appreciated that the current approach of looking for parameters or measuring differences associated with single components of complex conditions like CD or UC can only provide partial and incomplete answers. Considering both the number and the complexities laying ahead, a totally different approach to IBD biomarkers discovery seems imperative, one that takes into account both the intricacy of disease pathogenesis and the variability of the patient population. Novel conceptual approaches and high-throughput technologies are in existence that can not only analyze massive amount of data but also integrate clinical, biochemical, and molecular information. Thus, in this final chapter, we will examine key reasons of why current IBD biomarkers are suboptimal, consider new ways of thinking about biomarkers, and explore the potential to discover better and specific markers for diagnosis, treatment, and prediction for CD and UC.

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30.2 Biomarkers: Expectations and Reality

In 2015 the US Food and Drug Administration (FDA) and the National Institutes of Health (NIH) jointly sponsored a resource called BEST (Biomarkers, EndpointS, and other Tools) [4] and in 2016 formed a Biomarker Working Group that produced a document lastly updated in May of 2018. This document contains a list of desirable biomarkers and provides a detailed definition of each one of them, as summarized in Table 30.1. From this list, it is evident that the number and goals of the proposed biomarkers far exceed what physicians have at their disposal in practice, in both quantity and quality. This is so not only in the case of IBD but the majority of other chronic diseases that affect humanity, ranging from cancer to asthma, rheumatoid arthritis, psoriasis, liver, cardiovascular, kidney, and metabolic and neuropsychiatric diseases [5–13]. Therefore, the limitations existing in biomarker number and usefulness are universal, which implies that in most diseases, if not all, the approach taken to their discovery and validation is inadequate. A fundamental reason for this disappointing situation is that most biomarkers are based on simplistic differences between a given biological measurement in a diseased population and a healthy control population.

Let's take a look at the current situation in IBD. The most studied and utilized biomarkers in IBD are based on serologies of antimicrobial antibodies, such as pANCA, ASCA, OmpC, I2, Cbir1, ALCA, ACCA, AMCA, anti-L, anti-C, antigoblet cell, tropomyosin, and pancreatic antibody [14]. Based on their corresponding reports, their prevalence varies widely between 2% and 79% depending on the type of IBD, and many are also detected in low titers in healthy subjects, questioning the specificity and sensitivity of the antimicrobial antibodies [15]. Many other biomarkers have been described and used in IBD, including calprotectin, lactoferrin, S100A12, C-reactive protein, erythrocyte sedimentation rate, 6MP metabolites, and antibodies against biological agents [16]. Each one of them reflects vastly different events occurring in

Table 30.1 BEST biomarkers proposed by the FDA/NIH Working Group

Biomarker type	Biomarker definition
Susceptibility/risk biomarker	A biomarker that indicates the potential for developing a disease or medical condition in an individual who does not currently have clinically apparent disease or the medical condition
Predictive biomarker	A biomarker used to identify individuals who are more likely than similar individuals without the biomarker to experience a favorable or unfavorable effect from exposure to a medical product or an environmental agent
Diagnostic biomarker	A biomarker used to detect or confirm presence of a disease or condition of interest or to identify individuals with a subtype of the disease
Monitoring biomarker	A biomarker measured serially for assessing status of a disease or medical condition or for evidence of exposure to (or effect of) a medical product or an environmental agent
Pharmacodynamic/response biomarker	A biomarker used to show that a biological response has occurred in an individual who has been exposed to a medical product or an environmental agent
Safety biomarker	A biomarker measured before or after an exposure to a medical product or an environmental agent to indicate the likelihood, presence, or extent of toxicity as an adverse effect
Prognostic biomarker	A biomarker used to identify likelihood of a clinical event, disease recurrence, or progression in patients who have the disease or medical condition of interest
Reasonably likely surrogate endpoint	An endpoint supported by strong mechanistic and/or epidemiologic rationale such that an effect on the surrogate endpoint is expected to be correlated with an endpoint intended to assess clinical benefit in clinical trials but without sufficient clinical data to show that it is a validated surrogate endpoint

Adapted from FDA-NIH Biomarker Working Group [4]

CD and UC patients, and although some of them do have a reasonable clinical value such as calprotectin, they cannot inform about the overall status of the disease. On the other hand, they are expected to help in differentiating CD from UC and quiescent from active disease, informing about mucosal healing, and predicting disease course, relapse, and response to therapy. The reality is that existing blood, stool, urine, and breath biomarkers in IBD largely reflect only inflammation in one way or another [17], questioning whether the current biomarkers tell us something about individual events or the overall disease process. This state of affairs persists as novel putative biomarkers are added to an already long list, such as the recently described circulating and fecal microRNAs in CD and UC patients [18]. The overall result is a frustrating situation when it comes to therapeutic decisions that are still made mostly on clinical grounds rather than dependable biomarkers [19].

30.3 Existing Pitfalls in IBD Biomarker Discovery

One way or another, essentially all current IBD biomarkers reflect phenomena associated with the pathogenesis of CD or UC, primarily and independently of genetic, microbial, or immune derivation. Intrinsic to this approach is the assumption that IBD patients share common etiologies and mechanisms that lead to common manifestations recognized as the clinical phenotypes of IBD. This assumption is understandable, but it is wishful and naïve in light of the realities of IBD and the great diversity of individual patients. Well before present-day molecular techniques confirmed the variability and uniqueness of every patient, Sir William Osler stated the following while addressing the New Haven Medical Association on January 6, 1903: "Variability is the law of life, and as no two faces are the same, so no two bodies are alike, and no two individuals react alike and behave alike under the abnormal conditions which we know as disease" [20]. Today we have irrefutable proof of human biological diversity in both health and disease as demonstrated, just as one example, by the results of the Human Functional Genomics Project (HFGP) based on studies of 500 healthy adult subjects [21]. Measuring cytokines as an endpoint, this project clearly demonstrated how types and levels of cytokines vary depending on environmental factors (e.g., the season of the year), genetic background, and gut microbial composition [22]. The last report from the HFGP found that 11 different categories of host factors together explained up to 67% of interindividual variability in stimulated cytokine production in healthy subjects [23]. To further increase variability, each pathogenic component influences the others, like human genetics shaping the gut microbiota [24]; is intrinsically unstable, like the human microbiome [25, 26]; and is extremely complex, like the immune system [27]. When the immune system is activated, as characteristically occurs in CD and UC, one final outcome is the production of cytokines and antibodies. We forget that this fundamental process, i.e., protein production, is under genetic control; that protein levels represent molecular phenotypes with considerable variation between individuals, populations, and sexes [28]; and that proteins vastly differ in abundance among humans, even between twins [29]. Given these examples, how can we correctly interpret, for instance, the levels of antimicrobial antibodies in IBD? Perhaps two CD patients with a similar clinical phenotype have different capacities of producing ASCA, one high and one low: then the one with high ASCA is "confirmed" to have CD, while the other is questioned. Similarly, how can we correctly interpret the levels of pro-inflammatory cytokines in a colon involved by UC? Do high or low levels of interleukin (IL-)1β, IL-6, and tumor necrosis factor-α truly translate "higher and lower" degrees of inflammation?

30.4 Integrating Pathogenesis for IBD Biomarker Discovery

It is unlikely that major new strides will be made in the immediate future of the IBD therapeutic arena as long as we keep targeting one cytokine, one receptor, and one signaling molecule at a time. Although beneficial effects can be achieved, they will predictably be of partial effect and limited duration, and drug escalation or switch will eventually be needed. The reason is because complex diseases require complex therapies that take into account all components of the underlying pathogenic process [30], something

that we are nowhere near to do in IBD. Accepting these premises, one must assume that reliable IBD biomarkers must also reflect the integration of pathogenic components; in other words, we need more comprehensive biomarkers. Following this reasoning, the question emerges of how to integrate all components of CD or UC pathogenesis into a unified scheme. This can be done by drastically novel approaches based on integrated systems biology "omics" platforms. Systems biology can be defined as the computational modeling of complex biological systems, but a more precise and comprehensive definition is that found in the NIH website: "Systems biology is an approach in biomedical research to understanding the larger picture by putting its pieces together. It's in stark contrast to decades of reductionist biology, which involves taking the pieces apart" [31]. This is the essence of network medicine, as emphasized in a recent book: "Rather than trying to force disease pathogenesis into a reductionist model, network medicine embraces the complexity of multiple influences on disease and relies on many different types of networks. By developing technologies that comprehensively assess genetic variation, cellular metabolism, and protein function, network medicine is opening up new vistas for uncovering causes and identifying cures of disease" [32, 33]. Investigation of omics, i.e., the study of individual "omes" (a word conveying a sense of totality of any complex system), to discover better biomarkers is under way in a variety of conditions [34–36], including aging, psoriasis, and cardiovascular, liver, and neuropsychiatric diseases [37–41]. The power of integrating multi-omics has been just highlighted by the last report of the HFGP mentioned above [23], which reveals the potential of achieving disease risk biomarkers. In this study the authors show that that production of cytokines is predictable through the use of multiple baseline profiles and that interindividual variation in immune responses correlates with individuals' genetic risk of immune-mediated disease. In IBD the innovative concept of an "IBD interactome" has been recently proposed [42], and the value of integrating IBD-relevant omes is emerging in the literature [43–46], including for omics-based biomarker discovery in IBD [47]. The traditional and still current approach of searching for IBD biomarkers by studying individual pathogenic components can only yield isolated ome-related biomarkers (Fig. 30.1); in contrast, an approach based on the integration of pathogenic components, i.e., the IBD interactome, can deliver biomarkers that comprehensively reflect the whole disease process (Fig. 30.1).

Patient communities are becoming familiar with the potential of "precision" or "personalized" medicine, defined by the NIH as "an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person" [48]. Hopefully, the scientific and clinical communities of IBD investigators will welcome this trend and start using the powerful and sophisticated tools of omics-based systems biology to develop entirely new IBD biomarkers that faithfully reflect all aspects of the disease.

Summary Points

- Biomarkers are tools to help in the diagnosis, classification, therapy, and outcome prediction of complex diseases, as in the case of IBD.
- Traditional biomarkers' discovery is based on the separate evaluation of the molecular, cellular, clinical, pathological, and other components of the disease process.
- Numerous IBD biomarkers have been developed, tested, and used in clinical practice, but all fall short in regard to sensitivity and specificity.
- The main reasons for the inadequacy of current IBD biomarkers are the extreme complexity of the disease process, the intrinsic variability of the human population, and the highly individualized patient response to disease.
- To obtain reliable IBD biomarkers it is necessary to adopt bioinformatics-based methodologies that allow to integrate all pathogenic components into a comprehensive and unified process, i.e., the *IBD interactome*, whose analysis can deliver biomarkers reflecting the disease as a whole.

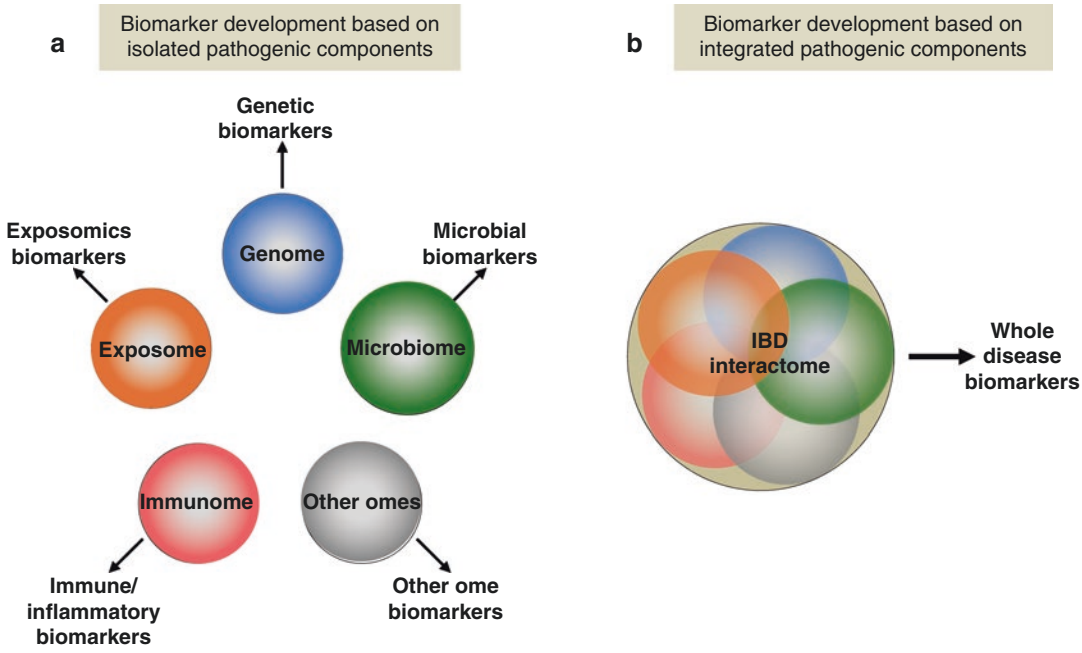

Fig. 30.1 Present and future approaches to biomarkers discovery in IBD. (**a**) The traditional and still current approach to identifying IBD biomarkers is to investigate each pathogenic component in isolation, which can only deliver biomarkers that reflect the status of individual genetic, microbial, immune, and other factors. (**b**) In contrast, an approach based on a fully integrated disease process where all pathogenic components and their interactions are taken into account, i.e., the *IBD interactome*, can deliver comprehensive biomarkers that reflect the IBD process as a whole

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