DiBiCol® – Development of a Multigene Diagnostic Test for the Differentiation of Inflammatory Bowel Disease Subtypes

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Abstract

Accurate diagnosis of inflammatory bowel disease (IBD) and differentiation between its two major subtypes, Crohn's disease (CD) and ulcerative colitis (UC), are crucial for planning optimum treatment strategies. However, as these related diseases share many pathological, histological and serological features, their differential diagnosis can be difficult in practice. We have been able to identify seven biomarkers which, following further evaluation, proved to be very effective in discriminating UC from CD and excluding irritable bowel syndrome. These biomarkers and a specially developed algorithm were incorporated into a quantitative polymerase chain reaction (qPCR)-based diagnostic test launched on the Swedish market in 2009 under the tradename DiBiCol® (InDex Pharmaceuticals AB, Stockholm). DiBiCol allows to quickly and reliably determine the correct IBD subtype, thereby ensuring a more appropriate treatment of patients.

Keywords

DiBiCol®, InDex Pharmaceuticals AB, inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, IBD unclassified, indeterminate colitis, irritable bowel syndrome, colonoscopy, quantitative polymerase chain reaction diagnostics

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Inflammatory bowel disease (IBD) is a chronic, relapsing, inflammatory disorder of the gastrointestinal (GI) tract that includes two main entities, Crohn's disease (CD) and ulcerative colitis (UC). CD is typically a patchy disease that can affect the GI tract anywhere from the mouth to the anus. In UC, the disease extends proximally from the anal verge to involve all or part of the colon. UC and CD are characterised by episodes of remission and exacerbations causing significant GI symptoms, including diarrhoea, abdominal pain, bleeding, anaemia and weight loss. The incidence of IBD in northern Europe is about 20 per 100,000 annually.[1,2] Although both diseases have a trivial negative impact on mortality, they have a substantial negative impact on the quality of life of affected individuals.

Diagnostic Challenges

There is a general consensus that IBD is the result of the combined effects of four factors: environmental influences, genetic variations, intestinal microbiota alterations, and disturbances in the innate and adaptive immune responses. A combination of all these factors is probably necessary for the disease to be clinically expressed. However, it seems that each individual patient has a different combination of factors leading to the disease, explaining why each patient displays their own clinical picture and response to therapy.[3]

In both CD and UC, the immunological balance at the intestinal barrier is severely impaired and shifted towards the pro-inflammatory side, with the expression of cytokines causing chronic mucosal inflammation. While CD is associated with an inflammatory process of T-helper 1 (Th1) and Th17 immune response with increased secretion of interleukin (IL)-12, tumour necrosis factor alpha (TNF-α), interferon gamma (IFN-γ) and IL-17, UC is associated with an atypical Th2 immune response with increased secretion of IL-5 and IL-13 leading to the cytolysis of epithelial cells.[4–6] Despite these differences, CD and UC can be phenotypically quite similar, as both diseases share many pathological, histological and serological features, which makes the differential diagnosis a challenging process.

So far, there are no standard biomarkers for IBD and the diagnosis is typically based on clinical symptoms, patient history and endoscopic and histopathological findings. However, the full structural changes detectable by endoscopy or histopathology often develop over a substantial period of time. The diagnosis may therefore be delayed by six to 12 months.[7] Overall, the diagnostic accuracy of pathologists in determining the IBD subtype through multiple biopsies is 60–74 %,[8] with uncertainty occurring not only in patients seen at the earlier stages of disease, but also in patients presenting with fulminant colitis and in those having already received medical treatment, as various treatments can change the typical disease pattern.[9]

Despite recent advances in medical therapy, surgery is required in 30–40 % of patients with UC and in about 25 % of patients with colonic CD (up to 80 % if the small intestine is involved). For patients with UC, surgery may be curative, whereas a recurrence of CD following surgery is common. A reliable differential diagnosis between UC and CD is particularly important in patients in whom a colectomy and

ileal pouch-anal anastomosis is considered, as this type of restorative surgery is generally not appropriate for CD.

In 10–30 % of patients presenting with IBD, no firm diagnosis can be made after endoscopic and histological evaluation, and a temporary diagnosis of 'IBD unclassified' (IBDU) is given.[8,10,11] (This range of 10–30% is an estimation drawn from various publications, observations and personal communications.) Clinical practice has taught us that a vast proportion (up to 80 %) of these patients are eventually reclassified as having either CD or UC and that up to 20 % are not actually affected by IBD.[12] However, reassessment of diagnosis may take up to 10 years, sometimes even longer. Complicating the issue further, a small subset of patients exists in which no firm diagnosis can be made, even following resection. These cases are classified as indeterminate colitis (IC) or 'colitis of uncertain type and etiology',[9] a condition which some believe may represent a separate clinical entity. If this proves to be the case, the need for a reliable test to provide a more refined diagnosis of IBD is even more evident.

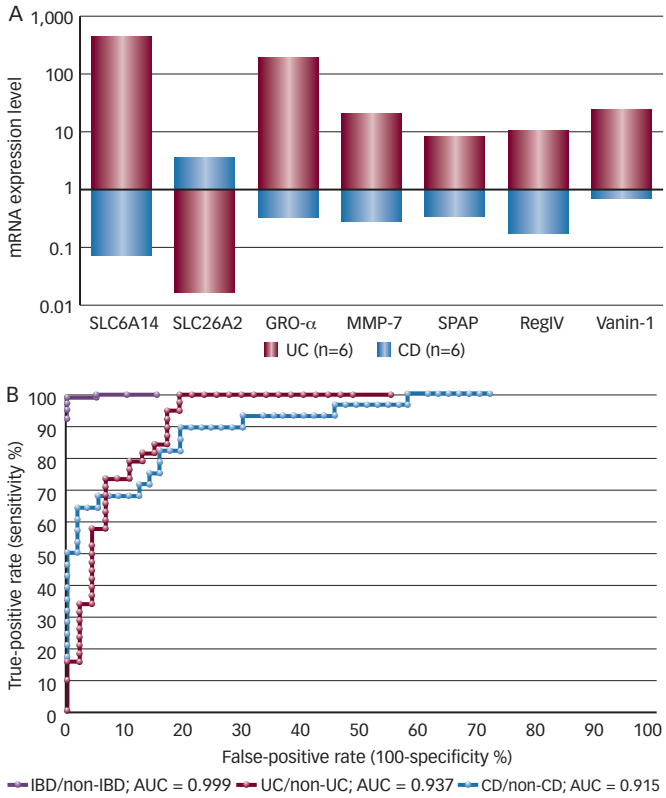
Gene Expression and Inflammatory Bowel Disease Classification

Biomarkers are measureable substances found in body fluids, stool or other bodily secretions and tissue samples, and can be used as tools for disease diagnosis and/or prognosis. Using IBD biomarkers can be cheaper, less laborious, less invasive and more objective than using an endoscopy- and/or biopsy-based approach.[13–15] However, as none of the current commercially available biomarker tests and/or assays are able to differentiate convincingly enough between CD and UC, new IBD biomarkers and more comprehensive bioinformatic algorithms with multiple biomarkers are urgently needed.[16–19]

In the late 1990s, when complementary DNA (cDNA)-based and oligonucleotide-based microarrays had become sufficiently far developed to enable the measurement of messenger RNA (mRNA) expression levels of hundreds of genes simultaneously, this technology became widely used to address a whole variety of diseases including IBD. The objectives were relatively straightforward: to find genes involved in the pathogenesis of IBD that could also serve as potential biomarkers for the differential diagnosis of CD and UC. Obviously a microarray-based approach offered the possibility of a high-throughput, but the use of such technology came with a price, insofar as microarrays query a large number of genes, many of which are ultimately not relevant to the specific disease process. This inevitably makes the rational interpretation of the huge amount of data generated a challenging task.

Perhaps a more manageable approach is offered by methods which, through a form of molecular subtraction termed 'subtractive suppression hybridisation',[20] enrich genes on the basis of their abundance and thereby generate cDNA libraries that contain a high proportion of dysregulated genes specific to the disease in question. This approach was successfully applied to cases of IBD, whereby resected biopsy specimens from the inflamed colon of patients were enriched for dysregulated genes. The enriched expression profiles were then screened against cases of CD and UC. This led to the identification of seven biomarkers which, through further evaluation, proved to be very effective in discriminating between CD and UC, and, equally, IBD from irritable bowel syndrome (IBS).[21] These biomarkers were identified as being solute carrier (SLC) 6A14, SLC26A2, small protein associated with

Figure 1: Opposite Gene Expression Patterns in Ulcerative Colitis and Crohn's Disease (A), which Formed the Basis of an Algorithm that Allows to Differentiate between Ulcerative Colitis and Crohn's Disease with a High Sensitivity and Specificity (B)



In Figure 1A*, note that the mRNA levels have been normalised to the levels in non-inflamed biopsies; material from six UC patients and six CD patients were used. AUC = area under the curve; CD = Crohn's disease; GRO-α = growth-related oncogene alpha; IBD = inflammatory bowel disease; MMP-7 = matrix metalloproteinase 7; mRNA = messenger RNA; RegIV = regenerating protein IV; SLC6A14 = solute carrier 6A14; SLC26A2 = solute carrier 26A2; SPAP = small protein associated with PDZ domain-containing protein-1; UC = ulcerative colitis.*

PDZ domain-containing protein-1 (SPAP), regenerating protein IV (RegIV), Vanin-1, matrix metalloproteinase 7 (MMP-7) and growth-related oncogene alpha (GRO-α). These genes are reported to be involved in biological processes such as transmembrane transport, inflammation, tissue repair and extracellular matrix turnover as well as carcinogenesis. By analysing their expression profiles in different patient biopsy samples by quantitative polymerase chain reaction (PCR), it was demonstrated that UC and CD have distinct expression profiles (see *Figure 1A*).

By developing a special algorithm based on the quantitative and qualitative differences between the biomarkers, it was possible to predict with a high sensitivity and specificity the probability of a patient having one of the diseases: UC, CD, or indeed neither of them, thereby also excluding GI disorders such as IBS (see *Figure 1B*). More importantly, the multigene analysis of IBDU cases enabled a diagnosis in the absence of any specific histopathological features.

Launch of DiBiCol® on the Market

After a number of test studies in which colonic biopsies from more than 300 patients presenting IBD-related symptoms were submitted to the multigene analysis, it became evident that this molecular method had a strong potential to be launched on the market as a diagnostic test. Such a diagnostic test was launched on the Swedish

Figure 2: Increase in Sales of DiBiCol® since its Launch

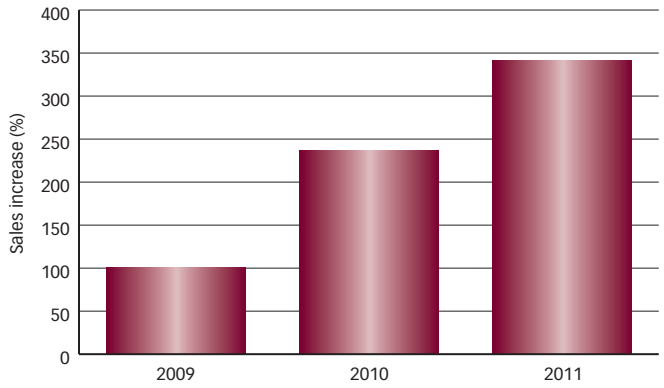
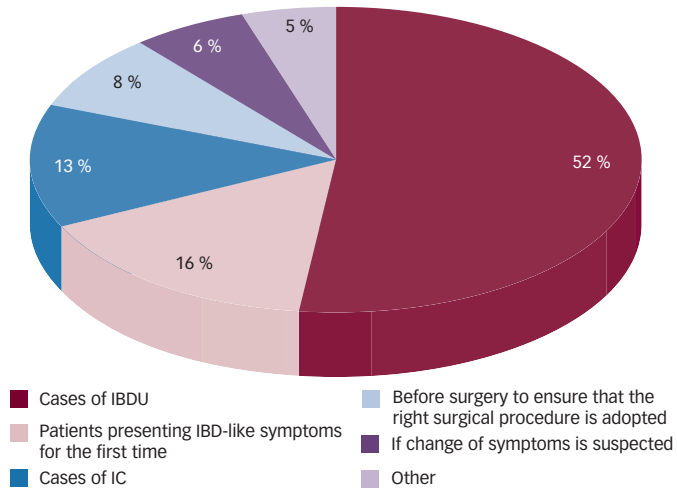


Figure 3: Most Physicians use DiBiCol® to Classify Patients with Inflammatory Bowel Disorder Unclassified and Indeterminate Colitis as well as Naive Patients

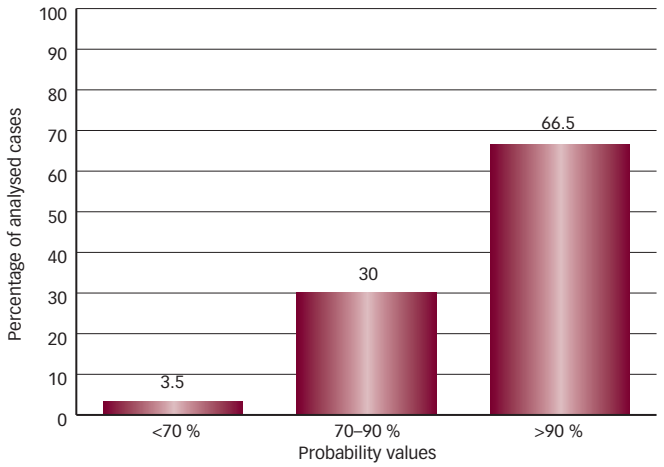


IBD = inflammatory bowel disease; IBDU = inflammatory bowel disease unclassified; IC = indeterminate colitis. Data compiled from over 30 clinics in Sweden since the market introduction of DiBiCol®.

market in 2009 under the tradename DiBiCol® (InDex Pharmaceuticals AB, Stockholm). During the time it has been commercially available, its sales have considerably increased (see *Figure 2*), so that several hundreds of patient samples were analysed in 2011.

In practice, the procedure can be described as follows: DiBiCol kits containing biopsy sampling tubes with RNA stabilising solution, outer transport tubes and instructions for use are sent to the requesting physician working in gastroenterology units throughout Sweden. During colonoscopy, the physician excises a pinch-biopsy from the inflamed mucosal surface of the colon and places it directly into the stabilising solution. Next, the tubes containing the biopsies are sent back to InDex Pharmaceuticals AB. As biopsies are stable for up to one week at room temperature, there is no need for urgent or specialised means of transport. Upon arrival, the biopsies are processed, RNA is extracted, cDNA is synthesised and finally quantitative polymerase chain reaction (qPCR) is performed. An algorithm processes the raw data and generates a probability value indicating whether the patient is afflicted either with CD, or UC, or neither of these two conditions. This information is then communicated back to the physician to help provide a safe, fast and reliable diagnosis. The whole procedure, starting from the arrival of

Figure 4: Two-thirds of Cases Analysed with DiBiCol® Received a Probability Value of at least 90 % for either Crohn's Disease, or Ulcerative Colitis, or Non-inflammatory Bowel Disorder



CD = Crohn's disease; IBD = inflammatory bowel disease; UC = ulcerative colitis.

the biopsies at the laboratory to the moment when the results are sent back to the physician, can take as little as eight hours.

Over the three years that have elapsed since the introduction of DiBiCol on the market, the vast majority of requests for the test were made by physicians for patients originally presenting with IBDU or IC (52 and 13 %, respectively; see *Figure 3*), suggesting that DiBiCol was useful in helping physicians giving a diagnosis in such patients. Other reasons to use the test were to diagnose naive patients (i.e., patients who do not have a medical history of IBD-related disease and thus are new 'recruits' to the clinics) as well as patients eligible for surgery. In the latter case, the outcome of DiBiCol is used by the treating physician and/or surgeon to ensure the most appropriate surgical procedure is used depending on whether the patient has been diagnosed with CD or with UC.

The DiBiCol results show that, in two-thirds of the analysed cases, a probability greater than 90 % could be given for any of the three possible outcomes of the algorithm (CD, UC or non-IBD), 30 % of cases fell in the range of a 70–90 % probability, and only 3.5 % of cases received a probability value of less than 70 % (see *Figure 4*). It should be remembered that these probability values indicate to what extent a patient is likely to suffer from CD, UC or neither of these two conditions. Assuming that DiBiCol has been correctly deemed to give an accurate prediction in 90 % of cases,[21] then the fact that two-thirds of the patients are receiving a diagnosis with a probability greater than 90 % is impressive.

Conclusion

Subtractive suppression hybridisation is a useful way of isolating dysregulated genes in specific patient material. mRNA-based biomarkers have been used to develop DiBiCol, a unique qPCR-based diagnostic test that enables to differentiate between CD and UC, two closely related, severe conditions of the GI tract. It is our conviction that the ability to correctly determine the IBD subtype using the DiBiCol diagnostic test allows the implementation of more appropriate treatment regimens and, as a result, saves time and financial resources for the clinic and improves quality of life for patients. ■

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