# A randomised controlled trial of Absorbatox™ C35 in Irritable Bowel Syndrome: A pilot study

JR KLOPPERS

# A randomised controlled trial of Absorbatox™ C35 in Irritable Bowel Syndrome: A pilot study

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*Dedicated to Gerard Rial Kloppers, my hero, mentor and beloved father.*

*SOLI DEO GLORIA!*

# ABSTRACT

**Background:** Irritable Bowel Syndrome (IBS) is one of the most common gastrointestinal disorders managed by primary care physicians and gastroenterologists. It is a recurrent and chronic disorder characterised by abdominal discomfort, bloating and altered defecation patterns. IBS casts significant burdens on patients' quality of life and has an enormous economic impact through direct costs in health care utilization and indirect costs through absenteeism from work. Many IBS sufferers have resorted to complimentary and alternative medicine (CAM) mainly because of the ineffective cure rate with conventional western treatment. It is estimated that 40% of IBS sufferers seek symptomatic relief from CAM. A lack of understanding of the pathophysiology mechanism has been labelled as the main cause for poor IBS management. Nevertheless, several hypotheses have been proposed, including abnormal motility, visceral hypersensitivity, inflammation and infection, neurotransmitter imbalance, and psychological factors. In addition, IBS patients are considered to be visceral hypersensitive to luminal factors and intestinal gas.

**Aim:** To assess the efficacy of Absorbatox™ C35, a natural, non-toxic zeolite, with enhanced ion exchange capacity, as well as water and gas adsorbing properties, in the treatment of IBS in a 6-week randomised, double-blind, placebo-controlled trial with parallel group assignment.

**Methods:** Ethical approval for the study was received (Ethical approval number NWU-0001-008-S5) and the necessary consent from trial candidates were received as per international guidelines. Sixty-seven (67) IBS candidates were recruited for participation. Only thirty-three (33) patients met the trial entry criteria. IBS candidates were diagnosed using the Rome III diagnostic criteria. Organic diseases were first excluded by a full blood count and a physical examination. Any alarming symptoms, that could be indicative of diseases other than IBS, were also excluded during the preliminary examination. A 2-week run-in phase evaluated baseline symptoms. Patients were randomly assigned using a simple computer generated random codes system. Patients received 750 mg Absorbatox™ C35 three times daily or Placebo for 4 consecutive weeks. Symptoms were evaluated using validated questionnaires. The primary outcome was assessed using a global symptom endpoint, "adequate relief" questionnaire. Patients were characterised as *overall* responders if they reported "adequate relief" in 50% of treatment weeks. Secondary outcomes included a 50-point reduction in total severity score according to the IBS Severity Scoring System (IBS-SSS). The IBS-SSS was used to assess separate symptom ratings, such as abdominal pain, bloating, "bowel habit satisfaction" and

disease "interference with life in general". Stool parameters, including consistency, frequency and urgency were also assessed. Statistical analysis was primarily based on intention-to-treat analysis. Secondary outcomes were analysed through descriptive statistics. Statistical significance level was pre-set at 0.05, which means that whenever $p < 0.05$, the null-hypothesis was rejected.

**Results:** Seventeen (17) patients received Absorbatox™ C35 and sixteen (16) received Placebo. Two patients from the Absorbatox™ C35 group did not return after randomisation, hence only 31 patients were included in the intention to treat analysis. A total of twenty-nine (29) patients completed the entire study. A dropout rate of 12.12% (4/33) was encountered. At the end of treatment (12/15) 80% and 50% (8/16) of patients were classified as *overall* responders in the Absorbatox™ C35 and Placebo groups respectively ($p = 0.085$). After three and four weeks of treatment the number of *weekly* responders was significantly higher in the Absorbatox™ C35 group compared to the Placebo group ($p = 0.02$ and $p = 0.016$ for week 3 and 4, respectively). Moreover, both Absorbatox™ C35 and the Placebo groups were associated with significant decreases in the total severity score ($p < 0.001$ and $p = 0.005$, respectively). Likewise, both groups were associated with significant decreases in clinical parameters like pain, distension, bowel habit satisfaction and disease interference with life in general. No significant differences were observed between the Absorbatox™ C35 and Placebo groups in terms of total severity score and separate symptoms ratings. However, after 20 days of treatment the severity of distension was significantly lower in the Absorbatox™ C35 group compared to Placebo ($p = 0.024$). This effect was not sustainable, as the subsequent assessment (after 30 days of treatment) revealed no statistical significance between the two groups ($p = 0.553$). Absorbatox™ C35 was associated with a higher incidence of smooth stool ($p = 0.049$), but no significant difference were observed between the treatment groups in terms of stool frequency and urgency. Adverse events were of similar nature in both groups ($p = 0.259$).

**Conclusions:** Although the placebo effect was largely present during the trial, Absorbatox™ C35 showed a trend towards better improvement in several endpoint measurements. The possible implications for future trials on Absorbatox™ C35 were summarised. A larger trial is recommended with adequate statistical power, which is to be conducted over an extended period of time, to obtain inter-subjectivity on the efficiency of Absorbatox™ C35 in IBS treatment. It was statistically estimated, that for the repetition of these findings under similar conditions, with an 80% and 50% response rate in Absorbatox™ C35 and Placebo respectively, 45 IBS patients would be needed in each treatment group in order to achieve statistical significance.

***Keywords:*** *Irritable Bowel Syndrome, Absorbatox™ C35, zeolite, randomised controlled trial, efficacy, placebo.*

# OPSOMMING

**Agtergrond:** Prikkelbare dermsindroom (PDS) is een van die algemeenste gastroïntestinale afwykings wat deur primêre sorgdokters en gastroënteroloë behandel word. Dit is 'n herhalende en chroniese afwyking wat deur abdominale ongemak, opgeblasenheid en veranderde defekasiepatrone gekarakteriseer word. PDS veroorsaak dat beduidende laste op pasiënte se lewenskwaliteit geplaas word en het 'n geweldige groot ekonomiese impak op direkte kostes in gesondheidsorgaanwending en indirekte kostes in die afwesigheid van werk. Baie PDS-lyers het hulle na komplimentêre en alternatiewe medikasie (KAM) gewend, hoofsaaklik weens die oneffektiewe genesingspotensiaal van die konvensionele Westerse behandeling. Daar word geskat dat 40% van alle PDS-lyers simptomatiese verligitng deur KAM soek. ' n Gebrek aan begrip vir die pato-fisiologiemeganisme word gesien as die hoofoorsaak vir die swak behandelingsuitkomste van PDS. Ten spyte daarvan, is verskeie hipoteses al voorgelê, wat abnormale motiliteit, ingewandshipersensitiwiteit, inflammasie en infeksie, neuro-oordraerwanbalans, en psigologiese faktore insluit. Daar is al voorheen aangemeld dat PDS pasiënte ingewandshipersensitief is vir luminale faktore en intestinale gas.

**Doel:** Om die effektiwitiet van Absorbatox™ C35, 'n natuurlike, nie-giftige zeoliet, met 'n potensiërende ioonruilingskapasiteit-, asook wateradsorpsie- en gasadsorpsie-eienskappe te ondersoek in die behandeling van PDS in 'n 6-weeklange ewekansige, dubbelblinde, plasebogekontroleerde proefneming met 'n parallelle groep indeling.

**Metodes:** Etiese goedkeuring is vir die studie verkry (Etiese goedkeuringsnommer NWU-0001-008-S5) en die benodigde toestemming, volgens internasionale riglyne, is van die proefnemingskandidate ontvang. Sewe-en-sestig (67) PDS-kandidate is vir deelname gewerf. Slegs drie-en-dertig (33) pasiënte het aan die insluitingskriteria voldoen. PDS-kandidate is gediagnoseer aan die hand van die Rome III diagnostiese kriteria. Organiese siektes is aanvanklik deur 'n volbloedtelling en 'n fisiese ondersoek uitgesluit. Enige waarskuwingsimptome wat op ander siektes as PDS kon wys, is deur die aanvanklike ondersoek uitgeskakel. 'n 2-week ingangsfase het die basissimptome geëvalueer. Pasiënte is ewekansig volgens 'n eenvoudige rekenaargegenereerde ewekansige kodesisteem toegedeel. Pasiënte het 750 mg Absorbatox™ C35 of Plasebo drie maal per dag vir 4 opeenvolgende weke ontvang. Simptome is aan die hand van gevalideerde vraelyste geëvalueer. Die primêre uitkoms is geassesseer deur van 'n globale simptoomdoel, "genoegsame verligting"-vraelys gebruik te maak. Pasiënte is as *algehele* respondente gekarakteriseer as hulle "genoegsame

verligting" in 50% van die behandelingsweke gerapporteer het. Sekondêre uitkomste het 'n 50 punt vermindering in die totale felheidstelling op die PDS-Felheidstellingsisteem ('IBS-SSS') ingesluit. Die 'IBS-SSS' is gebruik om afsonderlike simptoomskattings, soos abdominale pyn, opgeblasenheid, "ontlastingsatisfaksie" en die siekte se "invloed op die lewe oor die algemeen" te assesseer. Stoelgangparameters, wat konsistensie, gereeldheid en dringendheid insluit is ook geassesseer. Die statistiese analise is hoofsaaklik op 'n intensie-om-te-behandel-analise gebaseer. Sekondêre uitkomste is aan die hand van deskriptiese statistieke geanaliseer. Die betekenisdraende statistiese vlak is vooraf vasgestel op 0.05, wat beteken dat wanneer $p < 0.05$, word die nulhipotese verwerp.

**Resultate:** Sewentien (17) pasiënte het Absorbatox™ C35 en sestien (16) het Plasebo ontvang. Twee pasiënte uit die Absorbatox™ C35 groep het nie na die ewekansige verdeling teruggekeer nie, dus is slegs 31 pasiënte in die intensie-om-te-behandel-analise ingesluit. Nege-en-twintig (29) pasiënte het die totale studie voltooi. ' n Uitvalkoers van 12.12% (4/33) is behaal. Aan die einde van die behandeling is (12/15) 80% en 50% (8/16) pasiënte in onderskeidelik die Absorbatox™ C35- en Plasebo-groepe ($p = 0.085$) as *algehele* respondeerders geklassifiseer. Na drie en vier weke van behandeling was die getal *weeklikse* respondente aansienlik hoër in die Absorbatox™ C35-groep as in die Plasebo-groep ($p = 0.02$ en $p = 0.016$ vir weke 3 en 4, ondskeidelik). Verder het beide die Absorbatox™ C35- en die Plasebo-groepe aansienlike afnames in die totale felheidstelling ($p < 0.001$ en $p = 0.005$, onderskeidelik) getoon. Soortgelyk het beide groepe aansienlike afnames in kliniese parameters, soos pyn, opgeblasenheid, ontlastingsatisfaksie en die inmenging van die siekte met die lewe oor die algemeen, getoon. Geen beduidende verskille is tussen die Absorbatox™ C35- en Plasebo-groepe in terme van totale felheidstelling en afsonderlike simptoomkoerse waargeneem nie. Tog was die felheid van opgeblasenheid na 20 dae van behandeling aansienlik laer in die Absorbatox™ C35-groep as in die Plasebo-groep ($p = 0.024$). Hierdie effek was egter nie volhoubaar nie, aangesien die volgende assessering (na 30 dae van behandeling) geen beduidende statistiese onderskeid tussen die twee groepe ($p = 0.553$) getoon het nie. Absorbatox™ C35 is met 'n hoër voorkoms van gladde stoelgang ($p = 0.049$) geassosieer, maar geen beduidende verskil is tussen die twee behandelingsgroepe in terme van stoelganggereeldheid en -dringendheid opgemerk nie. Nadelige effekte was ook byna dieselfde in beide groepe ($p = 0.259$).

**Gevolgtrekkings:** Alhoewel die placebo-effek deur die behandeling duidelik aanwesig was, het Absorbatox™ C35 'n neiging tot groter verbetering in verskeie doelmaatstawwe getoon. Die moontlikhede vir toekomstige toetsing op Absorbatox™ C35 is opgesom. 'n Groter toetsgroep word aanbeveel vir genoegsame statistiese bewyse, wat oor 'n verlengde tydperk uitgevoer moet word, om 'n inter-subjektiewe beeld van die effektiwiteit van Absorbatox™ C35-

behandeling in PDS-behandeling te verkry. Daar is 'n statistiese skatting gedoen, dat daar vir die herhaling van die bevindinge onder soortgelyke voorwaardes, met 'n 80% en 50% respons-koers in Absorbatox™ C35 en Plasebo onderskeidelik, 45 PDS-pasiënte in elke behandelingsgroep benodig word om statisties beduidende resultate te verkry.

***Sleutelwoorde:*** *Prikkelbare dermsindroom, Absorbatox™ C35, zeoliet, ewekansige beheerde toetsing, effektiwiteit, plasebo.*

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***SOLI DEO GLORIA:*** **Glory to God alone**

**Throughout my life I have found comfort in the grace of the Lord, freedom in his son Jesus Christ and guidance from the Holy Spirit**

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# INDEX

# LIST OF FIGURES

# LIST OF TABLES

# LIST OF ABBREVIATIONS

| | |
|---|---|
| 5HT: | serotonin |
| ß: | Beta |
| α: | Alfa |
| K: | Kappa |
| μ: | Mu |
| б: | Gamma |
| $: | US Dollar |
| £: | British pound |
| €: | Euro (European currency) |
| ABTX: | Absorbatox™ C35 |
| AE: | Adverse event |
| AGA: | American Gastroenterological Association |
| BM: | Bowel movement |
| BM = 0: | No bowel movement |
| BM > 3: | More than 3 bowel movements |
| BMI: | Body mass index |
| CAM: | complimentary and alternative medicine |
| $CH_4$: | Methane |
| $CH_3SH$: | methanethiol |
| $CO_2$: | Carbon dioxide |
| DM: | Deutcsh mark |

| | |
|---|---|
| DM1: | Diabetes Mellitus Type 1 |
| DM2: | Diabetes Mellitus 2 |
| ESR: | Erythrocyte sedimentation rate |
| ESRD: | End stage renal diseases |
| FBC: | Full blood count |
| FDA: | Food and Drug Administration (USA) |
| FGID: | Functional gastrointestinal disorder |
| GORD: | Gastro-oesophageal reflux disease |
| $H_2$: | Hydrogen |
| HRQoL: | Health related quality of Life |
| IBS: | Irritable Bowel Syndrome |
| IBS-A: | IBS with alternating diarrhoea and constipation |
| IBS-C: | IBS with predominant constipation |
| IBS-D: | IBS with predominant diarrhoea |
| IBS-M: | Mixed IBS |
| IBS-QOL: | Irritable Bowel Syndrome Quality of Life instrument |
| ITT: | Intention-to-treat analysis |
| JCL: | Johan C Lamprecht (Study supervisor) |
| JDP: | Jesslee du Plessis (Study physician) |
| JRK | Jean Rial Kloppers (primary researcher, author of dissertation) |
| LCL: | Lactulose Dry powder (Laxette Dry®) |
| LPM: | Loperamide 2 mg tablets (Gastron®) |
| MBV: | Mebeverine 125 mg tablets (Merck-Mebeverine®) |
| MEG: | Magneto-encephalography |

| | |
|---|---|
| PET: | Positron emisson tomography |
| PI-IBS: | Post-infectious Irritable Bowel Syndrome |
| PP: | Per-protocol analysis |
| ns: | not statistically significant |
| NSP: | Non-starch polysaccharides |
| PGWB: | Psychological general well-being |
| PPI: | Proton pump inhibitor |
| QATMS: | Quantitative Assessment of Trial Methodology Scale |
| QOL: | Quality of Life |
| SD: | Standard deviation |
| SEM: | Standard error of mean |
| $SH_2$: | Hydrogen sulphide |
| SIBO: | Small intestine bacterial overgrowth |
| SSRI's: | Serotonin re-uptake inhibitors |
| RCT: | Randomised controlled trial |
| TCA: | Trcyclic antidepressants |
| tds: | three times daily |
| TMAZ: | tribomechanically micronised activated zeolite |
| UVK: | Ulrich V. Kruger (Recruitment Operator) |
| VAS: | Visual Analogue Scale |

# CHAPTER 1 INTRODUCTION

## 1.1 Problem Statement

Irritable Bowel Syndrome (IBS) is a common gastrointestinal (GI) disorder characterised by recurrent abdominal pain or discomfort, bloating and stool irregularities (constipation and/or diarrhoea) (Lesbros-Pantoflickova *et al.*, 2004). IBS tend to affect women two times more frequently than men (Payne, 2004), probably due to sexual or biological differences (Rahimi *et al.*, 2008).

In the United States, IBS is the most common disorder of all functional gastrointestinal disorders (FGID's) (Viera *et al.*, 2002) and the most common disorder diagnosed by gastroenterologists (Sandler, 1990). Once considered a Western predominant disorder, IBS is increasingly getting recognition in developing countries (Spiller, 2007b). Studies around the globe have demonstrated a prevalence of between 10% and 20% in the general population (Tack *et al.*, 2006b). However, most IBS sufferers (76.6%) are undiagnosed (Hungin *et al.*, 2005). Unfortunately, few studies are available on the prevalence of IBS in a South African population, but studies from Africa and Asia have shown that IBS is quite common among the general population (Olubuyide *et al.*, 1995; Ringel *et al.*, 2001; Segal, 1984).

As a chronic disorder, most patients suffer with their symptoms for many years. The symptoms of IBS tend to recur frequently and have a considerable impact on sufferers' lives, greatly reducing their physical, social and emotional well-being, as well as consuming considerable healthcare resources (Heading *et al.*, 2006). Previous studies reported inferior quality of life scores compared with gastro-oesophageal reflux disease (GORD), asthma, migraine and similar ratings with diabetes and end-stage renal diseases (Frank *et al.*, 2002; Gralnek *et al.*, 2000). Furthermore, IBS has a substantial impact on work productivity, in view of the fact that IBS patients often miss work days, spend fewer hours at work, undergo job losses, job changes, and even turn promotions down due to health related reasons (Hahn *et al.*, 1999).

Despite low rates of health care seeking behaviour, IBS accounts for 12% of primary care visits (Drossman *et al.*, 1997) and 28% of gastroenterology clinic visits (Thompson *et al.*, 2000). Little mortality, if any, is associated with IBS, a definitive cure does not exist and therefore the illness has a substantial detrimental effect on health care costs (Inadomi *et al.*, 2003).

Little understanding of pathophysiology mechanisms still remains the order of the day regardless of intensive research efforts in the past three decades (Andresen & Camilleri, 2006). IBS may be due to altered intestinal micro-flora, excessive intestinal smooth muscle motility, reduced bowel wall compliance and enhanced pain perception (Capello *et al.*, 2007). Also, environmental factors (psychological disturbances and stress), genetic links, previous infection, bacterial overgrowth in small intestine, food intolerance, altered bowel secretion and deregulation of serotonin pathways have all been proposed as possible aetiological factors in IBS (Rahimi *et al.*, 2008).

Bloating is one of the most difficult GI symptoms to treat (Lacy & De Lee, 2005), as much as 80 -90% of IBS patients suffer from this condition (Corazza *et al.*, 2006) and it is often considered the most bothersome (Lembo *et al.*, 1999). Medications, such as simethicone, charcoal, and pancrealipase utilised in the management of gaseous distension have proved disappointing, and no randomised controlled trials (RCT) have assessed its efficacy (Lacy & De Lee, 2005).

After oral ingestion, carbohydrates are fermented by bacterial flora to give water, short chain fatty acids and gasses, including $CO_2$, $H_2$, $CH_4$, $SH_2$ and $CH_3SH$. These gasses may impose severe symptoms on the IBS patient, as sufferers are known to have impaired transit and tolerance of intestinal gas loads (Azpiroz & Malagelada, 2005). Bacterial fermentation can ultimately result in colon distension and bloating (Camilleri *et al.*, 2002). As a result, antibiotics and probiotics have been under investigation for some of these symptoms (Farthing, 2004; Kim *et al.*, 2005)

Furthermore, luminal factors have also been indicated as aggravators of IBS symptoms. These include malabsorbed sugars, endogenous chemicals, such as short chain fatty acids and bile salts (Camilleri *et al.*, 2002).

The treatment options have not changed dramatically after numerous research efforts (Farthing, 2004). Traditional therapies (psychotherapy, bulking agents, anti-diarrhoeals, antispasmodics, and tricyclic antidepressants) are mainly symptom orientated and often non beneficial (Andresen & Camilleri, 2006). In addition, these therapies have lacked demonstrable efficacy in RCT's (Gilkin, 2005). A substance that acts on all of the pathophysiological sites is yet to be found.

Partly from lack of effective conventional therapeutics and in search of better treatment options, many patients with IBS in both the east and west have turned to complementary and alternative medicine (Bensoussan *et al.*, 1998; Leung *et al.*, 2006; Wang *et al.*, 2006). It is estimated that approximately 40% of IBS suffers seek symptom relief from alternative and complimentary medication (Langmead & Rampton, 2001).

These alternative therapies vary in efficacy and have mainly shown inconsistent results (Hussain & Quigley, 2006). Herbal remedies, including novel and traditional Chinese formulations, peppermint oil and acupuncture are some of the therapies that have been used before as complimentary and alternative medicine (CAM) (Videlock & Chang, 2007).

In this study, Absorbatox™ C35 [$(Na, Ca, K)_6Si_{30}Al_6O_{72}.nH_2O$] a natural, physico-chemically enhanced, non-toxic zeolite with strong adsorption and ion-exchange capacity (Grce & Pavelić, 2005) was assessed as a possible CAM agent in IBS. Since many biochemical processes are closely related to ion exchange, absorption and catalytic processes, it is believed that natural zeolites, like Absorbatox™ C35, could make a significant contribution to the pharmaceutical industry and medicine in the near future. Their unique structure enables them to absorb gas and water (Zarkovic *et al.*, 2003), making them especially attractive as a novel agent in IBS adjuvant therapy. It is documented in literature that, montmorillonite, which also belongs to the zeolite group and consist of similar chemical and physical properties than Absorbatox™ C35, has been shown effective ($p < 0.016$) in constipation predominant IBS (IBS-C) patients (Ducrotte *et al.*, 2005).

In addition the zeolite has the ability to adsorb bile acids (Simón Carballo *et al.*, 2001), harmful toxins (Ward *et al.*, 1993), gasses including $CO_2$, $CH_4$, $H_2$ (Ackley *et al.*, 2003), $NH_4$ (Mumpton, 1999) and also it has been shown to reduce bacterial contamination of the gut (Varel *et al.*, 1987).

The exact pharmacological action is however, not clear but the substance may play a role as an ameliorating agent in the adsorption of certain endogenous chemicals that can cause GI symptoms like diarrhoea, bloating, distension and abdominal discomfort.

## 1.2 Study aims

The aim of this pilot study was to explore the efficacy of a natural zeolite, Absorbatox™ C35, as a complimentary treatment in the management of IBS.

The objectives of this trial were:

- To establish whether Absorbatox™ C35 is effective in the management of IBS-D (diarrhoea predominant IBS), IBS-A (alternating IBS) and IBS-C (constipation predominant).
- To evaluate whether Absorbatox™ C35 can be used as adjuvant treatment in IBS-D, IBS-A and IBS-C.

- To evaluate whether Absorbatox™ C35 can relieve symptoms of pain/discomfort and bloating (also referred to as distension)
- To evaluate whether Absorbatox™ C35 can normalise the frequency of bowel habits in patients.
- To evaluate whether Absorbatox™ C35 can lower the sense of urgency.
- To evaluate whether Absorbatox C35 can stabilize stool form and altered defecation pattern.
- To evaluate whether Absorbatox™ C35 can reduce the usage of rescue medication.
- To establish whether Absorbatox™ C35 can minimise the incidence of concomitant heartburn (or dyspepsia).

**These objectives were assessed using the following endpoints:**

**Primary outcome:** Patients who report adequate relief from at least 50% of treatment weeks will be regarded as responders (Camilleri *et al.*, 2007; Camilleri *et al.*, 2000; Irvine *et al.*, 2006; Jones *et al.*, 1999; Kim *et al.*, 2005; Schoenfeld & Talley, 2006).

**Secondary outcome:** A 50-point reduction in symptom severity according to the IBS Severity Scoring System (Francis *et al.*, 1997; Bijkerk *et al.*, 2003). Stool frequency, urgency and consistency were assessed as stool parameters.

## 1.3 Study layout

The study was designed as a pilot randomised double-blind, placebo-controlled trial with parallel group assignment.

IBS patients, suffering from any bowel subtype (IBS-A, IBS-C and IBS-D) were recruited from various sites in the Potchefstroom district, North-West province, South Africa. Patients that met the inclusion criteria were randomised to receive a placebo (n=16) or active treatment (n=17) of Absorbatox™ C35. Both investigators and participants were blinded to treatment allocation during the study.

Prior to randomisation patients had to undergo a 2-week run-in period in which baseline symptoms were recorded. During this period patients did not receive any treatment. After the 2-week period and treatment allocation, participants were exposed to a 4-week (i.e. 30 days) treatment period. Patients had access to rescue medication to obtain relieve in case patients

did not respond to treatment. Throughout the 6-week study, participants had to complete a study booklet consisting of various questionnaires. These questionnaires were ultimately used to assess the treatment effect in each treatment group.

In addition, participants had to attend five study visits that were distributed over six weeks.

## 1.4 Human Resources

Various investigators and study staff formed part of this study. Refer to Table 1.1 for details.

**Table 1.1: Study staff of Absorbatox™ C35 trial.**

| Name | Text reference | Title | Responsibilities |
|---|---|---|---|
| **Rial Kloppers**<br>MPharm student | **JRK** | Primary researcher | General research functions<br>Study design, outlay & methodological procedures<br>Recruitment<br>Study visit conductor<br>Author of documents (Protocol, Informed Consent)<br>Author of dissertation |
| **Dr Johan C Lamprecht**<br>Registered Pharmacist | **JCL** | Study Supervisor | Supervising<br>Administration<br>Communication with Ethics Committees |
| **Dr Jesslee du Plessis**<br>Registered General Practitioner | **JDP** | Study physician | History taking and physical examination of IBS candidates |
| **Prof Jacques Snyman**<br>Researcher<br>Registered General Practitioner | **JS** | Co-Supervisor | Supervising |
| **Mr George John**<br>Researcher | **GJ** | Co-supervisor | Supervising<br>Administration |
| **Prof Faans Steyn** | **FS** | Statistician | Study sample and statistical power calculation<br>Statistical methods and analysis |

| | | | |
|---|---|---|---|
| **Ulrich V Kruger**<br>Academic Pharmacist Intern | **UVK** | Recruitment Administrator | Allocation of treatment groups |

## 1.5 Financial aspects

This trial was sponsored by the North-West University, Potchefstroom campus, South Africa and Absorbatox (Pty) Ltd.

The primary researcher (JRK) has previously received remuneration from the sponsoring company, Absorbatox (Pty) Ltd, for a project unrelated to this one. A bursary from the National Research Fund was awarded to the primary researcher (JRK).

## 1.6 Division of chapters

The study will be discussed in eight chapters as follows:

- ***Chapter 1:*** ***Introduction***
- ***Chapter 2:*** ***Irritable Bowel Syndrome***
- ***Chapter 3:*** ***Clinical Drug Trials in Irritable Bowel Syndrome***
- ***Chapter 4:*** ***Properties and Applications of Investigational Material***
- ***Chapter 5:*** ***Patients and Methods***
- ***Chapter 6:*** ***Results***
- ***Chapter 7:*** ***Discussion***
- ***Chapter 8:*** ***Synopsis***

## 1.7 Chapter restatement

It is evident from literature that IBS is associated with several burdens, with a significant impact on patients and society as a whole. Current treatment options are scarce and numerous sufferers have turned to complimentary and alternative medicine. These treatments, whether in the form of drugs or any other medical practices, have not been adequately exposed to robust randomised controlled trials.

Absorbatox™ C35, with ion exchange and adsorbing properties, might be an effective adjuvant in IBS management. A small sample size consisting of IBS patients diagnosed according to Rome III standards have been randomised into a double-blind, placebo-controlled trial to receive Absorbatox™ C35 or placebo. This pilot study must be viewed as hypothesis generating, rather than hypothesis testing and likely implications for future studies as a key focus.

Chapter 2 will focus on IBS as a disorder of misunderstood pathophysiology, on the epidemiology of IBS, the burden that is cast on sufferers' lives due the disorder, and a lack of proper treatment. It will also discuss novel future treatments, with emphasize on the serotonin modifying drugs. CAM used in IBS will be discussed briefly.

# CHAPTER 2 IRRITABLE BOWEL SYNDROME

## 2.1 Introduction

Irritable Bowel Syndrome (IBS) is a multi-factorial, heterogeneous disorder (Rothstein, 2000) and is one of over 20 functional gastrointestinal disorders (FGID) characterised by chronic or recurrent gastrointestinal (GI) tract symptoms (Ringel *et al.*, 2001). Furthermore, it is classified by the most widely accepted "Rome III diagnostic criteria" as a functional gastrointestinal disorder mainly because of the absence of any biological marker, infection, inflammation, structural or biochemical defects (Corazziari, 2004; Hadley & Gaarder, 2005).

Patients complain to family doctors, gynaecologists, surgeons, and gastroenterologists about abdominal pain and/or discomfort (Talley & Spiller, 2002). In the United States, IBS is the most common disorder of all FGID's (Viera *et al.*, 2002) and the most common disorder diagnosed by gastroenterologists (Sandler, 1990). The syndrome in general is accompanied by a substantial impact on individual patients, health care systems and society as a whole (Gilkin, 2005). IBS affects people around the world and is increasingly recognised in developing countries (Spiller, 2007b). Unfortunately, few studies are available on the prevalence of IBS in the South African population, but studies from Africa and Asia have shown that IBS is quite common among the general population (Olubuyide *et al.*, 1995; Ringel *et al.*, 2001; Segal, 1984). It is estimated that 25% of the general population in the United Kingdom have symptoms consistent of IBS diagnosis (Jones & Lydeard, 1992). It is clear that IBS tends to affect women more than it affects men, probably due to sexual or biological differences between men and women (Payne, 2004).

Abnormalities of GI sensation, motility, autonomic function, bacterial flora, the mucosal immune system and serotonin pathways have all been identified as possible mechanisms of IBS (Spiller, 2007b). However, the hallmark signs and symptoms of irritable bowel syndrome are abdominal pain or discomfort relieved by defecation and pain or discomfort associated with looser or more frequent stools (Viera *et al.*, 2002).

Several scientists have labelled the mucosal serotonin receptors as promising research areas. (Hunt & Tougas, 2002). Some studies have suggested serotonin transporter impairment in the GI wall (Spiller & Bennett, 2007). It is clear, however, that both central and peripheral mechanisms are involved in the genesis of symptomatology (Bueno, 2005). Therefore, standard

drug therapy is aimed at end-organ targets (predominant symptom) and relieving an associated affective disorder or modifying pain pathways in the central nervous system (CNS) (Bueno, 2005). Despite three decades of intensive research done on the pathophysiology of IBS, little is understood (Andresen & Camilleri, 2006) and the treatment options have not changed dramatically (Farthing, 2004).

At least 40% of IBS suffers tend to seek symptom improvement from alternative and complimentary medication (Langmead & Rampton, 2001). This may be attributed to the fact that there is no current comprehensive treatment or cure for IBS; therefore, every patient has to be treated individually (Snelling, 2006).

## 2.2 Epidemiology

Twelve percent of primary care patients' visits are due to IBS symptoms and between 25-50% of these patients are referred to gastroenterologists (Birrer, 2002). Patients, mainly, between the ages of 20-50 years (Ringel *et al.*, 2001) are responsible for 1.5 to 3.7 million physician office visits each year (Sandler *et al.*, 2002; Shih *et al.*, 2002). Furthermore, it is estimated that approximately 2.2 million prescriptions and 96 000 hospital discharges are due to IBS, in the USA alone (Saito *et al.*, 2002; Sandler, 1990). Although these findings may seem large, it is quite small considering the fact that most IBS sufferers (76.6%) are undiagnosed (Hungin *et al.*, 2005).

For many years IBS was considered a western predominant disease, with many studies from developing countries suggesting that IBS has a low prevalence. However, recent robust studies have shown that IBS is now getting more recognition than ever before and that the prevalence rates are picking up in these developing countries. In addition, the high prevalence among women in the western world is not seen in developing countries, this may probably be attributed to ethnic differences with regards to dietary and environmental factors. (Gwee, 2005; Kang, 2005). In contrast, a recent study from Malaysia, and elsewhere, has concluded that female preponderance is seen in the general population (Rajendra & Alahuddin, 2003). From twelve western community studies, eight stated female predominance, while one study found a female predominance with the Manning criteria but not with Rome I or II criteria (Kang, 2005). In eight eastern studies, only four described female predominance. One study found female predominance when the Manning criteria were used, but no gender difference was detected while using the Rome I and II criteria. Gender differences are seen between the east and west. In general, female preponderance was seen in most countries, but cases of no gender differences must not be completely neglected (Kang, 2005). A systematic review report, by

Saito and colleagues, stated that female predominance range between 2:1 and 1:1, in relation to male sufferers (Saito *et al.*, 2002). It is estimated that IBS are most likely to occur between the ages of 18 -34 years (Hungin *et al.*, 2003).

Probably one of the biggest limitations, to previous published prevalence studies, is that different prevalence rates have been reported as the diagnostic criteria changed from the Manning Criteria to the Rome 1 Criteria to the Rome II Criteria and finally to the Rome III Criteria (Andrews *et al.*, 2005). In the USA and Europe, 5-23% of adults have IBS and 60-70% of them are women (Talley *et al.*, 1991). In 1988, Danivat and colleagues from Thailand indicated that IBS has a low prevalence of 4% in both rural and urban communities (Gwee, 2005). In this particular study a questionnaire similar to the one utilised by Drossman and colleagues six years earlier was used, who found a prevalence of 22% in an American population (Drossman *et al.*, 1982). Although the prevalence is found to be higher in certain western countries, i.e., Canada, UK, and Italy all reported a prevalence of approximately 12%. Asian countries, like South China and Singapore reported a prevalence of 5.7% and 8.6% respectively. This is close to the values reported by Australian (6.95%) and European (9.6%) studies (Hungin *et al.*, 2003). In these studies the Rome II diagnostic criteria were used.

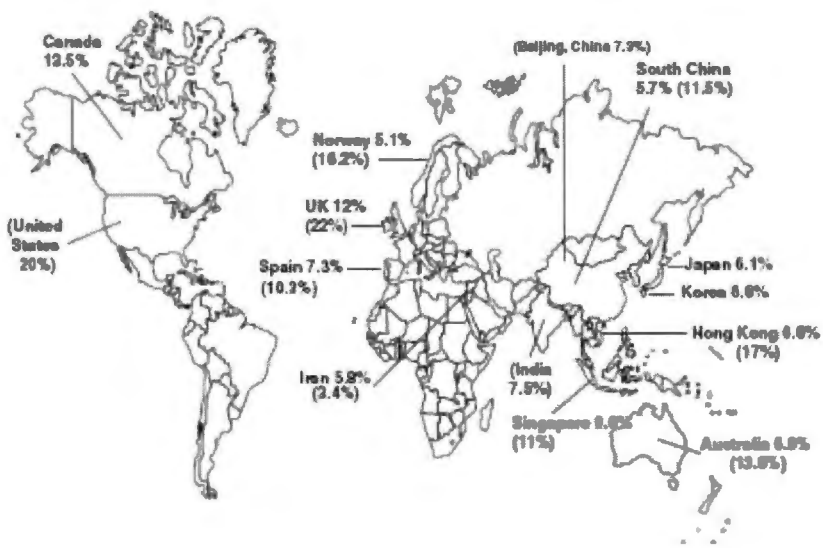


**Figure 2.1: World map of IBS prevalence year 2000–2004 by Rome 2 criteria with Manning criteria in parenthesis where available (adapted from Gwee, 2005).**

The older Manning criteria were utilised in various Western and Eastern countries, slight inferior rates are seen in Eastern countries but are not of any significance (Gwee, 2005). These countries are China with 7.3%, India with 7.5%, Singapore with 11% and South-China with 11.5%. Western countries that used the Rome II criteria were Spain at 10.3%, Norway at 16.2%, USA at 17% and UK at 22%. Several recent methodologically sound studies from Singapore have revealed that IBS is on the increase in the Eastern world, more than one would

expect (Gwee, 2005). This is probably due to industrialisation and urbanisation. In total, approximately 30 million people in North America meet the diagnostic criteria for IBS, at the time (Saito *et al.*, 2002).

## 2.3 The Burden of Irritable Bowel Syndrome

### *2.3.1 The impact on patients well-being*

Health related quality of life (HRQOL) is an emerging parameter that addresses the impact of chronic diseases on social, psychological, and physical levels of one's life (Gilkin, 2005).

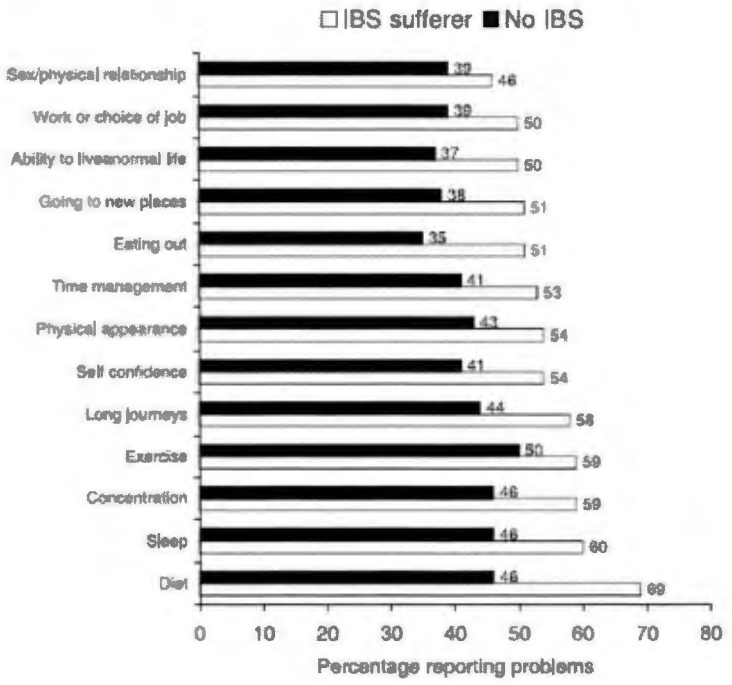


**Figure 2.2: Impact of IBS on the lives of individuals with IBS (adapted from Hungin *et al.*, 2003).**

In general, IBS has a substantial negative effect on sufferers' lives and has a severe impact on various levels of well-being (Quigley *et al.*, 2006). From a prevalence, symptom pattern and impact study by Hungin *et al.* (2005), it is now clear that IBS sufferers have an inability to concentrate and struggle with time management in comparison to non-IBS sufferers. IBS sufferers generally felt they had to cut down on usual activities and nearly one quarter of current IBS sufferers had missed social engagements. Self-confidence was found to be impaired in IBS sufferers in comparison to controls. Individuals with IBS are reluctant to enrol in long journeys, go out for a meal and undertake holidays as these were all reported as a problem to sufferers

(Hungin *et al.*, 2005). In a Canadian study, IBS sufferers reported a mean overall IBS-QOL, a diseases specific outcome measure, of 66.3 (0-100 scale, 0 indicates a poor QOL) with "food avoidance" and "health worry" being the most serious concerns (Paré *et al.*, 2006).

The SF-36, a HRQOL questionnaire, was completed by IBS sufferers, other GI disease sufferers and non-GI disease sufferers. IBS patients had a lower quality of life compared to asthma, migraine and gastro-oesophageal reflux disease (GORD) groups, but superior HRQOL in relation to rheumatoid arthritis and panic disorders (Frank *et al.*, 2002).

Individuals with IBS had lower psychological well-being levels, in relation to Inflammatory Bowel Diseases (in remission) and renal insufficiency in a general Swedish population (Simrén *et al.*, 2004).

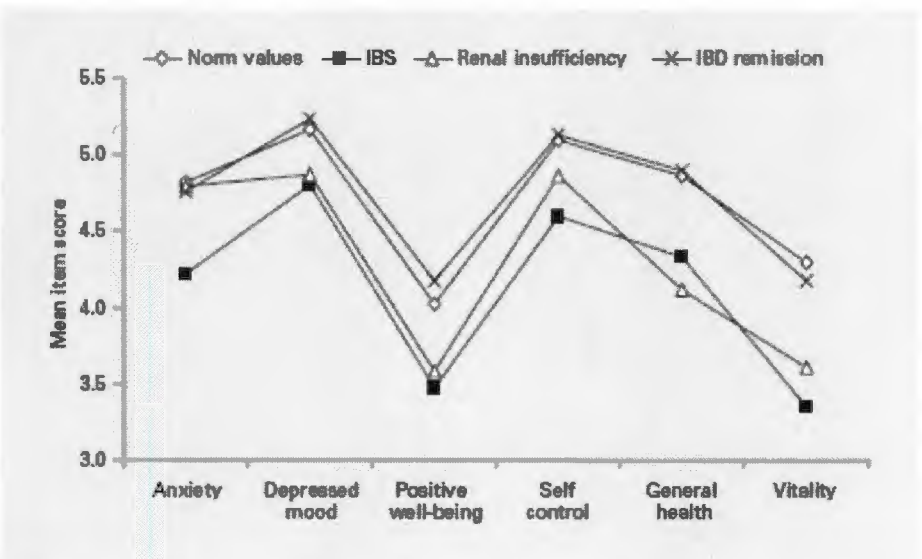


**Figure 2.3: Well-being as measured by the PGWB (psychological general well-being) in IBS, IBD (remission) and renal insufficiency and normal values from a Swedish population (adapted from Simrén *et al.*, 2004).**

Gralnek *et al.* (2000) studied the impact IBS has on the health related quality of life in 877 IBS sufferers. The SF-36 was utilised and they further compared the data with previously reported data of a general US population and with patients suffering from (GORD), diabetes mellitus, depression and end-stage renal diseases (ESRD). In all categories of the SF-36, IBS sufferers reported inferior levels of HRQOL compared to the general population. Patients with IBS had a worse QOL than GORD patients, similar score than diabetes and ESRD. However, IBS patients had significantly better mental health SF-36 scale scores than patients with depression. Energy/fatigue, role limitations caused by physical health problems, bodily pain, and general health perceptions were first and foremost influenced by IBS (Gralnek *et al.*, 2000). From these data it is clear that patients with IBS have a reduced QOL (Simrén *et al.*, 2004).

## 2.3.2 *The impact on employers*

IBS is known to be the second most common cause of work absenteeism (Pirk *et al.*, 2000). Costs due to productivity loss arising from absence from work are labelled as indirect costs (Simén *et al.*, 2004). In the US Householder Survey of Gastrointestinal disorders, IBS sufferers were shown to be three times more absent from work than the normal population, which is a total average, spend away from work, of 13.4 days per year (Drossman *et al.*, 1993). Hahn *et al.* (1999) concluded that IBS has a substantial effect on work, since patients from the US and UK reported missed work days, job losses, job changes due to health reasons, fewer hours at work and promotion turn downs. Nearly one third of the patients' surveyed reported at least 1 day off work in the previous 4 weeks due to IBS (Hahn *et al.*, 1999).

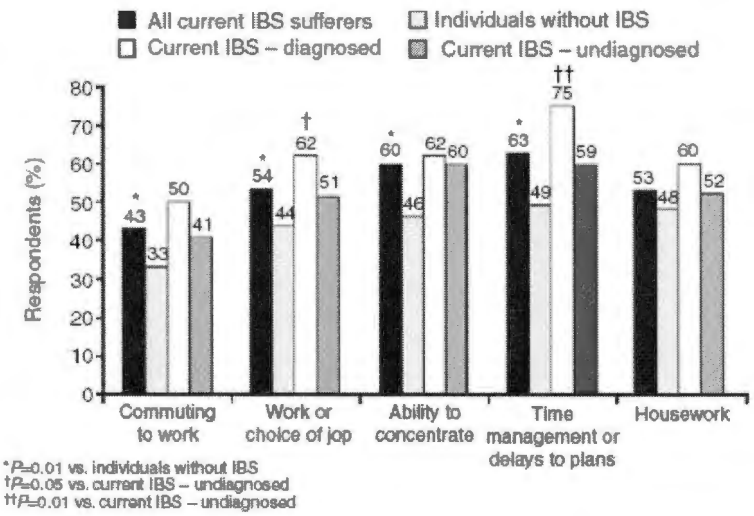


**Figure 2.4: Impact of irritable bowel syndrome on work activities (adapted from Hungin *et al.*, 2005).**

It is documented that the illness has an indirect cost of US $335 to US $748 per patient annually, with total annual costs of $205 million in the US (Inadomi *et al.*, 2003). A Canadian study documented a work absenteeism of 5.6% and a work productivity loss of 34.6%, after administering the WPAI (Work Productivity and Activity Impairment questionnaire) to IBS sufferers (Paré *et al.*, 2006).

In an international survey of 40 000 participants, IBS sufferers reported spending 3.4 days in bed in the previous 12 months, while non-sufferers spend 2.7 days in bed (Hungin *et al.*, 2003). Hungin and colleagues further documented that patients with IBS missed 5.5 days of work on average compared to 3.1 days in the general population. Patients also reported that they had to cut their work activities (10.2 days by IBS patients and 4.8 days by non-sufferers). A great deal of the days spend not at work were attributed to days spend with doctors and nurses, 8.4 days reported by IBS patients and 5.2 days reported by non IBS patients (Hungin *et al.*, 2003).

IBS affects work productivity in other countries as well. In a German study, work absenteeism was the second most common expenditure of all cost drivers identified (Pirk *et al.*, 2000). From the 141 subjects in France, 12.1% stated that they had to be absent from work at least once during the previous 6 months (Le Pen *et al.*, 2004). Before the survey these French IBS patients scored an average "degree of effect on work productivity" of 72 (VAS 0 -100, with 100 as the highest score) (Le Pen *et al.*, 2004).

### 2.3.3 The impact on Health Care and resource utilisation

Although little mortality is associated with irritable bowel syndrome, a definitive cure does not exist and therefore the illness has substantial detrimental effects on health care costs (Inadomi *et al.*, 2003).

It is estimated that 80 000 to 100 000 patients are hospitalised due to IBS related causes in the US each year. It was further stated that patients stay between 4.7 and 5.2 days in hospital before they are discharged from hospital (Shih *et al.*, 2002). The same study discovered that the average cost of IBS related hospitalisation was US $7882 per patient. The total cost of GI motility and sensory disorders in the eight most developed countries are estimated to exceed the US $40 billion margin, with direct cost (physician visits, diagnostic testing, investigations, medical treatment, hospitalisations and prescription medications) accounting for one third of the total cost (Fullerton, 1998; Quigley *et al.*, 2006).

In the US, the range of total annual direct cost per IBS patient lies between US $530 and US $8750 (Maxion-Bergemann *et al.*, 2006). Drug expenditures and outpatient treatment were the main cost drivers in a German study (Pirk *et al.*, 2000). The annual societal cost of IBS was estimated at an average of DM 2300 per patient (Pirk *et al.*, 2000). Investigators from France administered a questionnaire, which include resource consumption, quality of life and impact of IBS on productivity, to a sample of 253 French IBS (diagnosed with well-known diagnostic criteria) adults (Le Pen *et al.*, 2004). Seventy seven percent of the population had previously consulted a general practitioner and 43% a gastroenterologist. Twenty five percent reported hospitalisation, while 61% of patients reported that they were taking medication. These two components were also indicated as the main factors of medical costs. The average monthly medical costs were calculated at €71.8 per patient.

Hundred and sixty-one IBS patients from a primary care setting, defined by the Rome 1 criteria, were compared with 213 healthy controls. Akehurst *et al.* (2002) reported that on an average IBS patients cost the NHS £123 more per year than individuals from the control group. Furthermore, hospital inpatient episodes were indicated as the biggest feature of health costs,

secondly listed were outpatient attendances and GP prescriptions. Patients in the IBS group scored far more than their healthy counterparts in all sections.

In a systematic review report by Inadomi and colleagues, it was documented that the total annual direct costs related to IBS was estimated at £45.6 million (UK) and $1.35 billion (USA) respectively (Inadomi *et al.*, 2003). Direct resource consumption annually by IBS patients ranged from US $742 to US $3166 per patient.

Quigley *et al.* (2006) concluded that there is still an unmet need in the biggest part of the European world, with regards to familiarity with IBS, difficulties in diagnosis and the lack of effective treatment all which may be part of the detrimental costs associated with IBS.

## 2.4 Pathophysiology and Pathogenesis

### *2.4.1 Evolution of the pathophysiological hypothesis in IBS*

The term aetiology is considered inappropriate when dealing with a multi-factorial syndrome like IBS (Ringel *et al.*, 2001). The pathophysiology is not completely understood leading to limited treatment options and improper disease management (Öhman & Simrén, 2007). Terms like *spastic colon*, *neurogenic mucous colitis* and *irritable colon syndrome* were used previously. However, in 1967 it was De Lor that named the disorder *irritable bowel syndrome* (Zaman, 2002).

The Principles and Practice of Medicine, by Sir William Osler, published in 1892 were the first paper to describe *mucous colitis*, which we know as Irritable Bowel Syndrome today. Patients were described as hysterical, hypochondriacal, depressed, self-centred, neurasthenic, and subject to colicky abdominal pains (Christensen, 1992). According to Christensen (1992) Bockus and co-workers described 50 cases in which patients were found to be constipated, dyspeptic, introspective, exhausted, emotionally unstable and many showed signs of palpable and tender colon. At that time specific diagnostic criteria were non-existing and patients were diagnosed on the basis of a history of diarrhoea, abdominal pain and anxiety (Christensen, 1992).

In 1928, Ryle described fifty patients as having *spastic colon*, their symptoms mainly comprised of constant lower abdominal pain. These symptoms were aggravated by periods of anxiety, smoking, menses and defecation and were relieved by rest, hot baths and eating (Christensen, 1992). Thirty percent of Ryle's patients were constipated while 20% were complaining of diarrhoea, and another 20% had mucorrhoea (Christensen, 1992).

After much research done on the pathophysiology of IBS, the existing terms, like *irritable colon* and *colonic neurosis* (meaning autonomic disorder) were replaced by a more acceptable term: *Irritable Bowel Syndrome.*

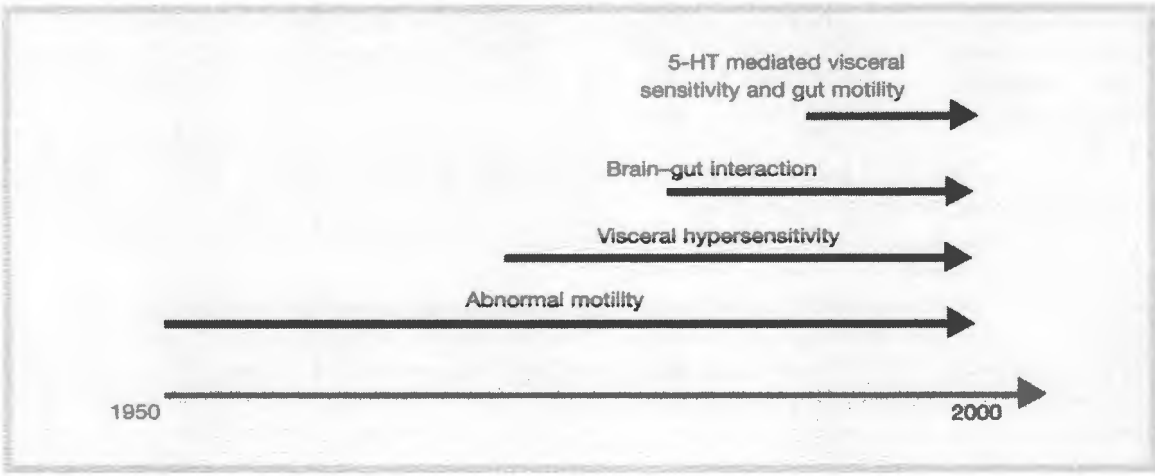


**Figure 2.5: Pathophysiological evolution Hypothesis (Zaman, 2002).**

Initial attention was focused on potential abnormalities of intestinal motility. Various models have been used to characterize a variety of abnormalities of colonic motility as well as enhanced response to stress and food (Gorard & Farthing, 1994). The barostat distension models were later introduced and have been used to assess intestinal wall sensitivity. These studies confirmed that IBS patients may have visceral hypersensitivity in comparison to healthy individuals (Bose & Farthing, 2001). More recently, scientists have described the enteric nervous system (ENS) as a plausible area of IBS symptom evolution, since ENS controls motility and secretory functions in the gastrointestinal system. Later the neurotransmitter, serotonin became a field of interest as it was clear that 95% of its receptors are located within the ENS (Zaman, 2002) and that it plays an important role in peristaltic, secretory, vasodilatory, vagal and nociceptive functions (Crowell, 2004). Research and focus have shifted from psychological and stress-related to an emphasis on motility disturbances to autonomic system imbalance and visceral hypersensitivity (Ringel *et al.*, 2001).

### *2.4.2 Current pathophysiological mechanisms of IBS as seen today*

Today irritable bowel syndrome is viewed upon as a disorder of deregulation of the brain-gut axis, involving abnormal function in the enteric, autonomic and/or central nervous systems (Öhman & Simrén, 2007). In addition, recent novel research focus areas are under investigation, they are immunology, infection and genetics, all presumed to play a role in the evolution of IBS (Barbara *et al.*, 2004).

### 2.4.2.1 *Abnormal motility*

For several years IBS was considered a gut motor disorder (Barbara *et al.*, 2004). In previous years, several abnormalities were described in manometric and electromyographic recordings, made in the small bowel and colon in IBS patients (Delvaux, 2004). Today the exact contribution of abnormal motility to the global IBS pathophysiology is still not known, since many controversial studies have been published in which some authors have found differences, between sufferers and controls, and other authors were not able to find any difference at all (Farthing, 1999). Various investigators, however, have ascribed the reason for the symptoms of abdominal pain, bloating, and altered bowel habits due to disturbances of intestinal motility (Gilkin, 2005). It is postulated that motility patterns may be aggravated in response to various stimuli, like food and balloon distension or psychological stress (Drossman *et al.*, 2002; Birrer, 2002). Furthermore, this disruption may last for 2.5 – 8 hours depending on the quantity and quality of the stimuli (Delvaux, 2004).

Evidence exists that patients with delayed and accelerated intestinal transit, manifested as constipation and diarrhoea respectively, may have a migratory motor complex abnormality (Lacy & De Lee, 2005). In addition, patients complaining of bloating have shown delayed ileo-colonic transit (Delvaux, 2004). Another group of sufferers have very high amplitude propagating contractions within the colon and this may be associated with periods of abdominal pain. Postprandial contractions were found to be more severe, since the colon contracts more vigorously during these periods (Lacy & De Lee, 2005). Consistent data exists that repetitive bursts of contractions occur at a regular rhythm within the IBS population. Unfortunately these patterns, called minute rhythm, were observed in healthy controls as well, and are considered non-specific of IBS (Delvaux, 2004).

The pattern of altered motility is only observed in 25% to 75% of IBS patients. Although insignificant motility patterns in IBS patients have been found in the proximal small intestine and stomach; hyperactivity has been observed throughout the ileum, colon, and rectum in response to certain stimuli, in comparison to healthy controls (Gilkin, 2005). It is now accepted that IBS is not primarily a gut motility disorder with few studies proving a link between motility patterns and pain attacks (Delvaux, 2004).

### 2.4.2.2 *Visceral hypersensitivity*

IBS sufferers appear to have an enhanced perception of visceral actions throughout the gastrointestinal tract, including the oesophagus, stomach, duodenum, and ileum (Drossman *et al.*, 2002). IBS sufferers were considered hypersensitive since the early 1960's, but results presented over the years are contradictory. IBS patients have shown lower pain thresholds than controls, when using a steadily ascending distension model, but appear to have similar

thresholds when the same volumes are presented in a random order (Talley & Spiller, 2002). Dutch investigators, Kuiken and colleagues (2005), have found lower pain/abdominal discomfort thresholds in IBS patients than in healthy controls. It is thoroughly proven that anticipation and hypervigilance play an important role in the perception of visceral sensation in IBS (Barbara *et al.*, 2004). Furthermore, evidence exist that visceral hypersensitivity of the lower and upper gastrointestinal tract in IBS patients may be attributed to a variety of perceptual alterations (Crowell *et al.*, 2005).

Most studies have found that IBS sufferers do not exhibit generalised hypersensitivity to noxious somatic stimulation (Crowell *et al.*, 2005). Although various animal models have aided in the understanding of visceral hyperalgesia, the application in man is notoriously difficult. However, a few novel models have been utilised in the experimentation of the brain's response to visceral stimuli such as magneto-encephalography (MEG), positron emission tomography (PET), and functional magnetic resonance imaging (fMRI) (Quigley, 2003). For instance, investigators have recently characterised IBS patients by a failure to activate the cingulate gyrus in response to painful stimulus (Quigley, 2003). In contrast to this finding, Mertz and colleagues (2000) have found a greater intensity of activation of the anterior cingulate cortex in IBS patients. Recently it has been postulated that hypersensitivity may be attributed to either one of two factors or both simultaneously. One, changes in rectal wall tension receptors and, two; altered CNS processing of rectal inputs (Kwan *et al.*, 2005). In an earlier study, Whitehead *et al.* (1999) had suggested altered receptor sensitivity in the rectosigmoid, a similar theory as found by Kwan and colleagues (2005).

The mechanism by which visceral hypersensitivity is exerted in IBS is still not completely known, and further investigation is needed (Crowell *et al.*, 2005). Investigators have claimed that visceral hypersensitivity may be considered as a biological marker of IBS (Öhman & Simrén, 2007), others have challenged this statement (Drossman *et al.*, 2002). Unfortunately studies addressing this issue were done under fasting and non-stimulating conditions. Sufferers, however, tend to complain more when exposed to stimuli of food, stress and the menstrual cycle. Therefore, recent studies placed emphasis on hypersensitivity during periods of gut stimulus (Öhman & Simrén, 2007). In various studies by Simrén and colleagues, investigators have found that certain foods can cause hypersensitivity in the GI-tract (Simrén *et al.*, 2001; Simrén *et al.*, 2007). In addition, rich foods increased rectal sensitivity more than food rich in carbohydrates. From these data it would appear that hypersensitivity is associated with both central and peripheral processing and that it is unrelated to bowel habit, gender and psychological factors (Öhman & Simrén, 2007).

Other factors that may contribute to the global IBS pathophysiological multi-factor model are stress, hormones and most recently found, mast cells (Öhman & Simrén, 2007; Santos *et al.*,

2005). Severe forms of stress, participation in war, have been found to result in visceral hypersensitivity (Dunphy *et al.*, 2003). Since female IBS patients tend to have more severe symptoms during menses, it is postulated that certain hormones may exaggerate symptoms and increase visceral hypersensitivity (Kim *et al.*, 2006). In addition, rectal sensitivity changes with menstrual cycle in female sufferers (Houghton *et al.*, 2002). The mast cells' imperative steps in inflammation and the role in IBS aetiology are plausible research areas. It has recently been found that people with mastocytosis show enhanced colonic sensitivity to balloon distension (Santos *et al.*, 2005).

It is widely acknowledged that the central nervous system plays an imperative role in both visceral sensitivity and altered motility, since abnormalities in intrinsic and extrinsic nervous system function may lead to alterations in motility, heightened visceral sensation, and abnormal secretion in the GI-tract, all of which may induce the symptoms of IBS (Crowell *et al.*, 2005)

#### *2.4.2.3 Inflammation and Infection*

Over 40 years ago, physicians recognised IBS-like (irritable colon at that time) symptoms after acute infective enteritis (Lacy & De Lee, 2005). It is well documented that approximately 30% of IBS patients have a history of preceding gastroenteritis, mainly caused by *Campylobacter*, *Salmonella*, *Shigella*, *Trichinella* (Birrer, 2002; Spiller, 2007a). Moreover, the risk of presenting with IBS symptoms a year after gastroenteritis is tenfold (Gwee *et al.*, 1996; Neal *et al.*, 1997). Interestingly enough, one study has shown that Post-infectious IBS (PI-IBS) may be related to non-GI or GI infections (McKeown *et al.*, 2006).

Studies have confirmed the hypothesis that bacterial gastroenteritis is a risk factor for IBS and the severity of the initial illness (duration of diarrhoea) is an independent risk factor for the magnitude of PI-IBS (Ji *et al.*, 2005). Investigators further documented that the clinical course of PI-IBS is variable over a one-year follow-up period (Ji *et al.*, 2005). Females are at greater risk than males, anxiety and depression and a history of stressful events 6 months prior to the precipitating infection are all risk factors presumed to contribute to the development of PI-IBS (Collins *et al.*, 1999; Ji *et al.*, 2005). Interestingly, vomiting during gastroenteritis has shown some protection against the development of PI-IBS, probably due to a reduced amount of toxin exposure that could damage the enteric wall (Collins *et al.*, 1999; Lacy & De Lee, 2005). However, countries with a high prevalence of enteric infections do not necessarily have high rates of IBS symptoms (Drossman *et al.*, 2000; Gwee *et al.*, 1996).

Patients who developed IBS after acute gastroenteritis have shown increases in enterochromaffin and lymphocytes cell counts, after three months compared to those who did not develop IBS (Spiller, 2007a). In addition, a variety of gut alterations were found in mucosal biopsies in patients with PI-IBS; increased expression of interleukin 1$\beta$ messenger RNA,

increased cellularity of lamina propia, and an increase in $CD3^+$ lymphocytes (Spiller *et al.*, 2000).

With regards to inflammation, various animal models have supported the concept that mucosal inflammation, which accompanies bacterial gastroenteritis or Inflammatory Bowel Disease (IBD), leads to deeper neuromuscular tissue alterations. In turn, this must persist after resolution of the inflammatory process before IBS can appear (Collins *et al.*, 1999). Investigators are suggesting that the inflammation process switched on by a prior enteric infection may play a larger role in PI-IBS than the presence of the infective agent itself (Collins *et al.*, 2001). Recent studies have reported increased inflammatory cells in the jejunum, ileum, and colonic mucosa of IBS patients. It is well known that inflammation, acute and previous, alters gut motility, sensitivity and secretion functions in such a way that IBS may be a result of it (Törnblom *et al.*, 2005). Furthermore, mucosal inflammation in the GI-tract leads to the release of cytokines, including interleukin-1$\beta$, which has been found to affect neuron sensitivity and eventually give rise to increased perception of pain due to release of mucosal prostanoids (Hunt & Tougas, 2002).

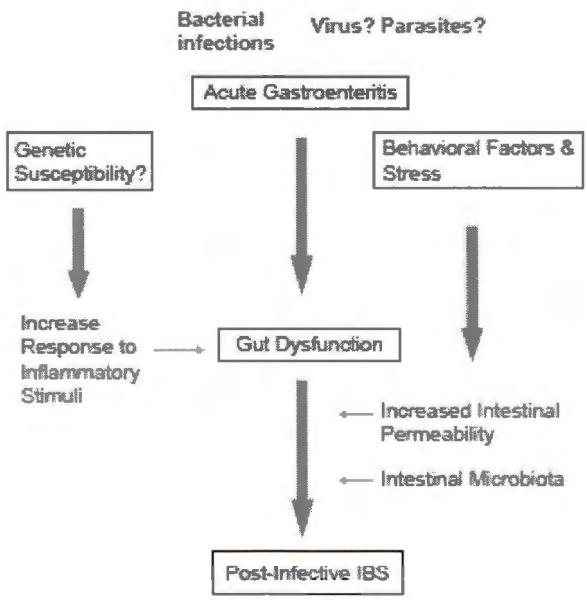


**Figure 2.6: Symptom Generation in post-infective IBS (Verdu & Collins, 2004).**

From this data, it is clear that researchers have spent money and time to prove existence of inflammation in the gut after an episode of gastroenteritis. Sadly, it seems that anti-inflammatory drugs like prednisolone appear to be ineffective in the alleviation of PI-IBS symptoms (Dunlop *et al.*, 2003).

The precise mechanism by which this type of IBS (PI-IBS) exerts its action is not known; but many hypotheses exist: 1) An infectious process may damage the enteric nervous system,

transiently or permanently, 2) An acute infectious process may affect the numbers and function of enteroendocrine cells and T lymphocytes and/or alter their function, 3) A chronic state of inflammation that can lead to transient/permanent alterations in motility and visceral sensitivity, after an infectious process, and 4) Local tissue destruction may occur during gastroenteritis and can lead to the release of chemical mediators, thereby altering gut function (Lacy & De Lee, 2005).

To conclude, PI-IBS is characterised by more loose stools, less prevalence of psychiatric illness, and increased serotonin containing enterochromaffin cells compared to idiopathic IBS. However, treatment options are those used to treat idiopathic IBS. The prognosis of PI-IBS is generally good, with resolution of symptoms within 6 years (Rhodes & Wallace, 2006), although some investigators have found that 50% of patients still had symptoms after five years (Spiller, 2007b).

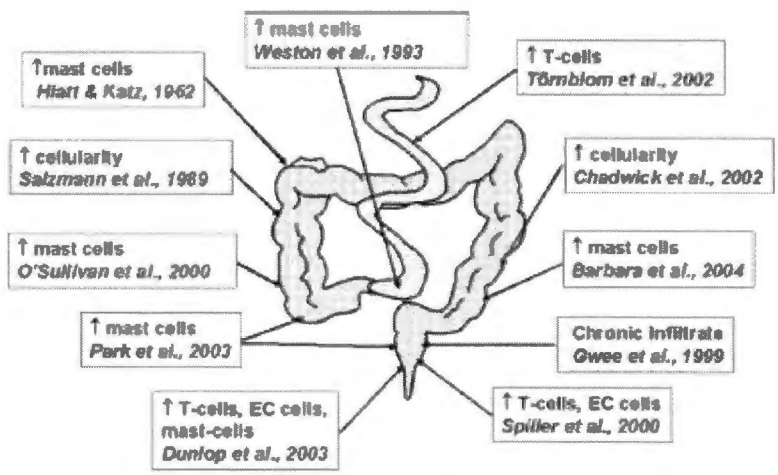


**Figure 2.7: Biopsies in the gut and the different findings (Tornblom *et al.*, 2005).**

### *2.4.2.4 Neurotransmitter imbalance*

A more recent theory exists that IBS may result from deregulation of the brain-gut neuro-enteric systems (Gilkin, 2005). To understand the neurotransmitters involved in IBS pathogenesis, it is important to understand which nervous system(s) control them. The enteric nervous system (ENS) functions semi-autonomously and controls intrinsic bowel motility and secretion function but is modified by the autonomic nervous system (ANS) (Hunt, 2002). Neurotransmitter imbalances in the ANS and ENS may explain the clinical differences reported in symptom subgroups of patients with IBS (Hunt, 2002).

Many substances including serotonin, substance P, neurotensin, and cytokines are potential participants in transmission of altered sensations, which might lead to pain. These mediators

may also function in the regulation of mood and behaviour (Ringel *et al.*, 2001), as well as visceral sensitivity, gut motility and secretions (Öhman & Simrén, 2007).

Of these neurotransmitters, serotonin is probably the most important one, since the majority of serotonin (5-HT) is found in the gastrointestinal tract. With regards to IBS, female patients versus controls have shown higher synthesis of 5-HT in several brain regions (Bose & Farthing, 2001). Although a small amount of 5-HT is released by enteric nerves, 90% of this bioactive amine is contained in enterochromaffin (EC) cells. The effects of serotonin are terminated by the enterocytes, which remove serotonin from the interstitial space through the serotonin re-uptake transporter (SERT) (Barbara *et al.*, 2004).

Scientists have classified seven different 5-HT receptors, however; $5\text{-HT}_1$, $5\text{-HT}_2$, $5\text{-HT}_3$ $5\text{-HT}_4$ have been extensively studied in the GI-tract. Activation of $5\text{-HT}_3$ results in enhanced motility, secretion, and sensation, whereas activation of the $5\text{-HT}_4$ receptor subtype has excitatory and inhibitory effects, like increased motility and secretion, and decreased visceral hypersensitivity (Talley, 2001). Studies have shown that increased 5-HT release or delayed 5-HT uptake occur within the PI-IBS (as mentioned before) and diarrhoea-predominant subgroups. Whereas, impaired release of 5-HT seems to be a characteristic of IBS with constipation (Atkinson *et al.*, 2006; Houghton *et al.*, 2003). Furthermore, altered EC cells and/or 5-HT signalling can result in abnormal gastrointestinal motility, visceral hypersensitivity and secretomotor abnormalities (Crowell, 2004). Therefore, alterations in 5-HT content and release, expression of 5-HT receptors or changes in SERT could be labelled as important contributors of IBS symptom generation (Barbara *et al.*, 2004).

**Table 2.1: Changes in entererochromaffin cells, serotonin and serotonin reuptake transporter in IBS (adapted from Barbara *et al.*, 2004).**

| Condition | Site | EC cells | 5-HT content | SERT | Reference |
|---|---|---|---|---|---|
| **IBS** | rectum | **increased** | - | - | Kyosola *et al.*, 1977 |
| **D-IBS** | serum | - | **increased** | - | Bearcroft *et al.*, 1998 |
| **Constipation** | colon | **decreased** | - | - | El Salhy *et al.*, 1999 |
| **PI-IBS** | rectum | **increased** | - | - | Spiller *et al.*, 2000 |
| **C-IBS** | rectum | - | **increased** | - | Miwa *et al.*, 2001 |
| **D-IBS** | serum | - | **increased** | - | Houghton *et al.*, 2003 |
| **IBS** | rectum | no change | - | - | Coates *et al.*, 2004 |

From these and other data, it is no wonder that novel pharmaceutical drugs in IBS are aimed to modulate various 5-HT targets and receptors in the GI-tract, of which 5-$HT_3$ and 5-$HT_4$ receptors are the most important. In addition, serotonin-modulating drugs have shown beneficial effects on the CNS-associated symptoms of IBS (Crowell, 2004). As a result drugs like alosetron, a 5-$HT_3$ receptor antagonist and tegaserod, a partial 5-$HT_4$ agonist were manufactured (Talley, 2001).

#### *2.4.2.5 Abnormal intestinal micro-flora*

A number of investigations on colonic fermentation suggest that colonic flora might be abnormal in IBS patients (Talley & Spiller, 2002). IBS may result from a dysfunctional interaction between the indigenous flora and the intestinal mucosa, which in turn leads to immune activation in the colonic mucosa (Quigley, 2007). In addition to this, some have focused on the possible role of small bowel bacterial overgrowth as a cause of symptoms (Crowell *et al.*, 2005), whereas, previous studies have reported lower numbers of *lactobacilli* and *bifidobacteria* among IBS patients (Verdu & Collins, 2004). Furthermore, investigators in this arena suggest that quantitative and/or qualitative changes in intestinal micro-flora may contribute to sensory-motor dysfunction of IBS (Barbara *et al.*, 2004). Although published studies are contradictory, it would appear that bacteria are one of many irritants within the GI-tract that may lead to persistent or long standing gut dysfunction (Camilleri, 2005).

#### *2.4.2.6 Genetic factors*

It is known that IBS patients commonly give a positive family history of abdominal discomfort/pain (Quigley, 2003). Scientists have elaborated on possible genetic factors that may be involved in the pathogenesis of IBS (Lacy & De Lee, 2005); however, data regarding this issue is still inconclusive (Camilleri, 2005).

#### *2.4.2.7 Abnormal gas handling*

Studies have linked bloating and distension to abnormal gas propulsion. Investigators have found that a certain subset of IBS subjects may have impaired anal evacuation, eventually leading to "gas trapping" (Crowell *et al.*, 2004). Interestingly enough, study results have failed to prove increased gas production among IBS patients (Talley & Spiller, 2002). The problem does not seem to be too much gas but rather abnormal responses to gas, since various data indicate that patients complaining of bloating have impaired transit and tolerance of intestinal gas loads (Azpiroz & Malagelada, 2005).

### 2.4.2.8 *To conclude*

"*IBS may be a central nervous system (CNS) disorder with centrally directed changes in gut sensory and motor activity or a primary gut disturbance with inappropriate input from the CNS*" (Birrer, 2002). Although the above mentioned literature may be considered abundant, it is clear that the pathogenesis is still inadequately understood, unfortunately leading to improper management (Barbara *et al.*, 2004). According to the conceptual model (Figure 2.8) by Ringel and colleagues, IBS should not be viewed upon as a disorder of physiological nor psychological origin, but rather a bio-psychosocial illness in which the deregulation of the brain-gut axis results in imbalances in the intestinal, central and peripheral neurological pathways (Ringel *et al.*, 2001).

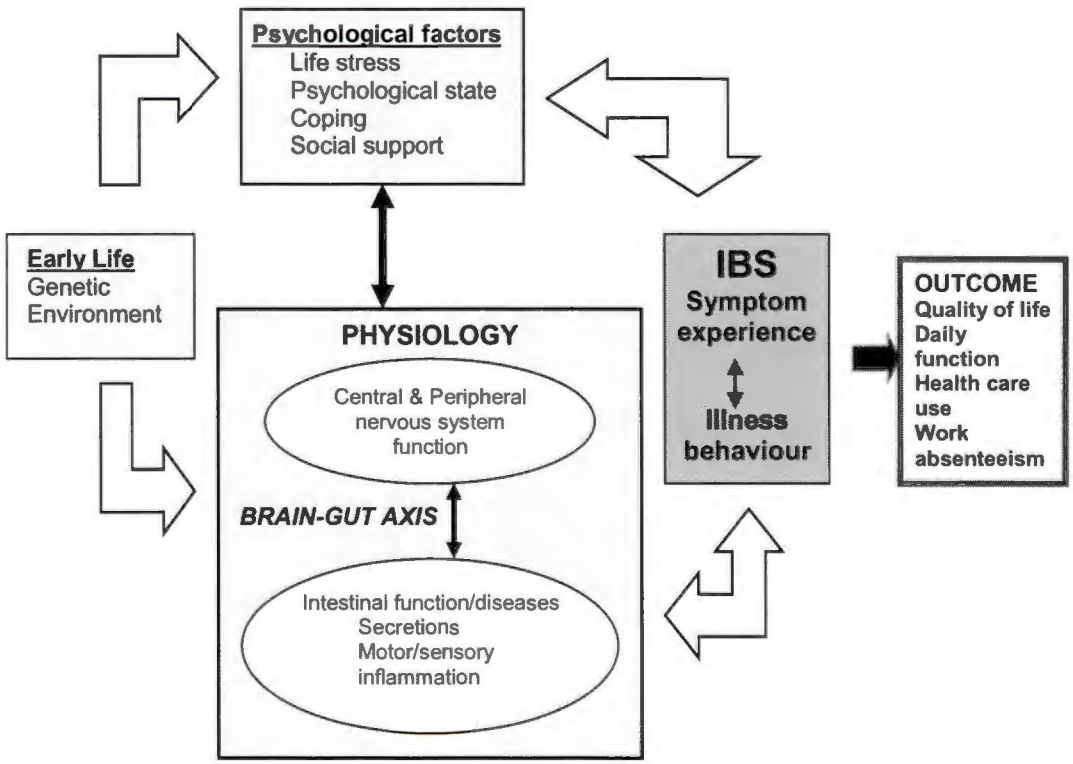


**Figure 2.8: Conceptual model of Irritable bowel syndrome (adapted from Ringel *et al.*, 2001).**

## 2.5 Clinical Presentation

Irritable bowel syndrome is characterised by a constellation of symptoms, including abdominal pain associated with one or more symptoms of altered bowel function (Bueno, 2005). Given the complexity of the symptomatology it is not surprising that previous investigators and physicians made statements like the one by Cumming.

*"The bowels are at one time constipated, at another lax, in the same person...How the disease has two different symptoms I do not profess to explain."*

W. Cumming, London Medical Gazette, 1849

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### 2.5.1 Different signs and symptoms

At present, from acquired knowledge, it is clear that the hallmark signs and symptoms of IBS are abdominal pain relieved by defecation and pain associated with looser or more frequent stools (Viera *et al.*, 2002). In addition to abdominal pain, diarrhoea or constipation, typical symptoms include bloating, flatulence, stool urgency or straining, the feeling of incomplete evacuation and increased amounts of mucus in stool (Andresen & Camilleri, 2006; Farthing, 1999). With regards to the altered bowel habit; patients usually present with predominant constipation, predominant diarrhoea, or alternating symptoms of constipation and diarrhoea (Harmon, 2007). There may be an accompaniment of symptoms, e.g. abdominal bloating may be accompanied by visible distension (Farthing, 1999).

An international survey of 40 000 subjects, indicated that the chief symptoms of IBS are abdominal pain (88%), bloating (80%), trapped wind (66%), tiredness (60%), diarrhoea (59%), tightness of clothing (58%), constipation (53%), and heartburn (47%) (Hungin *et al.*, 2003). Changes in bowel habits and abdominal pain/discomfort were the most common symptoms among 229 IBS patients. Constipation was more frequently reported than diarrhoea (Bellini *et al.*, 2005). In a study by Talley *et al.* (1995) 30% of patients reported a constipation-predominant bowel habit, 30% diarrhoea-predominant, and 30% a pattern that varies between diarrhoea and constipation. IBS may be accompanied by other clinical manifestations, associated with the GI-tract. Population based and large case studies have shown that one to two thirds of subjects with IBS have symptoms that overlap with functional dyspepsia. Other gut symptoms that were reported among IBS patients were heartburn, nausea, vomiting and early satiety (Cremonini & Talley, 2004).

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### 2.5.2 Extra-intestinal symptoms and co-morbid Disorders

Hungin and colleagues (2003) assessed the prevalence of co-morbid diseases among IBS sufferers. The results indicate that IBS sufferers are more prone to suffer from other diseases entities as well, in comparison to non-sufferers. Sufferers reported the following diseases (values in parentheses refer to non-sufferers): gastro-oesophageal reflux disease (GORD), 21% (7%); peptic ulcer, 13% (6%); dyspepsia, 13% (4%); depression, 25% (9%); asthma. 13% (7%). Furthermore, Whitehead and colleagues (2002) have found that certain non-GI disorders are more common among IBS patients than in healthy controls. Headache, back pain, fatigue,

myalgia, dyspareunia, urinary frequency and dizziness were some of the entities that were reported (Whitehead *et al.*, 2002). Psychological factors appear to play a major role in IBS symptomatology. The psychological component of IBS is so well recognised, that IBS was previously refer to as "neurogenic mucus colitis", and thought that IBS is secondary to a nervous disorder (Lacy & De Lee, 2005).

In Western populations, IBS patients experience more depression and anxiety than controls, six years after a formal IBS diagnosis (Jones *et al.*, 2006). In addition, Asian IBS patients also present with various psychological disturbances including anxiety, emotional upset, depression, hysteria, and panic disorder (Chang & Lu, 2006). Approximately 94% of IBS patients suffer from depression, anxiety or somatoform disorders (Holten, 2003).

### 2.5.3 *Symptom characteristics*

Symptoms of IBS patients usually wax and wane over long periods (Drossman *et al.*, 2002; Sandler, 1990). Over short terms (weeks to months) symptoms of IBS occur frequently (Drossman *et al.*, 2002), and may be continuous or intermittent (Birrer, 2002). Furthermore, it was established that individual symptoms occur sequentially rather than simultaneously (Hahn *et al.*, 1998) and high intensity symptoms occur more than 50% of the days over a six-week period (Mearin *et al.*, 2004). Hungin and colleagues established that 69% of sufferers report symptoms lasting for one hour, twice daily, for seven days of a month (Hungin et al., 2003). The dominant symptoms remain constant with 75% of IBS patients having the same set of symptoms over a five-year period (Lembo *et al.*, 1999). The frequency of symptoms is another vast variable among different IBS patients; some may have frequent exacerbations of symptoms, while others may experience long symptom free periods (Hahn *et al.*, 1998).

Abdominal pain, experienced by IBS patients may be located in the hypogastrium (25%), right side (20%), left side (20%), or epigastrium (10%). Pain is generally described as cramping or achy, but may also present as a burning, knifelike, dull feeling and can be diffused, focal or nondescript (Talley *et al.*, 1995). Although pain/discomfort experienced by patients is variable, it often occurs after a meal, during psychological stress, or at the time of menses (Hahn *et al.*, 1998). Symptoms are instable and patients characterised with diarrhoea or constipation predominant IBS, may shift to the alternating diarrhoea/constipation subtype, but rarely shift from D-IBS to C-IBS and vice versa (Mearin *et al.*, 2004). Furthermore, symptoms of IBS and functional dyspepsia overlap significantly and respond similarly to treatment. It has been argued that they are different manifestations of one condition (Videlock & Chang, 2007).

## 2.6 Diagnosis

For many years IBS was considered mainly a disease of exclusion (Christensen, 1992; Snelling, 2006). It is only been recently embraced that IBS should be viewed as a true disease entity and not as a diagnosis of exclusion (Lacy, 2003) but a confirmative objective diagnostic test is still unavailable; therefore the diagnosis of IBS is based on specific clinical features that are consistent with the condition (Drossman *et al.*, 2002; Hatlebakk & Hatlebakk, 2004). This makes IBS quite difficult to diagnose because it relies on the physician's clinical judgement and opinion (Camilleri *et al.*, 2002). Nevertheless, several symptom-based diagnostic criteria have been presented over the years and are found to be disease sensitive, whenever alarm symptoms have been ruled out (Holten, 2003).

### *2.6.1 Diagnostic criteria*

As early as the 1940's it was clear that IBS might be diagnosed after exclusion of organic diseases and identifying symptoms of colonic dysfunction. The main identifiable factor in patients with IBS was cramping lower abdominal pain (Christensen, 1992). Today physicians' remain unsure about the diagnosis (and treatment) of this disorder, and patients are still thinking they are the only ones who suffer from this disorder (Lacy & De Lee, 2005).

Gastroenterologists were slow to adopt the biopsychological model of IBS and other FGID's, as they became intrigued by the rapid technological advancements within the practice of gastroenterology. However, it was only in the 1970's that attention was given to the development of proper symptom-based diagnostic criteria specifically for the condition. In 1978, Manning and colleagues developed the first set of criteria to identify individuals with IBS. Although these criteria were extensively used in clinical practice, its specificity and sensitivity were questioned in various studies (Lacy & De Lee, 2005).

In 1988 Professor Aldo Torsoli initiated a consensus process at the International Congress of Gastroenterology in Rome. The meeting led to the establishment of the Rome Committee. This group of FGID specialists' mission was to address the concerns raised by the Manning criteria (Drossman & Dumitrascu, 2006). In 1992, the result came in the form of a new set of criteria, the Rome I criteria which recommend the diagnosis of IBS only in the presence of the main diagnostic criteria, that is abdominal pain or discomfort associated with chronic altered bowel habit and two or more of the supportive criteria. In contrast the Rome II criteria that appeared in 1998 recommend that the diagnosis of IBS should be based on the presence of two of the three main diagnostic criteria alone. The supportive criteria may then be used to further classify IBS into diarrhoea-predominant or constipation-predominant (Hammer & Talley, 1999). Differences between the Manning, Rome I and II criteria are stipulated in Table 2.2.

**Table 2.2: Comparison of symptom based criteria (adapted from Holten, 2003).**

| Symptom based criteria | Symptoms | Sensitivity | Specificity | Positive predictive value |
|---|---|---|---|---|
| Manning | ▪ Abdominal pain<br>▪ Pain relief with bowel movement<br>▪ More frequent stools with pain<br>▪ Looser stools with pain<br>▪ Mucus in stools<br>▪ Feeling of incomplete evacuation | **24-90%** | **70-100%** | **74%** |
| Rome I | >3 months of continuous or recurrent abdominal pain relieved with defecation or associated with change in stool consistency<br>PLUS: >2 of the following on 25% of days<br>▪ Altered stool frequency<br>▪ Altered stool form<br>▪ Altered stool passage<br>▪ Passage of mucus<br>▪ Bloating or abdominal distension | **65%-84%** | **100%** | **69-100%** |
| Rome II | Abdominal discomfort or pain for at least 12 weeks (not necessarily consecutive) in the preceding 12 months, and having 2 of the 3 following features:<br>▪ Relieved with defecation<br>▪ Onset associated with a change in frequency of stool<br>▪ Onset associated with a change in form (appearance) of stool<br><br><u>Supportive symptoms:</u><br>Fewer than 3 bowel movements per week<br>More than 3 bowel movements per day<br>Hard or lumpy stools | **49-65%** | **100%** | **69-100%** |

The new Rome III criteria for diagnosis of IBS were published in 2006 (Drossman & Dumitrascu, 2006; Videlock & Chang, 2007):

> **Criteria should be filled for the last 3 months with symptom onset at least 6 months before diagnosis.**

**Recurrent abdominal pain or discomfort (discomfort means an uncomfortable sensation not described as pain) at least 3 days per month in the last 3 months that is associated with two or more of the following:**

1. **Improvement with defecation**
2. **Onset associated with a change in frequency of stool**
3. **Onset associated with a change in form (appearance) of stool**

Abnormal stool frequency (<3 bowel movements per week or >3 bowel movements per day); abnormal stool form (lumpy-hard stool or loose watery stool); defecation; straining; urgency; a feeling of incomplete evacuation; and passing mucus and bloating are not part of the diagnostic criteria but are considered supportive symptoms (Videlock & Chang, 2007).

Major changes that were incorporated into the new Rome III criteria were: 1) Symptoms are now recommended to originate 6 months prior to diagnosis and be currently active for 3 months. This timeframe is less restrictive when compared to the Rome II criteria (12 weeks of symptoms over 12 months) and easier to understand and apply in research and clinical practice. 2) It is recommended that IBS-A (alternating IBS) should be reserved for patients with bowel habits that have changed over weeks to months. Patients with diarrhoea and constipation that may alternate within hours or days were classified as IBS-A according to Rome II, but should now be referred to as Mixed IBS (IBS-M). 3) IBS patients change subtypes frequently, with 29% moving from IBS-C to IBS-D within one year. Given this symptom instability, the terms "IBS with diarrhoea" and "IBS with constipation" are preferred over the previously used terms of "diarrhoea- and constipation-predominant IBS". However the bowel sub-typing used in the Rome II criteria is still acceptable (Drossman, 2006; Drossman & Dumitrascu, 2006; Videlock & Chang, 2007).

### *2.6.2 Differential diagnosis*

The differential diagnosis for patients with recurrent abdominal discomfort and bowel dysfunction is quite extensive and the goal of the evaluation should be to rule out organic diseases that mimic IBS (Zaman, 2002). These diseases that are known to mimic the clinical presentation of IBS are; thyroid dysfunction (diarrhoea and constipation), functional dyspepsia (abdominal pain), Crohn's disease or ulcerative colitis (diarrhoea, abdominal pain), celiac sprue (diarrhoea), polyps and cancers (constipation or abdominal pain) and infectious diarrhoea (Holten, 2003). Alarm symptoms are signs or symptoms requiring immediate attention and careful diagnostic evaluation to exclude diagnosis other than IBS (Hadley & Gaarder, 2005). The latter, also known as 'red-flags', should always be excluded before a positive diagnosis can

be made. They are; an age of more than 50, male patients, short history of symptoms, documented weight loss, nocturnal symptoms, family history of colon cancer, rectal bleeding and recent antibiotic use (Spiller, 2007b). The decision to perform tests for the exclusion of organic diseases depends on the patient's age, nature of practice (secondary or tertiary) and geographical location (e.g. older patients are at increased risk of colon cancer in the West; amoebiasisis common among the emerging economies). For example, patients older than 50 years should undergo the following laboratory tests: complete blood count; electrolytes; erythrocyte sedimentation rate; thyroid function tests; and stool studies, including faecal leukocytes, occult blood, ova and parasites. Furthermore, colon cancer and other colonic lesions should be ruled out with proper colon evaluation (e.g. physical examination and colonoscopy). A flexible sigmoidoscopy with biopsy may be considered in patients with diarrhoea to exclude colitis as a cause of symptomatology (Zaman, 2002). In addition, patients under the age of 50 with no alarm symptoms may forgo further testing. However, in certain cases further testing is necessary, for example; celiac sprue and lactose intolerance testing may be considered in patients with diarrhoea that improves or worsens with change in diet (Holten, 2003).

**Table 2.3: Differential diagnosis for IBS (adapted from Hatlebakk & Hatlebakk, 2004).**

| Differential diagnosis of Irritable Bowel Syndrome |
|---|
| Celiac disease |
| Food intolerance |
| Disaccharide intolerance |
| Inflammatory bowel disease |
| Bacterial overgrowth |
| Diverticular disease of the colon |
| Colorectal carcinoma |
| Bile acid induced diarrhoea |

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### *2.6.3 Summary*

Although the Rome criteria have undergone a lot of criticism in the past, symptoms remain the primary means in identifying and recruiting patients for research (Hammer & Talley, 1999). Furthermore, it is currently widely accepted that symptom-based criteria should be used and that excessive testing should be minimised when IBS is suspected in clinical practice (Lacy & De Lee, 2005). In general, any red flag symptoms and signs should be identified, which calls for a more invasive diagnostic approach, including a full colonoscopy. Faecal Calprotectin (f-calprotectin) is a highly stable glycoprotein present in neutrophilic leukocytes and increases whenever these cells are recruited to inflammation or neoplasia. It is analysed from only one

faecal specimen and was found to have a sensitivity and specificity of approximately 90% and 80%, respectively, making it a promising diagnostic parameter for inflammatory bowel disease and neoplasia. However, f-calprotectin could not distinguish properly between benign polyps, inflammation and colorectal cancer. Still, it can be used to exclude any organic pathology in suspected IBS patients. Serologic markers may be analysed to exclude celiac disease, at least in specialist care (Hatlebakk & Hatlebakk, 2004). Unfortunately, many IBS sufferers tend to think they have a serious undiagnosed underlying disease (Ringel *et al.*, 2001); therefore, a colonoscopy is sometimes worth performing to reassure the patient of the absence of any organic pathology. However, a colonoscopy is indicated in patients older than 50, when colorectal cancer becomes a relevant differential diagnosis (Hatlebakk & Hatlebakk, 2004).

In conclusion, if the Rome criteria are fulfilled, alarm signs or red flags are not present, and screening studies from a referral physician are negative, further testing is not needed (Drossman *et al.*, 2002).

## 2.7 Therapeutic options in IBS

### *2.7.1 Treatment goals*

*"From the diverse collection of symptoms it seems unlikely that a single medication can reliably treat all aspects of the syndrome"* (Farthing, 1999). Patients must be informed that progress may be slow and ongoing. A realistic strategy plan must be developed and the patient must be able to adhere to it (Harmon, 2007). Reductions in pain scores and normalisation of bowel habit were traditionally used as treatment goals. Recently the emphasis has been shifted to global scores of overall well-being, since IBS comprises of more than one symptom entity (Farthing, 2004). Moreover, the goal of treatment is to alleviate all symptoms of IBS. Treating altered bowel habits without addressing other IBS symptoms is an inappropriate strategy (Holten, 2003).

All guidelines on IBS treatment acknowledge that there is no clear treatment or cure for IBS, therefore the treatment needs to be individualised (Snelling, 2006) in cases of non-prescription remedy failure (Hadley & Gaarder, 2005). When this is taken in consideration, it is no wonder that so many IBS patients seek relief in complementary and alternative medicine (CAM) (Langmead & Rampton, 2001). Nevertheless, for effective treatment, it is essential that both healthcare provider and patient; 1) acknowledge the relationship between physiological and psychological factors underlying the symptoms and; 2) recognise the need for a bio-psychosocial approach to treatment (Ringel *et al.*, 2001).

## 2.7.2 Standard drug treatment

It is two decades ago that Klein published the devastating critical review of numerous clinical trials, it was reported that none of the existing therapies at that time could be considered plausible in the treatment of IBS (Klein, 1988). Since then many recommendation reviews have appeared and well-designed controlled trials are now possible (Bergmann, 1999; Corazziari *et al.*, 2003; Hawkey, 1999; Irvine *et al.*, 2006).

Despite a lack of understanding of the pathophysiology, numerous treatments have been used to try and treat IBS (Snelling, 2006). Classical therapies can be divided into two main categories, namely; those dedicated to treat predominant symptom (better known as end-organ therapy) and treatment aimed at relieving an associated affective disorder or modifying pain pathways in the central nervous system (Bueno, 2005).

### 2.7.2.1 End-organ therapy

**ANTI-DIARRHOEAL AGENTS:** Diarrhoea-predominant IBS is associated with acceleration of small bowel and proximal colonic transit. The synthetic opioid, loperamide decreases intestinal transit, enhances intestinal water and ion absorption, and increases anal sphincter tone at rest (Efskind *et al.*, 1996; Camilleri, 1999). However, loperamide were found ineffective against abdominal pain and distension (Jailwala *et al.*, 2000) and increased nightly abdominal pain has been documented (Efskind *et al.*, 1996). Generally loperamide is considered safe and is currently the preferred anti-diarrhoeal in IBS (Harmon, 2007). Loperamide is preferred above its structural counterparts, mainly because of two reasons; 1) it has a better side effects profile than codeine phosphate and diphenoxylate, since it does not cross the blood brain barrier and, 2) loperamide has a prolonged duration of action and a more rapid onset of action (Talley, 2003). The recommended dose is 2 to 4 mg up to four times daily (Dunphy & Verne, 2001). These agents are also recommended as a prophylactic in times of anticipated stress (Videlock & Chang, 2007).

Malabsorption of bile acids is a characteristic of functional diarrhoea (Smout *et al.*, 2000), and the presence of luminal irritants, including bile acids have been reported in IBS. Thus, cholestyramine a bile acid sequestering agent, has been successfully used as a second line agent in diarrhoea-predominant IBS associated with malabsorption of bile acids (Camilleri, 1999; Camilleri *et al.*, 2002).

**FIBRE & BULKING AGENTS:** The use of fibre in IBS has been extensively documented in the treatment of constipation-predominant IBS (20-30g/day) (Dunphy & Verne, 2001). Furthermore, recommendation of high fibre diets and the prescription thereof are seen in both primary and secondary care (Bijkerk *et al.*, 2004). It is believed that dietary fibre may enhance

the stools water-holding properties, increase gel-formation to provide lubrication, enhance bulking of the stool, and binding of agents such as bile (Friedman, 1991). Many types of fibre exist, but not all have been reviewed. Synthetic fibres are more soluble than natural fibres but are more likely to cause gaseous symptoms, whereas natural fibres, like psyllium and linseed, are bulking agents with lubricating properties (Jailwala *et al.*, 2000). Bijkerk and colleagues indicated that insoluble fibres are no better than placebo, whereas soluble fibres showed global improvements of symptoms and constipation, but other individual symptoms were left unchanged. It was also documented that fibre does not play any role in pain, associated with IBS. Contrary to what has been believed, it was found that insoluble fibre can actually exacerbate IBS symptoms (Bijkerk *et al.*, 2004). A trial of fibre however may be considered in IBS-C, since it is safe and cost effective (Hadley & Gaarder, 2005).

**LAXATIVES:** Osmotic laxatives are available over-the-counter and are widely used in the treatment of IBS-C and chronic constipation. These agents are well tolerated and are generally safe (Videlock & Chang, 2007), however evidence-based data on the efficacy of these agents in the treatment of constipation predominant and alternating bowel habits are lacking (Tack *et al.*, 2006a). Furthermore, osmotic laxatives including lactulose, sorbitol and milk-of-magnesia have been recommended as second line agents against IBS-C when dietary fibre and fibre supplementation have failed (Talley, 2003). Disappointingly, these agents may worsen abdominal cramps and exacerbate diarrhoea. Although, polyethylene glycol (PEG) solutions may be better tolerated, since less bloating is induced, no randomised controlled trials have tested its efficacy in this particular syndrome (Talley, 2003). Other laxatives that have been prescribed for IBS-C include stimulant laxatives (bisacodyl or cascara) and emollients (docuste), but long term use are discouraged (Johanson, 2004).

**ANTISPASMODICS:** IBS sufferers frequently use antispasmodics (also known as smooth muscle relaxants or myorelaxants). Although several drugs have been evaluated; most trials underwent strong criticism due to various methodological flaws, including small number of study participants, large numbers of dropouts and short duration of trials (Mearin, 2006). In a meta-analysis by Poynard and colleagues (2001), six smooth muscle relaxants were assessed (cimetropium bromide, hyoscine butyl bromide, mebeverine, otilium bromide, pinaverium bromide and trimebutine) in a total of 23 randomised controlled trials. The mean percentage of patients with global improvement was 38% in the placebo group and 56% in the myorelaxant group. Pain improvement in the placebo group was 41%, whereas improvement in the myorelaxant group was 53%. The authors concluded that myorelaxants are superior to placebo in the management of IBS (Poynard *et al.*, 2001). In a systematic review including 13 randomised controlled trials it was evident that antispasmodics were effective for abdominal pain in IBS patients (Jailwala *et al.*, 2000). The available scientific evidence suggests

antispasmodics to be useful in IBS, however new and more conclusive investigations are needed (Mearin, 2006).

**PRO-KINETIC AGENTS:** The recent systematic review of Jailwala *et al.* (2000) could not find significant support for the use of prokinetics in IBS. Prokinetics seems to be ineffective in relieving global symptom scores, but domperidone showed some efficacy in abdominal distension in a crossover trial.

#### *2.7.2.2 Central therapy*

**ANTIDEPRESSANTS:** In a recent meta-analysis twelve randomised controlled trials were identified that evaluated the use of antidepressants in functional gastrointestinal disorders. The studies mainly comprised of tricyclic antidepressants (TCA), including amitriptyline, clomipramine, desipramine, doxepin, and trimipramine. The investigators concluded that these agents appear to be effective; however, whether the improvement is independent of an effect of treatment on depression needs further evaluation (Jackson *et al.*, 2000).

Daily dosages of 10-25 mg that are below the therapeutic range for psychiatric treatment were found effective in most IBS patients (Clouse, 2003). In addition, the American Gastroenterological Association (AGA) mentioned the improvement of abdominal pain through TCA therapy, however inadequate evidence is reported to support global symptom improvement (Drossman *et al.*, 2002).

Amitriptyline may be an attractive agent if the patient suffers from coexistent sleep disturbances. Desipramine and nortryptyline are less sedating and may be considered in IBS patients, whenever anti-cholinergic and anti-histaminic effects become intolerable (Videlock & Chang, 2007). However, neither desipramine nor nortriptilyne are available from the South African pharmaceutical industry, and good substitutes with similar characteristics would be dothiepin and lofepramine (Gibbon *et al.*, 2005). Furthermore, it is emphasised that antidepressants should be reserved for patients with severe IBS symptoms (i.e. daily or persistent pain) (Lesbros-Pantoflickova *et al.*, 2004), especially patients with severe pain, with diarrhoea predominance (not for constipation since, constipation is a side effect of TCA) and/or co-morbid depression (Mearin, 2006).

The selective serotonin re-uptake inhibitors (SSRI's) have less side effects than the TCA and may benefit patients with constipation predominance as they accelerate oroceacal transit time; thus making this drug class attractive (Talley, 2003). Paroxetine and fluoxetine have been evaluated in high quality studies for the treatment of IBS, however data are limited and no consistent improvement have been found with SSRI's (Tack *et al.*, 2006a). In a recent 12-week prospective study, fluoxetine, sertraline and paroxetine were assessed for efficacy in IBS (Tosic

Golubovic *et al.*, 2006). Abdominal pain, depression, anxiety and other symptoms associated with IBS were significantly reduced after 12 weeks of SSRI therapy. Unfortunately, the lack of a placebo-controlled group and a small number of participants were highlighted as study limitations (Tosic Golubovic *et al.*, 2006). The investigators concluded that SSRI's are very effective in alleviating various symptoms associated with IBS; however, randomised, double-blind, placebo-controlled studies are necessary to confirm SSRI's as a treatment strategy in IBS patients (Tosic Golubovic *et al.*, 2006). Nevertheless, some investigators have recommended the use of SSRI's in IBS patients that have not responded to first-line treatments (Creed, 2006). Most recently, two antidepressants have been evaluated in a first of its kind, double blind, placebo controlled trial, in which citalopram (SSRI), imipramine and placebo were independently compared with each other. The authors found no significant differences in global adequate relief between the anti-depressants and placebo. However, it seems that imipramine was responsible for improvements in some bowel symptom scores and psychological function compared to citalopram and placebo (Talley *et al.*, 2008).

In general, antidepressants must be used on a continual rather than an as-needed basis therefore they are reserved for patients having frequently recurrent or continual symptoms (Camilleri *et al.*, 2002).

**ANXIOLYTICS:** Benzodiazepines and other anxiolytics have been used before for the treatment of IBS, however little evidence exists to support the use of these agents. The American Gastroenterology Association (AGA) recommends that these drugs should be prescribed with caution as physical dependence is associated with its use and when prescribed, it should be for a self-limited period of not more than 1-3 weeks (Drossman *et al.*, 2002).

## *2.7.3 Potential drug targets and novel pharmacological approaches*

This section will be restricted to previously evaluated drugs in humans, and drugs undergoing animal testing alone will not be discussed.

### *2.7.3.1 Serotonin modifying drugs*

A number of therapeutic targets have been studied in the field of IBS; however the serotonin modifying drugs are by far the most promising (Farthing, 2004). Serotonin is extensively distributed in the gut, as previously mentioned (refer to 2.4.2.4).

**$5HT_4$- RECEPTOR AGONISTS:** These receptors are located on neurons in the myenteric plexus, and are found on primary afferent neurons, smooth muscle cells, and enterochromaffin cells. Release of serotonin from enterochromaffin cells results in peristalsis, via $5HT_4$ receptors on the primary afferents (Talley, 2003). Various drugs are under investigation, both in animal and

human studies. Cisapride accelerates gastric emptying, but has little effect on the colon. Due to QT prolongation this drug has been removed from the pharmaceutical market, both in the UK, USA and elsewhere (Kamm, 2002).

Tegaserod, an aminoguanide indole derivative, is a partial $5HT_4$ receptor agonist that increases colonic transit, inhibits visceral sensitivity, stimulates the release of neurotransmitters and increases colonic motility (Hadley & Gaarder, 2005). In addition, it appears that tegaserod stimulates water and chloride secretion as well (Camilleri, 2001). Three large phase III randomized, double-blinded, and placebo-controlled trials were performed predominantly in females (>85%) with constipation-predominant irritable bowel syndrome. The results have shown superior efficacy in comparison to placebo as assessed by the subject's global assessment of relief with significant improvement in secondary endpoints such as abdominal pain, bowel frequency and consistency. The predominant side effect is transient diarrhoea (Camilleri, 2001). At first, Tegaserod, were considered safe as there has been no problem with arrhythmias and other cardiovascular events (Kamm, 2002), however post-marketing studies led to the withdrawal of the agent in several countries, including South Africa (Pasricha, 2007). After nearly 5 years of tegaserod marketing, Swiss regulators discovered 13 (including one death) ischemic cardiovascular events in a large population of more than 11 000 users compared with only one incidence in the placebo group. After these reports the Food and Drug Administration of USA (FDA) recommended its withdrawal, however the FDA stated that they will work with Novartis to create a controlled prescription system for tegaserod. These findings are considered obscure, since tegaserod is a moderate antagonist of $5HT_{1B}$ and $5HT_{2B}$ receptors; however, vasoconstriction results from activation not inhibition of $5HT_{1B}$ receptors (Pasricha, 2007).

Prucalopride, also $5HT_4$ receptor selective, enhanced visceral sensitivity, improved stool frequency, and modified pain and bloating in patients with constipation in phase 3 studies (Kamm, 2005)

**$5HT_3$- RECEPTOR ANTAGONISTS:** Since it came clear that $5HT_3$ receptors are present on visceral afferent nerves, it was hypothesised that such drugs may modify disorders of visceral sensitivity. Furthermore, $5HT_3$ receptors were identified in the spine of rats (Kamm, 2002). Blocking $5HT_3$ receptors leads to reduced contractility, slowed colonic transit and increased fluid absorption, and is therefore of clinical relevance in chronic diarrhoea (Talley, 2001) The $5HT_3$ receptor antagonist, alosetron was the result of a series of animal and human studies in the field of visceral sensitivity. Since then alosetron has been widely evaluated in several clinical trials, most of them reported positive effects compared to placebo (Kamm, 2002).

The drug decreases pain, urgency, and stool frequency in women with diarrhoea-predominant IBS (Camilleri *et al.*, 2000). In a meta-analysis, six large multi-centre trials using good methodological study design reported that alosetron 1 mg twice daily significantly improved global scores of pain and altered bowel function in non-constipated IBS patients. The average number of patients that had to be treated with alosetron for one patient to achieve improvement over placebo treatment was seven. One in four patients treated with alosetron may develop constipation. However, the efficacy of alosetron is unclear in male patients (Cremonini *et al.*, 2003).

Approximately one to two in 1000 patients reported serious events of ischemic colitis, which led to the withdrawal of alosetron in 2001. The drug was reintroduced in the USA in November 2002 and is currently available under restricted prescribing programme for women with severe diarrhoea predominant IBS. Mandatory physician training and certification as well as patient inform consent is needed before the drug is prescribed in the USA. Unfortunately, neither alosetron nor the programme is available in South Africa (Johanson, 2004).

**MIXED $5HT_4$- RECEPTOR AGONISTS — $5HT_3$- RECEPTOR ANTAGONISTS:** Renzapride is a compound that has both $5HT_4$ receptor agonist and $5HT_3$ receptor antagonist activity and is currently under clinical development for IBS-C. This promising agent may have pro-kinetic properties through $5HT_4$ receptor activation, while minimising the primary side effect of $5HT_3$ receptor antagonists, constipation (Bradesi & Mayer, 2007). Preliminary data showed that renzapride relieves abdominal pain and discomfort in IBS-C, and for male and female patients with mixed symptoms of diarrhoea and constipation (Tack *et al.*, 2006b).

#### *2.7.3.2 Alpha2 adrenoceptor agonists*

In a small study that enrolled 44 IBS-D patients, 67% of the patients treated with clonidine 0.1 mg twice daily compared with 46% in the placebo group achieved satisfactory relief. Clonidine further succeeded in improving altered bowel function without effecting normal gastrointestinal transit. The proposed mechanism, which was demonstrated in earlier studies, is thought to be associated with the $\alpha 2$ receptor and its effect on human colonic and rectal motor and sensory functions. Overall this study suggests beneficial effects of $\alpha 2$ adrenergic agents in IBS-D (Camilleri *et al.*, 2003). Although, the observed adverse effects (drowsiness, dizziness and dry mouth) decreased after one week of treatment these agents' usefulness may be limited (Bradesi & Mayer, 2007). For these reasons, gut selective $\alpha 2$ adrenergic receptor agonists may be favoured above clonidine in the future (Camilleri, 2004).

#### 2.7.3.3 *Anti-cholinergic agents*

Acetylcholine is the main excitatory neurotransmitter in the gastrointestinal tract. Newer anti-cholinergic drugs have been developed to specifically target the muscarinic type-3 receptor ($M_3$) in the intestinal smooth muscle in order to decrease the non-specific anti-cholinergic adverse effects of dry mouth and increased heart rate (Andresen & Camilleri, 2006). The $M_3$ antagonist zamifenacin significantly reduced colonic motility without non-specific anti-cholinergic effects in a study of 36 patients (Houghton *et al.*, 1997).

#### 2.7.3.4 *Neurokinin receptor modulators*

The neurokinin receptor 1 (NK1) and its peptide ligand substance P (tachykinin) are widely distributed in the central nervous system, but also in visceral fibres and enteric sensory neurons. NK1 antagonism could lead to alleviated anxiety and improvements in major depression. Secondly and most importantly, NK1 antagonists have anti-inflammatory and anti-secretory properties in the bowel. Preliminary data from NK1 receptor antagonist, CJ-11974 that is derived from aprepitant, used for the inhibition of chemotherapy-induced emesis, showed beneficial effects with regards to motor and sensory dysfunctions in IBS (Camilleri, 2004). Unfortunately, published data supporting the use of NK1 receptors in the treatment of IBS is limited (Bradesi & Mayer, 2007).

#### 2.7.3.5 *Chloride channel activators*

Volume regulated $Cl^-$ channels such as the $Cl^-$ channel type 2 ($ClC_2$) and $ClC_3$ are found in the gastrointestinal tract and the liver of mammals and are responsible for various cellular functions. Lubiprostone is a $ClC_2$ activator that has been approved by the Food and Drug Administration (FDA) in January 2006 for the treatment of chronic idiopathic constipation after several clinical trials have shown positive effects on stool consistency, frequency and straining. In addition, lubiprostone has minimal side effects, of which nausea and transient diarrhoea are the commonest. This drug may be used for IBS-C in the near future (Andresen & Camilleri, 2006).

#### 2.7.3.6 *Opioid agents*

The three major opioid receptors (K, μ, б), found throughout the peripheral and central nervous system, are plausible targets for novel drugs in the treatment of IBS. μ-Agonists such as codeine or morphine are known to modulate visceral nociception and to slow down gastrointestinal transit, resulting in constipation and central side effects. Asimadoline a K-receptor agonist was shown to reduce sensation in response to non-noxious distensions when applied to colon. It was found that asimadoline had no significant effects on gastrointestinal or colonic transit (Andresen & Camilleri, 2006). Fedotozine, another selective K-agonist improves

abdominal symptoms (pain and bloating) in IBS at a dose of 30 mg three times daily compared to placebo treatment (Farthing, 1998). Decreasing afferent neuronal activity is the proposed mode of action (De Schryver & Samsom, 2000). It is likely to see more opioid restricted receptor antagonists and agonists for the treatment of IBS in the near future (Andresen & Camilleri, 2006).

#### *2.7.3.7 Cholecystokinin-A antagonists*

Cholecystokinin (CCK) is known to stimulate colonic smooth muscle in response to meals. Loxiglumide is a CCK antagonist that has been found to reduce colonic transit and increase the number of bowel movements in geriatric patients with chronic constipation (De Schryver & Samson, 2000). Dexloxiglumide, an enantiomer of loxiglumide and twice as potent, improved abdominal pain and discomfort in female IBS-C patients compared to placebo in a phase II trial. However, in two large phase III trials inefficacy for IBS symptom relief was reported (Andresen & Camilleri, 2006).

#### *2.7.3.8 Drugs that modify intestinal bowel flora and anti-inflammatory drugs*

Recently there have been attempts to modify a possible inflammatory component in IBS including attempts to modify the bowel flora (refer to 2.4.2.5) (with antibiotics and probiotics) as well as treatment with anti-inflammatory drugs (Farthing, 2004).

**PROBIOTICS:** Probiotics are non-pathogenic bacteria that may offer beneficial effects for IBS sufferers. Given the fact that IBS has a multi-factorial aetiology, these agents may be promising as they have various modes of action. Probiotics can alter pathogenic bacterial adherence to the bowel wall through physical barriers, alter epithelial surface glycosylation patterns and increase mucin production. Other modes of action include secretion of antimicrobial peptides and modulation of the immune system (Borowiec & Fedorak, 2007). Probiotics may influence IBS mechanisms through immune function, intra-luminal milieu and motility. Numerous inflammatory mediators have been altered after probiotic administration. The *lactobacillus* and *bifidobacterium* species have demonstrated anti-inflammatory changes in interleukin and tissue necrosis factor-$\alpha$ concentration (Camilleri, 2006).

Inconsistent results have been published in studies, little population groups have been used and methodological flaws have been made. Nevertheless, there are well-designed randomised controlled trials that have claimed probiotics to be effective in the management of at least some of the symptoms of IBS (Camilleri, 2006). Before the report by Borowiec & Fedorak (2007), 15 double-blind, randomised-controlled trials had been published of which 11 showed probiotics as beneficial in treating IBS. *Lactobacillus-* and *Bifidobacterium* species have been extensively evaluated in literature, both single and in combination with other strains. A large number of

treated patients have reported global improvement with various *Lactobacillus* species; however these effects are strain and species dependant. *L. plantarum* showed reductions in flatulence and pain scores (Borowiec & Fedorak, 2007). One study, considered to be the most significant study ever on probiotics and IBS, has indicated positive results with *B. infantis* (alleviated various bowel symptoms of IBS) and these results were correlated by a pathophysiological mechanism (Whorwell *et al.*, 2006). VSL#3 another well-documented probiotic, consisting of *Lactobacillus*, *Bifidibacterium* and *Streptococcus* strains, was effective in reducing flatulence and bloating scores (Kim *et al.*, 2003; Kim *et al.*, 2005).

It seems that further research is needed before probiotics can live up to their therapeutic expectations in IBS (Barbara & Corinaldesi, 2000). However, the safety profile, growing understanding of probiotic mechanisms of action, successful pilot studies and brand new evidence suggests that probiotics may be a plausible alternative treatment modality in IBS (Borowiec & Fedorak, 2007; Penner *et al.*, 2005).

**ANTIBIOTICS:** A significant number of IBS patients that meet diagnostic criteria were found to have concomitant small bowel bacterial overgrowth. Recent studies showed that IBS symptoms could be alleviated with antibiotic treatment and that this improvement correlated with normalisation of the lactulose breath test results. When neomycin was employed, 25% of patients recovered from their IBS symptoms, but side effects and low efficacy has led to the search for alternative antibiotics (Pimentel *et al.* 2003). Other antibiotics that shared the same low efficacy rate as neomycin were doxycycline and amoxicillin–clavulanate. Rifaximin an unabsorbed oral antibiotic were administered to IBS patients for 10 consecutive days. Eighty-seven patients either received rifaximin 400 mg three times daily or placebo and after the 10 day course patients were followed up for 10 weeks. Recipients of rifaximin reported increased improvement in overall IBS symptoms, especially bloating for 10 weeks after discontinuation of the therapy (Pimentel *et al.*, 2006).

It is necessary to emphasise that antibiotic treatment in IBS can only be fully recommended as soon as the relation between bacterial overgrowth and IBS symptoms are clarified (Bradesi & Mayer, 2007). In addition, the high prevalence of small bowel bacterial overgrowth in IBS is controversial, since other studies using the glucose hydrogen test, which is more sensitive for the detection of bacterial overgrowth than the lactulose hydrogen breath test, have reported a small prevalence (Andresen & Camilleri, 2006). In summary, short-term therapy with either antibiotics or probiotics seems to reduce symptoms among IBS patients. However, in the long term, safety issues will favour the probiotic approach; results of long-term studies with these agents are eagerly awaited (Quigley, 2007).

**ANTI-INFLAMMATORY DRUGS:** It is widely accepted that PI-IBS is associated with increased serotonin-containing enterochromaffin cells and lymphocytes in the rectum. In addition, animal studies have suggested that steroids may suppress changes in neuromuscular function, due to PI-IBS. A recent study aimed at improving PI-IBS symptoms and reducing the number of enterochromaffin cells, by administering prednisolone 30 mg/day to PI-IBS sufferers. Twenty-nine patients with PI-IBS were enrolled in a RCT to receive prednisolone or placebo for 3 weeks. Disappointingly, the prednisolone group did not reduce the number of enterochromaffin cells neither did it improve any PI-IBS symptoms compared to the placebo group. The role of anti-inflammatory drugs in IBS awaits further investigation (Dunlop *et al.*, 2003). According to Crentsil (2004), budesonide a corticosteroid with a high mucosal activity and low bioavailability might be promising and is worth investigating in diarrhoea-predominant IBS.

#### *2.7.3.9 Other potential novel drugs in the treatment of IBS*

**GUANYLATE CYCLASE C (GC-C) AGONISTS:**

GC-C is a trans-membrane protein located in the gut epithelium and may be a valuable target for treating chronic constipation. Intestinal fluid secretion increases and colonic fluid absorption decreases whenever GC-C is activated. Linaclotide, a GC-C agonist was safe, well-tolerated, decreased stool consistency, and increased stool frequency in human phase 1 studies. Furthermore, a recent RCT in 36 women showed that linaclotide significantly accelerated ascending colon emptying and colonic transit at 48h, improved stool consistency, stool frequency, ease of passage at 1000 µg compared to placebo (Bradesi & Mayer, 2007).

**SOMATOSTATIN ANALOGUES:** Ocreotide has been shown to have certain anti-nociceptive properties that are not inhibited by opioid receptor antagonism. Furthermore, this somatostatin analogue has other gastrointestinal effects including reduction in gastrointestinal secretion and retardation of gastrointestinal transit. Various investigators have labelled ocreotide as a promising candidate in future treatment of IBS (Farthing, 1998). Further investigation is awaited to clarify this statement.

**CORTICOTROPIN-RELASING FACTOR RECEPTOR ANTAGONISTS:** The corticotropin-releasing factor (CRF) is considered to play an important role in the brain-gut axis in times of stress. It is accepted that a relation between IBS and an increased gastrointestinal response to stress exists, therefore making the CRF antagonists (e.g. antalarmin) plausible agents in treating IBS. CRH-9-41, a CRF antagonist significantly suppressed the colonic motility induced by electrical stimulation in IBS patients. In addition, no suppression of plasma adrenocorticotropic hormone and serum cortisol levels were reported. However, the clinical relevance of these agents is limited by the fact that there is currently no oral formulation available (Andresen & Camilleri, 2007).

Other drugs that are in some form of development for IBS application are 2,3-benzodiazepine (dextofisopam) and novel antidepressants that are both serotonin and noradrenergic reuptake inhibitors (e.g. duloxetine) (Videlock & Chang, 2007).

### 2.7.4 Complimentary and alternative treatment

*"The terms alternative and complimentary medicine (CAM) denote theories and practices of medicine which deviate from the conventional, the former when they are used instead of, and the latter when they applied as an adjunct to standard management"* (Langmead & Rampton, 2001).

Since conventional therapies leave up to 25% of sufferers without relief of symptoms (Francis & Whorwell, 1997), it is not surprisingly that many IBS sufferers have turned to alternative therapies (Koloski *et al.*, 2003). In addition, the budget of the National Centre for Complimentary and Alternative Medicine in the United Kingdom rose from $2 million in 1993 to a devastating $70 million in 2000 (Langmead & Rampton, 2001).

Many patients prefer alternative therapy above conventional therapy, as they view them as natural, safe and time tested (Videlock & Chang, 2007). Between 11% and 43% of patients with gastrointestinal disorders use alternative or complementary techniques, and many consider them beneficial (Spanier *et al.*, 2003). Herbal medicines are now used by up to 50% of the Western population, in a substantial minority of instances for both the treatment and/or prevention of digestive diseases (Langmead & Rampton, 2001). However, CAM has shown limited proven efficacy in previous controlled trials (Drossman *et al.*, 2002). One exception of the latter is a standardised Chinese Herbal formulation that has been found significantly efficient in a randomised controlled trial (RCT) compared with placebo. The formulation was effective in relieving bowel symptoms (Bensoussan *et al.*, 1998).

Several over-the-counter remedies are available, of which peppermint oil is the most familiar of all. Eight randomised controlled trials have indicated its efficacy in the management of IBS symptoms (Pittler & Ernst, 1998), including abdominal discomfort, bloating and IBS symptoms in general (Hussain & Quigley, 2006). The most distressing side effect is heartburn (May *et al.*, 2000), this can be debilitating since heartburn is already a symptom of the disorder that is being treated (Hungin *et al.*, 2003). A meta-analysis of five randomised controlled studies has supported its use. However, in view of the flawed methodological design and small number of subjects, the efficacy of peppermint oil has not been completely established as an agent for the treatment of IBS (Pittler & Ernst, 1998). The investigators concluded that well designed studies are needed to shed light on this issue (Pittler & Ernst, 1998).

Numerous scientists have ascribed the effect of CAM due to the high placebo response seen in IBS (Patel *et al.*, 2005; Vožeh, 2005); as a matter of fact CAM has been referred to as the new placebo (Hussain & Quigley, 2006).

Other therapies in addition to conventional therapies that have been previously evaluated were acupuncture (Hussain & Quigley, 2006), oral cromolyn sodium (Spanier *et al.*, 2003) and various traditional and herbal therapies (Langmead & Rampton, 2001). Since it is unclear what must be included under the heading "Complimentary and Alternative Medicine" (Hussain & Quigley, 2006), some authors have placed probiotics among this confusing term (Spanier *et al.*, 2003; Hussain & Quigley, 2006)

Although a lack of scientific evidence exists for these agents (Drossman *et al.*, 2002), its popularity is increasing (Langmead & Rampton, 2001) and clinicians should be thoroughly acquainted with these treatments and should establish whether there is any evidence for their efficacy (Videlock & Chang, 2007).

### *2.7.5 Behavioural treatment in IBS*

Since there is an overlap of psychological disorders and IBS, behavioural therapies have been studied as treatments in IBS (Talley *et al.*, 1996).

The classification of these therapies may be confusing, since some authors refer to them as psychological therapies and group them as hypnotherapy, psychotherapy and behaviour therapy (Spanier *et al.*, 2003), whereas Brandt an colleagues have used the term behavioural treatment (Brandt *et al.*, 2002). However, for the purpose of this paper it will be referred to as behavioural therapies and different types are listed out in Table 2.4 (Brandt *et al.*, 2002).

**Table 2.4: Different behavioural therapies for treatment of IBS (adapted from Brandt *et al.*, 2002).**

| Type of therapy | Description |
|---|---|
| 1 Relaxation therapy | Based on the hypothesis that stress stimulates the autonomic nervous system and exacerbates IBS symptoms. Patients learn techniques to release tension and relax. |
| 2 Biofeedback | Patients learn to sense changes in rectal distension and to regulate bowel habits using biofeedback tools |
| 3 Hypnotherapy | Based on the hypothesis that hypnotherapy may induce a lasting state of relaxation and may positively affect gut motility and intestinal smooth muscle contraction |
| 4 Cognitive therapy | Based on the hypothesis that patient's response to stressful events exacerbates IBS symptoms. In cognitive therapy, patients accept responsibility for their IBS symptoms, learn to identify stressful events and actively seek solutions to these events. |
| 5 Psychotherapy | Based on the hypothesis that IBS symptoms may be a manifestation of traumatic life events. Psychotherapy provides insight about these life events and the association between these events and the IBS symptoms. Through this insight, patients may experience long-term resolution of IBS symptoms. |

Given the fact that previous studies lacked proper blinding and overall high quality methodology, no proven efficacious therapy among these practices exist. However, there are some data that suggests that relaxation therapy, hypnotherapy, biofeedback, cognitive therapy, and psychotherapy may improve individual symptoms of IBS. Furthermore, improvement in individual IBS symptoms correlated with improvement in psychological symptoms in most trials (Brandt *et al.*, 2002). Gut-directed hypnotherapy (GDH) is a type of hypnosis that has been used for the past twenty years (Whorwell *et al.*, 1984). GDH is based on the use of hypnotic induction, using progressive relaxation and other techniques, followed by imagery directed towards control and normalisation of gut function (Wilson *et al.*, 2006). This therapy further aims at teaching patients to conduct autohypnosis, in order to manage their own symptoms without reliance on primary and secondary care; however refreshment courses may be needed. A recent systematic review suggests that hypnotherapy may be effective in the management of IBS of long duration with severe impact on quality of life, however randomised placebo controlled trials of high internal validity are needed (Wilson *et al.*, 2006). These studies are highly prone to selection bias as enrolled patients generally view this therapy favourably. Several studies are further hampered by the absence of a true control group (Spanier *et al.*, 2003). For the time being, this form of therapy should be restricted to severe forms of refractory IBS that is practised in specialists care settings (Wilson *et al.*, 2006).

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### 2.7.6 *Dietary considerations and other interventions*

This is yet another controversial field, since results have been published (Burden, 2001). While true food allergy is extremely uncommon, food intolerance is found to be quite common among sufferers (Spanier *et al.*, 2003). Exclusion diets to identify intolerance in IBS should be revaluated (Burden, 2001), as severe restrictions in diet may impair quality of life of IBS patients even further (Mearin, 2006). However, food that has been found to provoke symptoms is wheat, dairy products and coffee/tea (Burden, 2001). Carbohydrate intolerance in IBS is probably much more common than carbohydrate malabsorption (Spanier *et al.*, 2003). High fibre diets are considered one of the primary treatment strategies of IBS, however little support for this recommendation was found in a systematic review by Bijkerk and colleagues (Bijkerk *et al.*, 2004).

The Environmental Nutrition, a newsletter on food and nutrition recommends the following to help manage symptoms: 1) Eat a variety of foods from all food groups, including whole grains, vegetables, fruits, lean meats, poultry and low-fat dairy products; 2) Eat small, frequent meals, as they are often better tolerated; 3) Eat at the same time each day to help normalise bowel movements; 4) Experiment with fibre cautiously, increasing it gradually with lots of fluid; 5) Avoid problem foods, such as gas producers (e.g. beans, cruciferous vegetables), or greasy or fatty foods and carbonated drinks; 6) Exercise regularly for stress relief and to help stimulate normal bowel contractions (Golub, 2005).

**Table 2.5: Guidelines for the dietary treatment of patients with IBS (adapted from Burden, 2001).**

| **Aim**<br><br>*To help patients to control their symptoms of IBS* | |
|---|---|
| **Objectives** | |
| Dietary assessment | *This is essential for all patients referred with IBS to determine their present dietary intake. A 7-day diet history supported by a food and symptoms diary. Any unusual or abnormal eating practices need to be assessed in relation to the patient's symptoms.* |
| Symptoms | *Inquiry with regard to symptoms suffered and the history of their onset is essential. It is important to determine if the onset of symptoms is related to any changes in the individual's eating pattern.* |
| Regular meals | *A regular meal pattern should be encouraged with all patients* |
| Caffeine | *The amount of tea/coffee consumed should be assessed. Excessive intakes should be avoided. A lethal dose of caffeine is between 3 and 10 g, and therefore 22 cups of coffee containing 143 mg of caffeine could potentially provide a lethal dose. However, symptoms of IBS may well be exacerbated by much less.* |
| Non-starch polysaccharides (NSP) | *An assessment of the type and quantity of NSP's consumed should be made. The addition of bran should be encouraged along with large proportions of insoluble fibre in the diet unless the individual feels this is of direct benefit in symptom control. More emphasis should be placed on increasing the proportion of foods containing a higher concentration of soluble NSP's.* |
| Fluid | *An assessment of the fluid intake of patients is essential and critical if a patient's predominant symptom is constipation. If an increase in soluble NSPs is recommended, this is essential and also important in patients who have a high dietary intake of insoluble NSPs.* |
| Wheat | *The form in which wheat is ingested needs to be assessed. A trial of a wheat-free diet may be helpful for some patients to relieve symptoms.* |
| Lactose sensitivity | *A trial of a milk-free or lactose-free diet should be tried in those patients in whom diary products are associated with symptoms. If patients continue on this diet, their calcium intake should be assessed and the supplements recommended if necessary.* |
| Sorbitol/Sorbitol-fructose malabsorbtion | *If a dietary assessment reveals patients are taking large quantities of sorbitol in slimming mixtures, this should be discouraged, especially if their predominant symptoms are pain or diarrhoea.* |
| Food intolerance | *Exclusion diets should only be tried when patients complain of multiple food intolerances and single food avoidance has not helped control symptoms.* |

In general, clinicians should encourage regular exercise, provision of appropriate time for eating and defecation and a balanced diet should be recommended (Thompson, 2002). The cause of IBS is still unknown but it is unlikely that food is responsible for all the symptoms, therefore

dietary modification in the treatment of IBS patients has to rely upon the assessment of the individual's symptoms in relation to a detailed evaluation of their habitual intake (Burden, 2001).

In addition some sweeteners (e.g. sorbitol, fructose) have laxative-like effects just like antibiotics and antacids and may exaggerate symptoms in some patients (Maxwell *et al.*, 2002; Thompson, 2002).

### 2.7.7 Conclusion

Most IBS sufferers require no drugs. Nevertheless, certain complaints may impair social and occupational functioning to such an extent, that a drug, sometimes more than one, might be needed to control intermittent symptoms (Thompson, 2002). It should be emphasised that seeking one single therapy to treat the diverse group of symptoms is impossible (Farthing, 1998). In general, treatment choices for IBS depend on the pattern and severity. Pattern being either constipation or diarrhoea (or alternating between diarrhoea and constipation) and severity usually reflects whether symptoms are mild or severe (Clouse, 2003). Research to date has focused on treating disturbed gastrointestinal motility, altered perception and psychological factors, the three main mechanisms contributing to the development if IBS. Several promising agents are in the developmental stage and include antispasmodics, such as $M_3$ receptor antagonists, $NK_2$ receptor antagonists and calcium channel blockers that may be beneficial in IBS with diarrhoea. Prucalopride and tegaserod, selective $5HT_4$ receptor agonists, improved colonic transit and reduced symptoms in patients with IBS-C (De Schryver & Samsom, 2000). Unfortunately some of these drugs have been withdrawn or are under restricted prescribing programs, due to post-marketing side effects (Bradesi & Mayer, 2007). Patients with increased visceral hypersensitivity may find relief in drugs that alter visceral perception, such as $5HT_3$ receptor antagonists, K–agonists, ocreotide and leuprolide (De Schryder & Samsom, 2000). The most promising candidates for IBS treatment appear to be the NK antagonists and corticotropin-releasing factor, since they may act at multiple sites along the brain-gut axis (Parischa, 2007).

Psychological treatments may be considered if all other treatments have failed (Wilson, 2006). Many authors assigned the serotonin modifying drugs (e.g. alosetron and tegaserod) as steps in the right direction in treating IBS, since global improvement of symptoms have been extensively reported (Johanson, 2004). Unfortunately, neither of these drugs is registered for use in South Africa.

For the time being physicians should follow current algorithmic guidelines, with the exception of some of the interventions as they are not always available in all countries (tegaserod and

alosetron is not available from the South African Pharmaceutical Market as mentioned - refer to the algorithm by Hadley and Gaarder, 2005, figure 2.9).

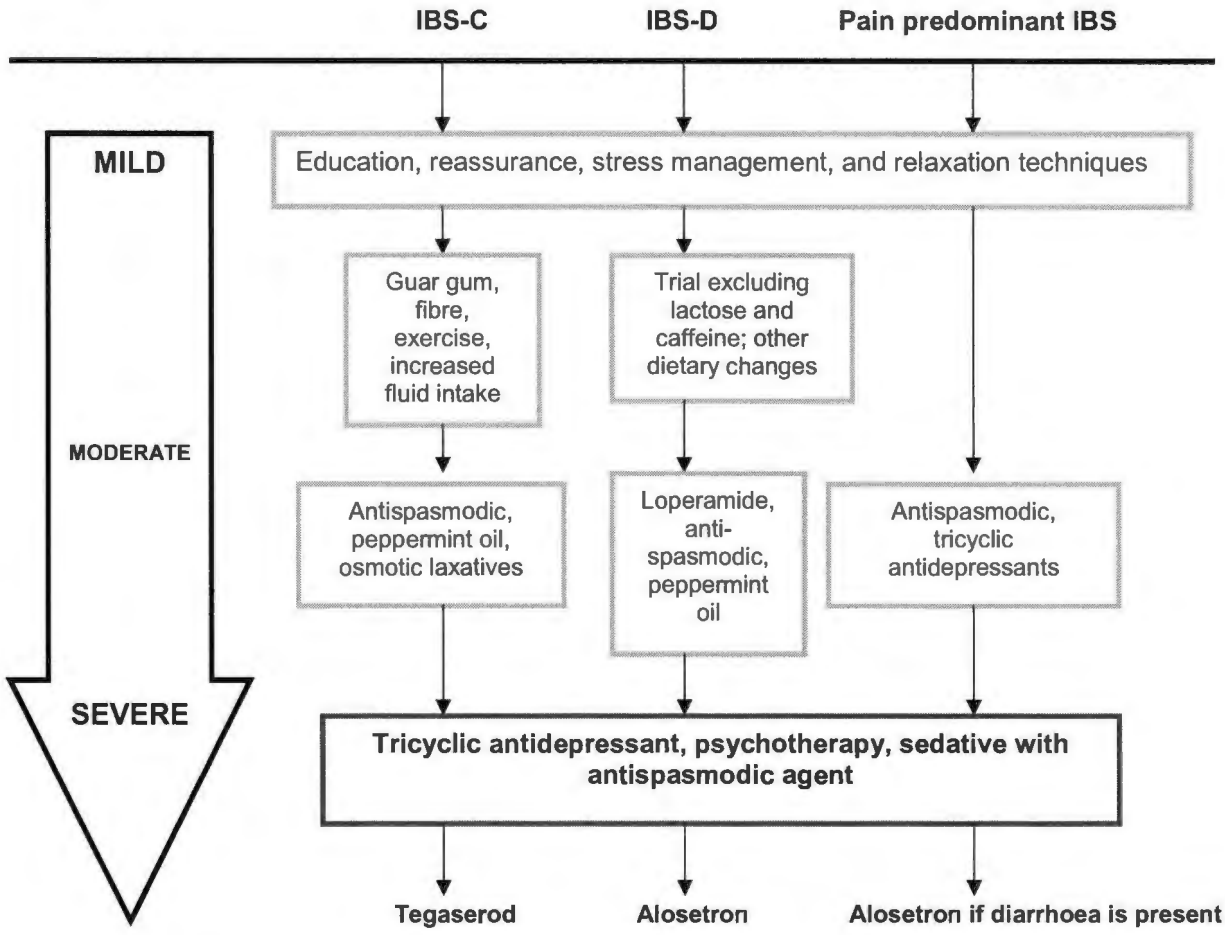



**Figure 2.9: Algorithm for the management of IBS (adapted from Hadley & Gaarder, 2005).**

# 2.8 Irritable Bowel Syndrome in special population groups

## *2.8.1 Gender related*

More women than men have IBS. Studies in the UK, US and Europe indicated that women outnumber men by ≥2:1. In community based studies the female:male ratio among persons with IBS-like symptoms, vary from 1:1 to 2>1. The female:male ratio in primary health care in the United States is 3:1, however in gastroenterology clinics this ratio increases to 4:1 or 5:1. Data from UK and Canada, suggest that 75% of patients referred to specialist clinics for IBS symptoms are female (Payne, 2004).

These differences, seen in a wide spectrum of health care, may be attributed to various factors, mainly listed as physiological and psychological components. Physiological factors responsible for differences in prevalence may be attributed to gender differences in gastrointestinal transit time, visceral sensitivity, central nervous system pain processing, and specific effects of oestrogen and progesterone on gut function. Additional factors include gender related differences in neuroendocrine, autonomic nervous system, and stress activity, which are related to bowel function and pain. Psychological factors that may play a role in difference in prevalence between men and women are gender related differences in somatization, depression, and anxiety as well as history of sexual abuse. (Chang & Heitkemper, 2002; Toner & Akman, 2000).

It is proposed that a model is needed to conceptualise the influences of both sex-linked biology and gender relations on IBS (Payne, 2004). Furthermore, men and women respond differently towards drugs and are not only limited to prevalence. For instance, the therapeutic effect of serotonergic agents has different benefits between men and women. Although these differences have been observed numerously, less is known about the potential differences in responsiveness to non-drug therapies (Chang & Heitkemper, 2002).

### 2.8.2 Adolescents and children

IBS is not only restricted to adulthood, and is emerging as a recognised disease in both adolescents and children. Although recently updated symptom based criteria and knowledge of the brain-gut axis has grown, the diagnosis is still based on a physical examination and a patient history. Treatment varies in the sense that few medications have proven efficacious in this population group. Still, the child with IBS is best evaluated and treated in the context of a bio-psychological model of care to relief symptoms and disability, similar as adult patients. Newer treatments recently implemented in the armamentarium of treatment are relaxation and distraction. In addition, research is awaited to address the prevalence, diagnosis and treatment of IBS among children and adolescents (Nakayama & Horiuchi, 2006; McOmber & Shulman, 2007).

### 2.8.3 The elderly

It is suggested that IBS decreases by age, but available data indicate that 10% to 20% of elderly people in the general population suffer from IBS. An increasing age is unlikely to alter the pathogenesis, but as the perception of pain changes with age IBS symptoms may decline, as patients get older. However, clinicians have hypothesised that the disease is still very common and under recognised in people older than 65. Treatment options are hampered by high frequency of reported adverse effects and a lack of support for efficacy of treatment in this

age group. Therefore, treatment remains empirical, symptomatic and largely unsatisfactory (Bennett & Talley, 2002; O'Keefe & Talley, 1991; Sasaki *et al.*, 2006).

Furthermore, as constipation are over diagnosed and over treated in the elderly, IBS should be considered in the differential diagnosis whenever symptoms present in the form of abdominal pain and altered bowel habits (Morley, 2007).

## 2.9 Prognosis

The clinical manifestation of symptoms may never be alleviated but can vary in intensity and lessen with age. Patients that have post-infectious IBS may see symptom resolution sooner than others (McLaughlin, 2003).

One hundred and twelve patients were diagnosed in Olmsted County, Minnesota, USA during the period of 1961-1963 and were followed-up for 29 years. Study investigators concluded that when diagnosed using current criteria, IBS is associated with a good prognosis and the diagnosis is unlikely to change into organic disease. Furthermore, a positive physician-patient relationship can result in IBS patients having reduced health services usage (Owens *et al.*, 1995). Harvey and colleagues (1987) assessed a total of 104 IBS diagnosed patients for 5 years. Each patient received current treatment including aggressive high fibre diets and bulking agents. Eighty five percent of patients were found virtually symptom free in a few weeks time, and 68% were still symptom free after 5 years. Response to treatment was better in men than in women, in those with constipation than diarrhoea, when symptoms had initially been triggered by an episode of acute diarrhoea, and in patients with a relatively short history. In conclusion, a few simple investigations, sympathetic explanation, and appropriate treatment will ensure that most patients with IBS have a good prognosis (Harvey *et al.*, 1987).

Chapter 3 will elaborate on clinical trials in IBS. It will shed some light on the conduct of IBS trials and the challenges that are faced by clinical trialists.

# CHAPTER 3 CLINICAL DRUG TRIALS IN IRRITABLE BOWEL SYNDROME

## 3.1 Clinical trials in general

### 3.1.1 Background

Clinical trials are the gold standard by which new treatments are tested and judged. Several different trials are conducted: 1) Treatment trials test new drugs, treatments, and devices or assess new interventions, such as a new surgical or radiological approach. 2) Primary prevention trials seek to detect better ways to prevent a first onset of disease, whereas secondary prevention trials seek optimal way of controlling the progression of a disease or side effects of treatment. 3) Screening trials investigate a variety of approaches to disease detection or the identification of health conditions or pre-clinical predictors of increased risk of developing a disease. 4) Quality of life trials (also known as supportive trials attempt to identify approaches to improving the lived experience, among those with a disease) (Fullerton & Sadler, 2004).

The first refernce to a clinical trial are found in the book of Daniel, in the Holy Bible. Daniel compared the health effects of a vegetarian diet with those of a royal Babylonian diet over a ten-day period. The study had numerous methodological flaws compared to contemporary standards but was done more than 2000 years ago and had actually been influential for over two millennia (Enkin, 2002). James Lind did the first clinical trial, as it is known today, aboard a ship in 1747. He identified 12 sailors with scurvy (condition caused by lack of vitamin C), each having similar symptoms and characteristics. The group of sailors was placed in a separate compartment whilst receiving a common diet. After a while, the sailors were allocated into six groups of different interventions. Each of the six pairs received one of the following treatments:

1) Twenty five drops of elixir of vitriol (copper or iron sulphate) 3 times a day on an empty stomach;

2) Two spoonfuls of vinegar 3 times a day;

3) A quart of cider each day;

4) A half pint of seawater every day;

5) A medicinal paste of garlic, mustard, myrrh, and balsam of Peru; or

6) 2 oranges and 1 lemon daily.

Both sailors receiving citrus fruit were ready for duty after only six days. These results were later considered as groundbreaking. James Lind's study with the sailors is an example of direct comparison of two or more treatments in human beings who have the same disease – what is known today as a clinical trial (Waning & Montagne, 2001).

In essence, the randomised controlled trial (RCT) is a study in which people are allocated at random to receive one of several clinical interventions (Jadad, 2002). Today the RCT is considered one of the simplest, most powerful and revolutionary tools of research. Furthermore, it is widely accepted that the best method for determining the true therapeutic benefit of a new unknown treatment is the randomised, controlled clinical trial (Waning & Montagne, 2001).

Clinical investigations that involve the development of new therapies proceed in phases (Fullerton & Sadler, 2004). Phase I trials are the first studies to be conducted in humans to evaluate a new drug. These studies will commence after the safety and potential efficacy has been established in animals. As the investigators bear no knowledge regarding the effects of the new drug, phase I trials tend to focus primarily on safety and are mostly conducted on healthy volunteers. After the safety of a new drug has been documented in phase I trials, investigators can proceed to conduct phase II trials. These are trials in which the new drug is given to small groups of patients with a given condition. The aim of phase II trials is to establish the efficacy of different doses and frequencies of administration. Even though these trials focus on efficacy, it can provide additional information on the safety of new drug (Jadad, 2002). From as few as 25 to as many as a few hundred patients are required to complete a Phase II trial (Fullerton & Sadler, 2004). If the drug is judged to be ineffective or excessively toxic, no more trials will be conducted. However, if the drug produces a good response and participants tolerate its adverse effects, the investigators may consider phase III trials. Phase III trials are designed and conducted once a new drug has been shown to be reasonably effective and safe in phase II trials. Most of the times, phase III trials are effectiveness studies, as they seek to compare the new drug with an existing drug or intervention known to be effective. Some studies seek to monitor adverse effects of a new drug after it has been approved for marketing (post-marketing surveillance) and are referred to as phase IV trials. Phase IV trials are large studies and are not RCT's (Jadad, 2002).

## 3.2 Ethical considerations

### 3.2.1 History of ethical review

The Nuremberg Code (World War II) is widely accepted to have defined the foundation of discussions on the ethics of clinical research. Since then many international efforts have been released to produce guidelines that have universal applicability and can deal with issues relevant within the existing research environment, with future ethical challenges. The Declaration of Helsinki, an international effort first published in 1964 and revised several times, clarified and interpreted the principles as outlined by the Nuremburg Code. It also acknowledged the crucial relevance of clinical research as an important societal strategy for improving human welfare. Other international efforts (e.g. the Belmont report) and guidelines for particular types of clinical research (especially studies involving investigational new drugs), developed by governmental agencies have appeared since then. These groups have augmented the more general policies and procedures established for all types of research involving human subjects (Markman & Markman, 2007).

It is recommended that every health district should have a local research ethics committee (REC) to provide independent advice to participants, researchers, employers and care organisations on the extent to which proposals for research studies should comply with the recognised ethical standards. The REC main purpose should be to review the study to ensure that the dignity, the rights and safety of research participants are protected under all circumstances (Green & Pace, 2006).

### 3.2.2 Ethical research guidelines

Each participant must be adequately informed of the aims, methods, anticipated benefits and potential risks of the study before consent is given. The participant should be aware of the right to abstain from participation, or to withdraw participation at any time without reprisal. As research is susceptible to bias, it is recommended that the treatment is compared to a proper control. An ethical dilemma presents itself when the control group also requires treatment. Providing the control group with the best standard therapy and comparing its benefits, risks, and effectiveness to the new treatment overcome this. However, this does not exclude the use of placebos in studies where no proven treatment exists (Green & Pace, 2006).

The use of placebos in RCT's has been the main topic of debate over the past decade in ethical human research (Miller & Brody, 2002; Resnik, 2008; Senn, 2002). Placebo-controlled trials are generally more reliable, providing their own check of internal validity and more conclusive proof of efficacy. They do not always mean that the participant receives no treatment because other

medications may be permitted in both groups and rescue medication can be prescribed for specific circumstances (Green & Pace, 2004). However, studies that have used placebo run-in periods instead of baseline observation periods are heavily criticised. Participants are given placebo in a single-blind fashion and therefore some have labelled this method as a violation of consent (Senn, 2002).

Randomisation is recommended, since both researchers and participants have their own expectations and hopes that may influence study outcomes. Researchers should not choose which participants receive treatment or which receive the control, but a valid method of randomisation should be used. Therefore, the researchers and participants must be blinded to study treatment. However, randomisation and reasons for it should be thoroughly discussed with potential participants before their consent (Green & Pace, 2004).

Moreover, estimating the correct sample size is essential when planning a trial. The investigator should ensure that there is sufficient power to detect a treatment effect as statistically significant. If a trial with negative results has insufficient power, a clinically important effect may be missed. Such a trial may be deemed unethical because participants are potentially at risk without apparent benefit (Green & Pace, 2004; Irvine et al., 2006).

### 3.2.3 Avoiding exploitation

People that may benefit from clinical research include the researchers, research sponsors or institutions, as well as people with the disease or condition and especially the participants directly involved. Exploitation happens when **participants** do not receive a fair share of the benefits of research. For example, a phase II clinical trial of a new diabetes medication that requires the participants in the control group to receive a placebo only would be exploitative, because there are inexpensive, effective treatments available for diabetes and not taking medication for diabetes has serious health consequences. Furthermore exploitation can also occur when a **population** (e.g. country) does not receive a fair share of the benefits of research. If a new drug is being tested in an impoverished developing country and the drug will be so expensive that few people living in the country will have access to the drug when testing is complete, it is considered as exploitation (Resnik, 2008).

### 3.2.4 Conclusion

Sadly, evidence exist that the mere documentation of regulatory requirements, such as submittal of data to an ethical review committee, has seemed to have gained far greater importance than ensuring that the research team is both respecting the rights of human beings and carefully monitoring each participant's course on the research programme (Markman & Markman, 2007). The aims, design, and methodology of the study must be justifiable, verifiable,

scientifically valid and ethical (Green & Pace, 2006). Changing the primary outcome at the time of analysis is considered unethical. Secondary data analysis that leads to some sort of positive results may be regarded as hypothesis generating and not hypothesis testing (Veldhuyzen van Zanten *et al.*, 1999). Although environmental health investigations usually do not have professional obligations to provide medical care to research participants, they have ethical obligations to avoid exploiting them. Withholding interventions from research participants is ethical, provided that it does not lead to exploitation of individuals or groups. To avoid exploiting individuals or groups, investigators should ensure that research participants and study populations receive a fair share of the benefits of research (Resnik, 2008). Patients need to learn why clinical trials benefit us all, but according to Senn (2002) deception of participants as in the use of placebo run-ins has no part in modern society (Senn, 2002).

## 3.3 Challenges in study design of Irritable Bowel Syndrome Clinical Trials

Researchers are faced with numerous challenges when designing and planning therapeutic drug trials in IBS. Clinical trials in IBS are difficult to design because, unlike organic disease entities, irritable bowel syndrome (IBS) lacks a biological marker and its diagnosis is based on symptom criteria (Corazziari *et al.*, 2003).

The condition being treated is polymorphous, there are many possible endpoints, and most therapies have only been marginally better than placebo (Spiller, 1999). Furthermore, it was quite difficult to evaluate past clinical trials, since inadequate patient definitions were used. The high placebo response seen in IBS patients enrolled in trials is also bothersome. The research nurse (or investigator) will reassure and often-correct misconception, during the frequent attendances, and in fact is providing a crude form of cognitive behavioural therapy; hence the placebo effect will remain high. In addition, the dropout rates might be increased whenever the investigators are instructed not to provide reassurance. For this reasons, the placebo effect is often considered to be unavoidable. Researchers are further challenged by selecting the most appropriate study duration, and the often the least favourable part of IBS trials, recruitment (Corazziari *et al.*, 2003; Spiller, 1998).

## 3.4 Recommendations for study design

This section is a summary of mainly two recommendation reports, one by a European collaboration led by Corazziari (2003) and the other by Irvine and colleagues (2006), whom are predominantly from North-America. As evidence-based medicine becomes more recognised, the general requirement of a trial in IBS should incorporate the principles of best and usual clinical practice as much as possible. This is to ensure that the study results are relevant to the real practice situation (Veldhuyzen van Zanten *et al.*, 1999).

### *3.4.1 Patient population and characteristics*

Because of noticeable instability of subgroups over time, it is preferred that studies include a broad spectrum of patients, as defined by the primary Rome diagnostic criteria. The most important patient characteristics to report include age, gender, race, symptom severity, duration of disease, prior treatments for the study condition, as well as the response thereof, and the use of concomitant medications, including over-the-counter drugs. Depending on the hypothesis, it is generally recognised that both genders should be sufficiently represented in the trial (Irvine *et al.*, 2006).

### *3.4.2 Clinical Trial Design*

Although many controversies exist within various fields of FGID's trial designs, it would appear that researchers agree that the double-blind, placebo-controlled trial with parallel group assignment is the most robust, when assessing the efficacy of a new treatment (Corazziari *et al.*, 2003; Irvine *et al.*, 2006; Veldhuyzen van Zanten *et al.*, 1999).

Crossover trials have been used before in FGID trials, however its design may pose several complications. Order or sequence effects are well-recognised problems in crossover designs, often referred to as period-by-treatment interaction, and occurs when the response in the second period is partly dictated by the response in the first period. Given the known variability in severity of symptoms, it is unlikely that a return to baseline takes place in most cases. Irvine and fellow workers also mentioned that the crossover design is best avoided (Irvine *et al.*, 2006).

Novel designs may lead the future in FGID trials, for instance if all participants are started on the active treatment to assess their improvement. Then, if improvement indeed occurs the responders are randomised in either placebo or active treatment. This design is generally referred to as the double-blind, randomised-withdrawal design and have been successfully

utilised in inflammatory bowel disease (Hawthorne *et al.*, 1992; Veldhuyzen van Zanten *et al.*, 1999). To date there have not been similar designs used in IBS trials.

### 3.4.3 Baseline observation versus placebo run-in

A baseline observation period is regarded relevant for various reasons, including the following (Corazziari *et al.*, 2003):

1) To assess the severity and duration of irritable bowel syndrome so they can be compared between active and placebo groups, and;

2) So that participants can become familiarised with data systems of data collection

A lack of knowledge about the natural history of IBS and the unpredictable fluctuation of symptoms make the choice of duration of the baseline period difficult. Most RCT's in IBS have employed a 2-week baseline phase and it seems to be appropriate (Camilleri *et al.*, 2003; Corazziari *et al.*, 2003; Kim *et al.*, 2005; Leung *et al.*, 2006). The majority of previous RCT's in IBS have used a placebo-run in period. Patients who significantly improved were excluded to reduce the high placebo response. Although this is considered acceptable by regulatory agencies (Irvine *et al.*, 2006), the placebo run-in is discouraged and open to selection bias: 1) dropouts due to rapid improvements in symptoms; 2) different responses of dropouts relative to those entering the trial; 3) study refusal due to an unwillingness to receive no active medication for a period of time; and 4) unpredictable prolongation of the placebo effect during the trial period (Corazziari *et al.*, 2003).

To conclude, the disadvantages of a placebo run-in appear to outweigh the benefit and it is best avoided (Irvine *et al.*, 2006). Moreover, the placebo run-in is usually introduced in a single-blinded fashion (participant blinded), considered to be a violation of consent and is therefore recommended to eliminate them completely from all clinical trial designs (Senn, 2002). Nevertheless, baseline observations are recommended (Irvine *et al.*, 2006).

### 3.4.4 Duration of treatment

Earlier reports suggested that the duration of an IBS clinical trial should be longer than 3 months (Spiller, 1998) or between 8-12 weeks (Veldhuyzen van Zanten *et al.*, 1999), more recent evidence show that a trial of 1-3 months is adequate for efficacy trials (Corazziari *et al.*, 2003; Irvine *et al.*, 2006). Investigators are encouraged to perform a follow-up on patients after treatment has been stopped, but it may not be necessary for short acting drugs (Veldhuyzen van Zanten *et al.*, 1999).

### 3.4.5 Timing of intervention

The timing of all interventions should be carefully considered, since there might be spontaneous improvement amongst participants after recent investigations. For instance, when diagnostic tests rule out any underlying diseases patients will often be reassured. Although there are no profound literature guidelines available on the optimal timing of diagnostic tests it is worthwhile to avoid testing immediately before randomisation (Veldhuyzen van Zanten *et al.*, 1999). However, another report emphasised that delaying randomisation for too long may encourage patients to withdraw from the trial. Since both the placebo and active treatment groups will be affected in a similar way by these changes there will be no bias introduced to either of the groups. Probably the best practical solution would be to do all diagnostic testing before the 2-week baseline observation period (Corazziari *et al.*, 2003).

### 3.4.6 Outcome measures

*"The success of a clinical trial is judged by achieving a statistically significant difference on a clinically relevant primary endpoint"* (Camilleri *et al.*, 2007).

Outcome in IBS treatment trials can be assessed by using either a global scale, a validated symptom questionnaire, or a quality of life instrument (Bijkerk *et al.*, 2003). The primary outcome variable provides the basis for judging the success or failure of an intervention. The Food and Drug Administration (FDA) has recommended that investigators classify the criteria for responders and non-responders prior to study procedures. Most trials also include secondary outcome variables to 1) strengthen the results through concordance between individual symptoms and the primary outcome measure, 2) address the mechanism of the intervention, 3) assess the safety or 4) cost effectiveness of the treatment, and 5) identify variables that predict which patients are most or least likely to benefit (Irvine *et al.*, 2006).

Although there is a growing interest in IBS research, there is yet no consensus agreement regarding the preferred outcome measure in IBS clinical trials (Bijkerk *et al.*, 2003), however there has been significant progress in the design of clinical trials during the last decade (Camilleri *et al.*, 2007). The Rome III Committee recently reviewed two binary (adequate relief and satisfactory relief) and one integrative (IBS Severity Scoring System) measures as primary endpoints. The adequate relief question "In the past seven days have you had overall adequate relief of your IBS symptoms" offers a dichotomous yes/no response. According to the committee, the adequate relief is responsive, reproducible and moves in the same direction as other meaningful measures, thus displaying strong reliability and validity. It has also been previously accepted by the FDA as a clinically relevant indicator of symptom improvement for the approval of novel drugs. The satisfactory relief uses a similar construct as adequate relief;

however, less psychometric evidence has been published with satisfactory relief. The adequate relief appears to be the most robust of all. The review by the Rome III Committee suggests that at present this measure should be recognised by regulatory authorities as a standard endpoint, and it should be acceptable as a primary endpoint in trials in IBS until future research demonstrates it should be replaced by a better endpoint (Camilleri *et al.*, 2007).

A European-based panel independently rated five IBS symptom scales and five IBS health related quality of life (HRQoL) scales. The adequate relief questionnaire scored best and is considered easy to interpret and appropriate for use. Moreover, the adequate relief scales, demonstrate responsiveness, as well as face and construct validity. The panel concluded that the Adequate Relief question is the measure of choice when assessing global symptomatology as an outcome in IBS studies. The IBS Severity Scoring System by Francis *et al.* is preferable, when a more detailed IBS symptom assessment is required and may be appropriate as a secondary endpoint (Bijkerk *et al.*, 2003). In addition, the IBS-SSS is the only IBS severity scale that has been shown to be responsive to treatment effects (Irvine *et al.*, 2006). Finally the IBS Quality of Life measurement scale, developed by Patrick and colleagues, can be used to determine changes in health-related quality of life (Bijkerk *et al.*, 2003; Patrick *et al.*, 1998). It seems that various instruments exist, however; the ones used by patients are preferred to physicians' symptom ratings (Drossman, 2005).

Furthermore, as there is no consensus on the preferred outcome measure; the definition of a responder also remains a topic of debate (Irvine *et al.*, 2006). However, recommendations by collaborative committees and groups are thoroughly described in literature (Corazziari *et al.*, 2003; Drossman, 2005; Irvine *et al.*, 2006). Typically in IBS clinical trials a responder rate of 60% compared to a placebo rate of 45% is typical, giving an effect size difference of 15% (Drossman, 2005). Whatever the case may be, investigators should define a responder prior to study interventions, thus enabling the trial to determine which patients achieve a previously stipulated clinical endpoint of response, and what proportion these patients compose of all participants. This rate would then be compared to the response rate of the placebo condition (Drossman, 2005). In a condition in which there is very little successful treatment, a 50% improvement in the primary efficacy endpoint seems to be a reasonable definition of a responder. In addition, a 10-15% improvement of the global outcome measure over placebo could be considered as a clinically therapeutic gain (Corazziari *et al.*, 2003). In various trials responders were classified as patients who reported, "yes" to adequate relief or satisfactory relief on at least half of the weeks in the treatment trial. When using an integrative symptom questionnaire participants are asked to report severity prior, and then after treatment have started. A responder can then be defined as a participant who reports 50% decrease in IBS symptom severity (Irvine *et al.*, 2006).

## 3.5 Recruitment of participants for IBS trials

Irritable Bowel Syndrome is a common problem in the general population, in patients in primary care (general practice, family medicine, and primary specialist care) and in secondary care (hospital, usually ambulatory care) settings, and is also seen in tertiary referral centres (Jones, 1999). Primary physicians manage and treat most patients that seek medical care. Nevertheless, previous treatment trials have been conducted on patients referred to specialists (Longstreth *et al.*, 2001). As recruitment continues to be an immense challenge to RCT's (Chin Feman *et al.*, 2008), the source of recruitment should never be neglected when study results are interpreted (Jones, 1999; Longstreth *et al.*, 2001). It is generally thought that patients from primary care have less severe IBS and less prominent psychosocial factors than patients in tertiary care (Longstreth *et al.*, 2001). Differences in disease severity and associated cofactors manifested by patients recruited from different sources into clinical trials could significantly influence their response to treatment. This variation could easily hamper the generalisation of study results. For instance, it was recently found that both IBS and Inflammatory bowel disease (IBD) sufferers from Internet recruitment had significantly poorer quality of life than subjects recruited from clinics (Jones *et al.*, 2007). The hypothesis that IBS patients from different recruitment resources may vary in characteristics was previously investigated by Longstreth and colleagues (2001). To describe and compare the characteristics of different groups of IBS, patients were recruited from three different resources and examined. Primary care patients were anxious, smokers and daily alcohol drinkers who had sought recent care for IBS and tried antispasmodic drugs. Their symptoms were intermediate in severity between those of the other two groups. Patients recruited from advertisements, were the oldest, most highly educated, most often depressed, and were least likely to have sought care recently for symptoms. These participants were moderate in symptom severity. Gastroenterologist patients tended to be anxious and had nearly all sought care recently for symptoms, which were the most severe (Longstreth *et al.*, 2001). According to Jones both primary- and secondary care are appropriate settings when recruiting IBS participants for treatment trials. The recruitment strategy, however, should be carefully considered in primary care. With regards to secondary care, it is probably the ideal setting for doing a classic drug randomised controlled trial but may be problematic in the view of uncertain generalisation and transferability of hospital research to primary care (Jones, 1999).

An analysis of recruitment methods used in a single centre IBS trial was recently conducted. Chin Feman *et al.* (2008) evaluated the effectiveness of physician referred newspaper advertisements; flyers, audio and video as well as relatively new methods, not previously extensively reported, such as Internet ads, ads in mass-transit vehicles and movie theatre previews. The investigators reported the fraction of cost each method consumed and the

fraction of recruitment each method generated. Furthermore, using a novel metric, referred to as *the efficacy index*, the investigators were able to compare the effectiveness of each method. Physician referrals and flyers were the most effective recruitment method in the trial. However, all the methods contributed in a synergistically manner. A relatively small number of patients are willing and eligible to participate in clinical trials but the large number of trials competing to accrue patients, delay the flow of innovative treatments to the public; thus making recruitment a troublesome barrier to overcome (Fullerton & Sadler, 2004).

## 3.6 Entry criteria for IBS clinical trials

### *3.6.1 Diagnostic criteria*

It is widely accepted that the first necessity in the conduct of IBS trials is to utilise well-defined diagnostic criteria. The Rome II criteria have become standard of preference (Corazziari *et al.*, 2003; Drossman, 2005).

### *3.6.2 Inclusion and exclusion criteria*

Second to proper diagnostic criteria, is ensuring appropriate exclusions. Previous trials have restricted themselves by including participants who have been experiencing two or more IBS episodes per week. This is to rule out any floor effects; if a patient is doing well there is little room to detect an improvement. Most importantly, the generalisation of projected trial findings to patients outside the trial is an important factor to consider (Drossman, 2005).

Generally, patients eligible for inclusion should have 1) a negative physical examination; 2) normal full blood count and sedimentation rate; 3) absence of ova and parasites in the stool; 4) no fever; 5) negative findings at colonoscopy or sigmoidoscopy and double-contrast enema, performed after the onset of irritable bowel syndrome symptoms within the previous five years (Corazziari *et al.*, 2003).

Various literature has recommended extensive laboratory testing before the inclusion of IBS patients in a clinical trial (Bergmann, 1999). However, these tests may hold some implications from a financial point of view. A recent consensus view is that the minimum evaluation should include a full blood count, imaging of the relevant part of the gastrointestinal tract within the previous five years, and other investigations determined by symptoms and family history (Corazziari *et al.*, 2003). Emerging evidence suggests that screening IBS patients for gluten enteropathy may also be desirable (Irvine *et al.*, 2006).

Alarm symptoms/conditions (weight loss, nocturnal predominant symptoms, progressive deterioration of symptoms, family history of colorectal cancer or inflammatory bowel disease) have been listed as rigorous exclusion criteria however, such cases may be considered eligible if a proper work-up has excluded organic disease and confirmed a diagnosis of IBS (Corazziari *et al.*, 2003).

The main indications for exclusion are (Corazziari *et al.*, 2003):

- *Patients over 50 years of age who have not had a colonoscopy and patients of 50 years or younger who have not had a colonoscopy or sigmoidoscopy after the onset of IBS symptoms and within the previous 5 years;*
- *Patients with relevant abnormalities on physical examination;*
- *Patients with an abnormal blood count or elevated sedimentation rate;*
- *Clinically evident disturbed behaviour and major psychiatric disorders;*
- *Female patients whose symptoms are suggestive of an underlying gynaecological disorder;*
- *Patients with suspected lactose intolerance, and;*
- *Patients with celiac disease.*

In conclusion, it is advisable to include as broad a spectrum of IBS patients as possible as defined by the Rome criteria. Restricting the study population must be justified and inclusion and exclusion criteria must be specified (Irvine *et al.*, 2006). Furthermore, IBS may be considered if the patient complains of abdominal pain and altered bowel habits in the absence of a structural or biochemical marker. Furthermore, extensive and expensive testing procedures are sometimes invaluable when patients, without alarm symptoms, fulfil the Rome criteria (Holten, 2003).

## 3.7 Statistical analysis and data reporting

It is widely accepted that the success of a clinical trial is judged by achieving a statistically significant difference on a clinically relevant primary endpoint (Camilleri *et al.*, 2007). Whether the study is negative or positive, the conclusions should be based on the primary outcome, which is stated in the protocol before the study commences. A participant that responds in a sustained fashion is superior to patients that are responding from time to time. It is further

acknowledged that the statistical analysis should primarily be based on an intention-to-treat (ITT) analysis. Secondary outcome measures should be analysed through descriptive statistics. In conclusion, the results of all outcome measures should state the estimated effect of the intervention (difference between active and placebo treatment) and a 95% confidence interval (Veldhuyzen van Zanten *et al.*, 1999). Statistically significant differences between study groups can also be expressed using a P value. It may be worthwhile to weigh the number needed to treat (NNT) against the number needed to harm (NNH). These calculations allow the researcher to more quantitatively assess the benefits and risks of any given therapy (Irvine *et al.*, 2006).

The study must have sufficient power to detect a minimal clinically important difference (MCID). Previous studies have used a power of 80% (type II error of 20%) and type I error of 5% using a two-sided test. Dropouts should be kept at a minimum, in the range of 10-20% (Veldhuyzen van Zanten *et al.*, 1999).

The Consolidated Standards of Reporting Trials (CONSORT) statement is designed to help minimise confusion and promote clarity in reporting the methods and results of randomised trials. Clinical trialists and investigators should adhere to the CONSORT guidelines when reporting study results of clinical trials (Irvine *et al.*, 2006; Shapiro, 2001). Emphasis has been placed on the necessity of a detailed flowchart that describes the progression of participants through the study (Refer to figure 3.1).

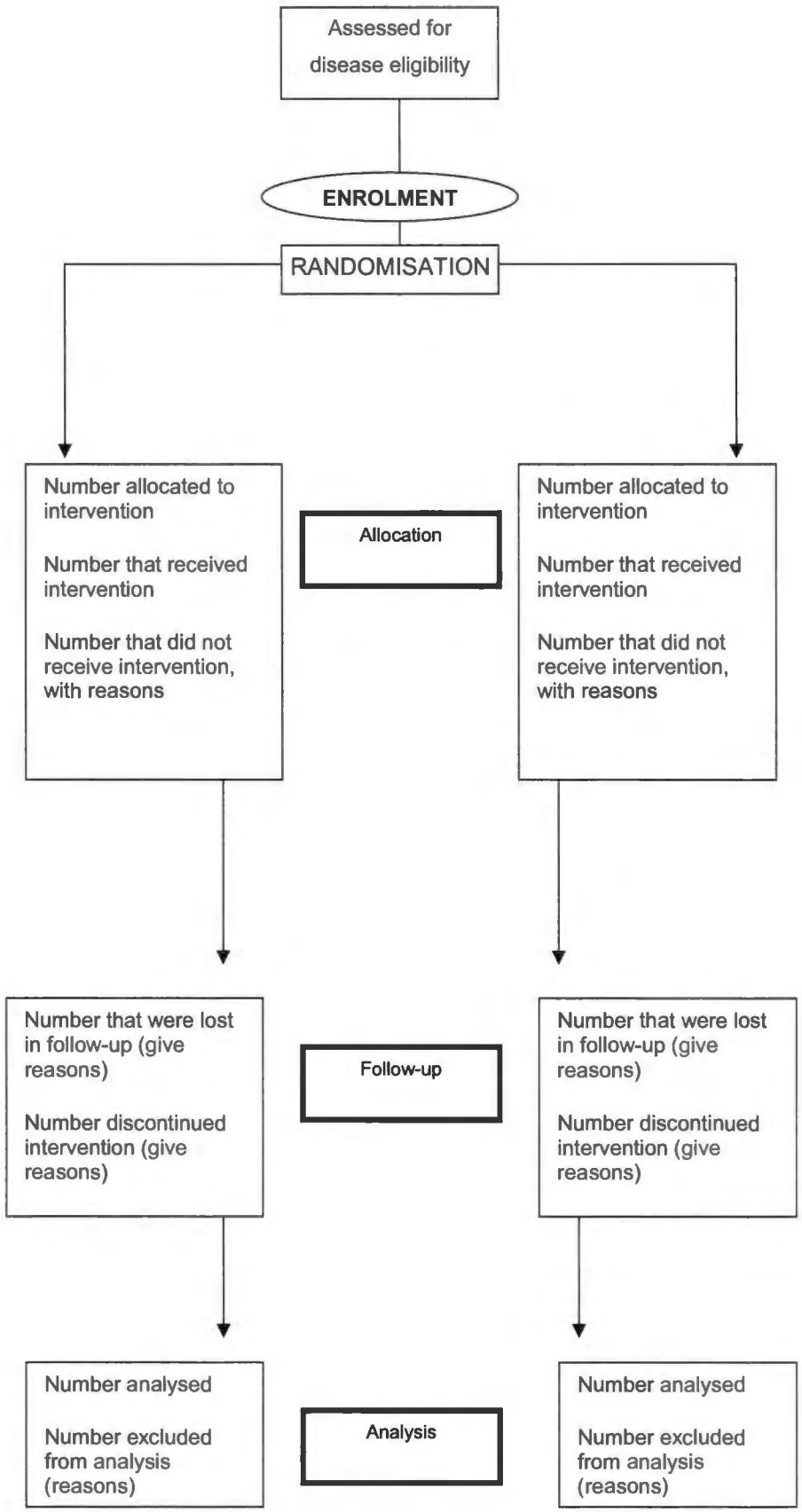



**Figure 3.1: The Consort E-flow chart (Consort, 2008)**

Because most readers base their assessment solely on the information in the abstract (conference or journal) clear, transparent, and sufficiently detailed abstracts related to randomised controlled trials are important. Recently several items have been proposed to be included in conference and/or journal abstracts. In general the abstract must have a structured format. Furthermore, it should include trial objectives, trial design (e.g. method of allocation, blinding), trial participants (i.e. description, numbers randomised, and numbers analysed), interventions intended for each randomised group and their impact on primary efficacy outcomes and harms, trial conclusions, trial registration name and number, and source of funding (Hopewell *et al.*, 2008). The table that follows lists the items that should be included in trial reports. The table can be found on the Consort website, http://www.consort-statement.org/

**Table 3.1: The CONSORT checklist of items to include when reporting a randomised controlled trial (Consort, 2008).**

| PAPER SECTION & TOPIC | ITEM | DESCRIPTION |
|---|---|---|
| *TITLE & ABSTRACT* | 1 | How participants were allocated to interventions (e.g. "random allocation", "randomised" or "randomly assigned"). |
| *INTRODUCTION*<br><br>Background | 2 | Scientific background and explanation of rationale |
| *METHODS*<br><br>Participants | 3 | Eligibility criteria for participants and the settings and locations where the data were collected |
| Interventions | 4 | Precise details of the interventions intended for each group and how and when they were actually administered |
| Objectives | 5 | Specific objectives and hypotheses |
| Outcomes | 6 | Clearly defined primary and secondary outcome measures and, when applicable, any methods used to enhance the quality of measurements (e.g. multiple observations, training of assessors). |
| Sample size | 7 | How sample size was determined and, when applicable explanation of any interim analyses and stopping rules |
| Randomisation<br><br>(Sequence generation) | 8 | Method used to generate the random allocation sequence, including details of any restrictions (e.g. blocking, stratification) |
| Randomisation<br><br>(allocation concealment) | 9 | Method used to implement the allocation sequence (e.g. numbered containers or central telephone), clarifying whether the sequence was concealed until interventions were assigned |

| | | |
|---|---|---|
| Randomisation<br><br>(implementation) | 10 | Who generated the allocation sequence, who enrolled participants, and who assigned participants to their groups |
| Blinding<br><br>(masking) | 11 | Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to group assignment. If done, how the success of blinding was evaluated. |
| Statistical methods | 12 | Statistical methods used to compare groups for primary outcomes; Methods for additional analyses, such as subgroup analyses and adjusted analyses |
| *RESULTS*<br><br>Participant flow | 13 | Flow of participants through each stage (diagram is strongly recommended). Specifically, for each group report the numbers of participants randomly assigned, receiving intended treatment, completing the study protocol, and analysed for primary outcome |
| Recruitment | 14 | Dates defining the periods of recruitment and follow up |
| Baseline data | 15 | Baseline demographic and clinical characteristics of each group. |
| Numbers analysed | 16 | Number of participants (denominator) in each group included in each analysis and whether the analysis was by "intention-to-treat". State the results in absolute numbers when feasible (e.g. 10/20, not 50%). |
| Outcomes and estimation | 17 | For each primary and secondary outcome, a summary of results for each group, and the estimated effect size and its precision (e.g. 95% confidence interval). |
| Ancillary analyses | 18 | Address multiplicity by reporting any other analyses performed, including subgroup analyses and adjusted analyses, indicating those per-specified and those exploratory. |
| Adverse events | 19 | All important adverse events or side effects in each intervention group. |
| *DISCUSSION*<br><br>Interpretation | 20 | Interpretation of the results, taking into account study hypotheses, sources of potential bias or imprecision and the dangers associated with multiplicity of analyses and outcomes |
| Generalisation | 21 | Generalisation (external validity) of trial findings |
| Overall evidence | 22 | General interpretation of the results in the context of current evidence |

## 3.8 Placebo effect (response) in IBS treatment trials

In the year 1801, Haygarth conducted a study that may have been the first placebo-controlled trial. At the time metal rods were used for many illnesses, since they were perceived as having therapeutic characteristics through their supposed electro-magnetic influence on the body. Haygarth treated five patients with imitation wooden rods and found that four gained relief. The following day, another five patients were treated with the metal rods and similar results were found: four out of five patients gained relief. He concluded, *"the wonderful and powerful passions of the mind are often overlooked in the cure of diseases"* (Greenberg *et al.*, 2005).

Various therapeutic RCT's for IBS have revealed a placebo effect of about 40% - 60% and are similar to those found in depression, erectile dysfunction, chronic pain and dyspepsia. This high placebo response among IBS sufferers has challenged investigators testing pharmacologic or psychological treatments (Longstreth & Drossman, 2002; Patel *et al.*, 2005). Scientists have even considered referring to this inappropriately understood phenomenon as "meaning effect" rather than "placebo effect" (Longstreth & Drossman, 2002). In addition, some authors have challenged the true existence of placebo effects in clinical trials (Conboy *et al.*, 2006).

A recent meta-analysis, determined to establish the magnitude and identify which contributing factors influence the magnitude, evaluated 11 variables as potential predictors of the placebo effect. Of the 7 101 patients included in the analysis (from different studies), 3 352 received placebo. Forty four percent responded to placebo and response rates were calculated to vary from as low as 16% to as high as 71%. Studies using the Rome entry criteria showed lower placebo response rates in comparison to those using Manning or other criteria. Thus, stringent entry criteria are recommended to minimise placebo response in IBS trials. In contrast to other studies, a greater number of office visits were associated with decreased placebo effects. Although insignificantly small, the use of a run-in phase showed a trend towards a decrease in the placebo response. Study heterogeneity and the use of published-only studies were listed as study limitations (Patel *et al.*, 2005).

Although a high placebo effect is evident in conventional therapies, data is lacking for complimentary and alternative medicine (CAM). Dorn and colleagues (2007) recently addressed the latter in a meta-analysis. According to the investigators the placebo effect is present among IBS sufferers irrespectively of the type of intervention administered, and is not particularly enhanced in CAM (Dorn *et al.*, 2007). Furthermore, placebo response rates increased as duration of treatment and office visits increased, this is in contrast to what were reported by Patel and colleagues (Dorn *et al.*, 2007; Patel *et al.*, 2005).

Scientists have most recently created the so-called "Placebo Quality Checklist" (PQC), to enable investigators to develop and assess placebos that are more suitable to the verum. It will also assist critical readers and investigators to interpret the findings of RCT's with more care (Brinkhaus *et al.*, 2008).

## 3.9 Ten Recent Clinical Drug Trial Studies in IBS

This section is a brief overview of recent drug trials, with small sample sizes. Emphasis was given to the type of study design, the interventions used, the rescue medication available (if any) and statistical methods. This section does not focus on efficacies of trial drugs.

- The efficacy of renzapride, a potent serotonin ($5HT_4$) – receptor agonist and a $5HT_3$ receptor antagonist, were evaluated in 17 constipation-predominant IBS patients. The investigators made use of a single-blind dose-escalating comparison design. After a 12-day baseline period, all patients received placebo for 4 weeks. This phase was followed by an active treatment phase, beginning with renzapride 2 mg o.d. for 4 weeks, increasing to 2 mg b.d. for a further 4 weeks. Statistically significant accelerated segmental colonic transit was seen in renzapride (2 mg b.d.) over placebo in caecum/ascending colon ($P$ = 0.019) and descending colon ($P$ = 0.022). Renzapride also reduced abdominal pain, increased the number of pain free days and improved stool consistency. The authors concluded that renzapride, particularly at the 2 mg b.d dose, stimulated gastrointestinal transit and improved symptoms compared to placebo. The study provided proof of concept and suggested further investigation of renzapride in patients with constipation-predominant irritable bowel syndrome (Tack *et al.*, 2006b).

- Camilleri and colleagues (2003) evaluated the efficacy of and tolerability of the alpha-2 adreno-receptor agonist, clonidine, in patients with diarrhoea-predominant IBS in a double-blind, randomised, parallel group, placebo-controlled trial. After a 2-week run-in phase, patients received 0.05, 0.1, or 0.2 mg clonidine or placebo twice daily for 4 weeks. Loperamide tablets were provided as rescue medication and participants were allowed to use them in times of intolerable diarrhoea. Overall satisfactory relief were assessed on a weekly basis and were labelled as the primary endpoint. Secondary endpoints, stool frequency, consistency, and ease of passage; gut transit and fasting and post-prandial gastric volumes were secondary endpoints. Stool parameters were assessed with a daily diary. Intention-to-treat principles were used as analysis. Forty four D-IBS patients participated. The proportion with satisfactory relief of IBS was 0.46, 0.42, and 0.67 with placebo, 0.05 mg, and 0.1 mg clonidine respectively. Data suggest

that to have a power of 80% to detect +/- 20% increase in the proportion of patients achieving satisfactory relief with clonidine 0.1 mg twice a day relative to placebo, the study would need 95 patients per treatment arm. However, clonidine 0.1 mg twice a day for 4 weeks appears to be effective in relieving bowel dysfunction and global symptomatology. Further investigation is warranted (Camilleri *et al.*, 2003).

- A total of 34 patients, that met Rome 2 criteria, were randomised to receive a synbiotic preparation (oligofructose/Lactobacillus, Bifdobacterium Bb 12) or placebo for 8 weeks. Abdominal pain, flatulence, bloating and Hospital Anxiety and Depression (HAD) scores were used as parameters during analysis. Both active and placebo groups showed significant improvement in pain frequency ($p < 0.05$) and pain severity ($p < 0.02$). In addition, improvement was seen in both groups for the HAD scores ($p < 0.05$). Other parameters were left unimproved. The investigators concluded that it is likely that a "placebo effect" has presented in this study since significant improvements were seen in both treatment groups. However, there is no evidence of benefit from an 8-week synbiotic treatment (Barker *et al.*, 2003).

- In 2001, twenty eight IBS patients (constipation-predominant and alternating types), that met Rome II criteria entered a 10-week study to establish the efficacy of naloxone (10 mg twice daily), an opioid antagonist compared to identical placebo. A randomised, double-blind, placebo-controlled trial was performed. After a 2-week baseline-screening phase, participants were randomised (active or placebo) to receive 8 weeks of treatment. 'Adequate symptomatic relief' was recorded in six of 14 on naloxone and three of 11 on placebo. The severity of symptoms was recorded by means of the IBS-Severity Scoring System developed by Francis *et al.* (1997), during the study. Although the differences were not significant, improvements in severity grading and mean symptom scores for pain, bloating, straining and urgency to defecate were greater with naloxone than placebo. These preliminary results suggested that naloxone is well tolerated and beneficial in patients with irritable bowel syndrome (constipation and alternating bowel types). However, a larger clinical trial is needed to provide sufficient statistical power to assess efficacy (Hawkes *et al.*, 2002).

- A total of 60 participants were recruited during a 10-month period for a randomised, double-blind, placebo-controlled trial. The aim of the study was to evaluate the efficacy of Traditional Chinese Medicine (TCM), tong-xie-ning in IBS patients that suffers from diarrhoea-predominant subtype. After initial gastroenterological screening, all participants entered a 2-week baseline period. After randomisation patients were treated for three weeks followed by a 4-week follow-up period. Outcome measures included were pain (degree, frequency and duration) and stool parameters. These

outcomes were assessed on a weekly basis by means of visual analogue scales. Investigators estimated that for adequate power (80%) to detect a 40% difference on symptom scores at $\alpha = 0.05$ level (1-tailed test), 26 patients were needed in each group. Intention-to-treat analysis was followed. Quantitative data were expressed as mean ± standard deviation (SD) using an unpaired Students *t* test. Statistical significance was pre-set at 0.05 using a 2-tailed test. Significant improvements were observed in IBS related pain (p=0.001) and stool parameters in tong-xie-ning (TXNG) compared to placebo. Investigators concluded that TXNG may offer an improvement of symptoms to diarrhoea-predominant IBS and, according to TCM terms, patients with stagnation of the liver Qi attacking the spleen. The herbal formulation, TXNG are most effective in relieving abdominal pain and diarrhoea. Further investigation is warranted (Wang *et al.*, 2006).

- The use of peppermint oil in irritable bowel syndrome has been studied with variable results probably due to the presence of patients affected by small intestinal bacterial overgrowth, lactose intolerance or celiac disease that may have symptoms similar to IBS. Therefore, 57 patients with irritable bowel syndrome according to the Rome II criteria, with normal lactose and lactose breath tests and negative antibody screening for celiac disease, were treated with peppermint oil or placebo (two enteric-coated capsules twice per day) in a 4-week RCT. Symptoms were assessed before therapy, after four weeks of treatment and 4 weeks after the end of therapy. Symptoms that were assessed included abdominal bloating, abdominal pain/discomfort, diarrhoea, constipation, feeling of incomplete evacuation, etc. The intensity and frequency of each individual symptom were scored on a 0 to 4 scoring card. With $\alpha = 0.05$, $\beta = 0.80$ and considering a 10% value of withdrawals and dropouts, a minimum number of 25 patients were therefore required in each group. The $X^2$–test was used to compare the percentage of patients with remission of the IBS symptoms in the group of peppermint oil versus that of the placebo. The Students *t*-test for paired data was used to test the changes in the symptoms score between T0 (at the beginning of treatment) and T4, and T8 within the group with peppermint oil and within the group with the placebo. The Mann-Whitney *U*-test (2-tailed) was used to compare the symptoms score between peppermint oil and the placebo at T0, T4, T8. A $P$ value $\leq 0.05$ was considered statistically significant. Investigators concluded, 4 weeks of treatment of peppermint oil improves abdominal symptoms in patients with irritable bowel syndrome (Cappello *et al.*, 2007).

- Post-infectious irritable bowel syndrome (PI-IBS) is associated with increased serotonin-containing enterochromaffin cells and lymphocytes in rectal biopsies. Twenty-nine

patients were randomised in a double-blind, placebo-controlled trial of 3 weeks to evaluate whether steroids, such as prednisolone, reduce the number of enterochromaffin cells and improve the symptoms of PI-IBS. Mucosal enterochromaffin cells, T lymphocytes and mast cells were assessed in rectal biopsies before and after treatment, and bowel symptoms were recorded in a daily diary. Investigators found that enterochromaffin cells did not change significantly after either prednisolone or placebo. Although lamina propria T-lymphocyte decreased significantly after prednisolone ($P$ = 0.003), but not after placebo ($P$ = 0.1) this was not associated with any significant treatment related improvement in abdominal pain, diarrhoea, frequency or urgency. From these results, it would appear that prednisolone is ineffective in reducing the number of enterochromaffin cells or cause an improvement in symptoms in patients with PI-IBS (Dunlop *et al.*, 2003).

- Various trials have assessed the therapeutic role of antidepressants but with inconclusive evidence. Most recently, this issue was investigated in a double-blind randomised controlled trial. Fifty four patients who fulfilled the Rome II criteria for diarrhoea predominant irritable bowel syndrome (IBS-D) were randomly assigned to receive 10 mg amitriptyline daily or placebo. A computer generated block randomisation table were used to assign patients to either placebo or amitryptiline. Organic causes were ruled out by standard laboratory and radiological tests that included full blood count, renal and liver function tests, thyroid function test, stool examination and serological testing for celiac disease. To exclude lactose intolerance, participants underwent a lactose free diet for 14 days. Those who found relieve were then excluded. Rectosigmoidoscopy was also performed. Analysis was performed using SPSS for Windows, version 15.0. Numerical variables, including the total score, were compared between the amitriptyline and placebo groups by an independent-samples t-test. Categorical variables were compared using a chi-squared test. Response and complete response to treatment was compared between the two groups using an independent-samples t-test. After 2 months, the active group showed greater reduction in the incidence of loose stools and feeling of incomplete defecation. Patients receiving amitryptiline showed greater complete response compared to placebo. Adverse effects were similar in the two groups. Vahedi and colleagues concluded that amytryptiline may be effective in the treatment of IBS-D (Vahedi *et al.*, 2008).

- A South African based study has recently assessed the effects of probiotic supplement on the symptoms and immunoglobulin levels of patients (n = 24) with IBS fulfilling the Drossman criteria. Immunoglobulins IgG, IgA and IgM were measured and symptoms were rated before (on day 0) and after treatment (on day 28). Immunoglobulins did not

change significantly from day 0 compared to day 28, in either of the treatment groups. The improvement in clinical symptoms form day 0 to day 28 in the probiotic group was highly significant (p = 0.0001). In addition, the participants from the placebo group found slight improvement from the treatment (p = 0.0473). Although the methodology was out of date with contemporary trial guidelines, the results of this study indicated that *Lactobacillus acidophilus*, *Bifidobacterium longum* en *Bifidobacterium bifidum* might be effective in treating IBS patients with altered gastrointestinal function (Viljoen *et al.*, 2004).

- Carmint™, a herbal drop containing three medicinal plants with sedative, calmative, spasmolytic and anti-flatulent properties, was investigated for efficacy for the relief of abdominal pain and bloating in IBS patients. Patients were randomised to receive 30 drops of either Carmint™ (n = 14) or placebo (n = 18) three times a day, plus loperamide (2 mg twice daily) if they had diarrhoea-predominant IBS (n = 10) or 1 spoonful of psyllium powder once a day if they had constipation-predominant or alternating IBS (n = 22). Randomisation was done using a random number table, with odd numbers for Carmint and even numbers for placebo. Weekly questionnaires elaborated on severity of abdominal pain/discomfort; severity of bloating (4 point scales, with 0 = none, 1 = mild, 2 = moderate, 3 = severe); frequency of abdominal pain/discomfort; frequency of bloating; frequency of hard or loose/watery stool; number of days without defecation; and, number of days with more than three bowel movements per day. Statistical methods used were the following; data were entered into a computer using ACCESS and were analysed using SPSS 11.5. Efficacy analysis by intention-to-treat included all randomised patients. Patients' demographic, baseline characteristics and clinical information were summarised. *T*- and Mann-Whitney tests were used to analyse the severity and frequency of abdominal pain/discomfort and bloating. The *t*-test analyses of the results showed that the severity and frequency of abdominal pain/discomfort were significantly lower in the Carmint™ group than the placebo group at the end of the treatment ($P = 0.016$ and $P = 0.001$, respectively). Also, the severity (P = 0.02) and frequency of bloating (0.002) were significantly improved. Small sample size was mentioned as study limitation. However, this pilot study suggest that Carmint™ with loperamide or Carmint™ with psyllium might be effective in IBS patients suffering from abdominal pain/discomfort and bloating (Vejdani *et al.*, 2006).

The following chapter will discuss the properties and possible applications of the zeolite that is chemically related to Absorbatox™ C35.

# CHAPTER 4

# PROPERTIES AND APPLICATIONS OF INVESTIGATIONAL MATERIAL

## 4.1 Introduction

Absorbatox™ C35 is the patented name of an aluminosilicate belonging to the zeolite family. This particular zeolite has an empirical formula of (Na, Ca, $K)_6Si_{30}Al_6O_{72}.nH_2O$. Although chemically related to clinoptilolite, the substance is greatly enhanced by means of physicochemical procedures and has unique properties when compared to the zeolite found in nature. The product is protected by international trademarks and patents (patent no. PCT/IB2006/053178 pending). The company has commercialised a similar form of Absorbatox™ C35 and claims that the agent has the capability of adsorbing toxins, nitrates and heavy metals in humans. However, the product is not indicated to diagnose, treat, cure or prevent any diseases. The product is manufactured by Brunel Farmaseutika (Pty) Ltd. and is currently available in South Africa from Absorbatox (Pty) Ltd. It is not a scheduled substance and is pharmacologically classified as an A32.2 substance (referred to as "Other") (Absorbatox, 2008).

Clinoptilolite is the most common natural zeolite found in sedimentary rocks of volcanic origin (Armbruster, 2001). Their chemical structure classify them as hydrated aluminosilicates, comprised of hydrogen, oxygen, aluminium and silicon, arranged in an interconnecting network (Zarkovic *et al.*, 2003). This non-toxic zeolite has monoclinic crystal symmetry, strong adsorptive and ion exchange capacity, and has been widely utilised by the industrial, agricultural and environmental industry (Grce & Pavelić, 2005).

Clinoptilolite deposits provoked strong commercial interest because tuffs are often rather pure and can be mined with simple techniques. A typical mining company in the USA, Canada and Europe has less than 50 employees and produces 20 000 to 50 000 tons per year from open pits. Clinoptilolite rocks consist of 60 – 90% clinoptilolite with the remaining being mainly feldspars, clays, glass and quartz. Depending on quality and specification the price ranges between 50 and 300 US$ per ton (based on 2001 figures) (Armbruster, 2001).

Since many biochemical processes are closely related to ion exchange, absorption and catalysis, it is believed that natural and synthetic zeolites could make a significant contribution to the pharmaceutical industry and medicine in the near future. Their unique structure enables

them to absorb gas and water, making them additionally attractive from a pharmaceutical point of view (Zarkovic *et al.*, 2003).

It is well established that some zeolites have antidiarrhoeal (Rodríguez-Feuntes *et al.*, 1997), immunostimulatory and antioxidative (Pavelić *et al.*, 2002), antibacterial and antifungus (Maeda & Nosé, 1999), antacid (Rodríguez-Feuntes *et al.*, 2006) as well as glucose adsorbent-like properties (Concepción-Rosabal *et al.*, 1997). In fact, former discoveries of promising properties has led to the trademarks of Neutacid™ and Enterex™, an antacid- and antidiarrhoeal drug respectively currently registered in Cuba (Rodríguez-Feuntes *et al.*, 2006).

A safety report published in the International Journal of Toxicology did not mention any toxicity of particular concern with clinoptilolite (Elmore, 2003). In toxicology studies, involving mice and rats, the administration of clinoptilolite during a period between 6 and 12 months caused no changes that could be considered a toxic effect due to the treatment (Pavelić *et al.*, 2001). These properties make the compound even more promising for future health indications. However, although zeolites, like clinoptilolite, have been extensively used in industry, agriculture and environmental programmes, their effects in appropriate animal models and possible medical applications are largely undiscovered and await further investigations (Martin-Kleiner *et al.*, 2001).

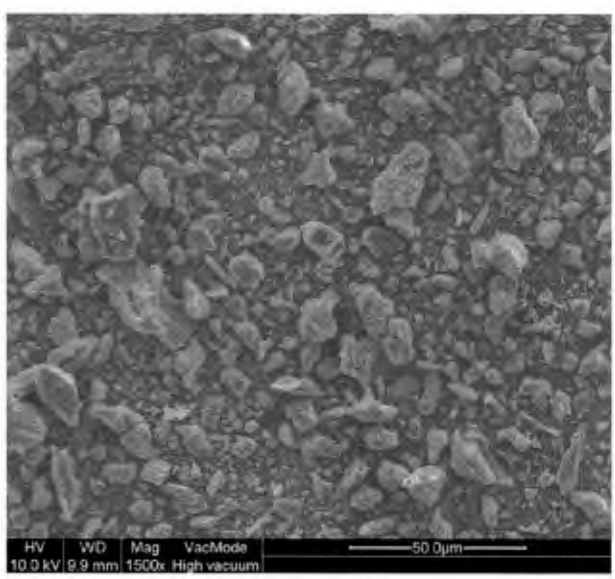



**Figure 4.1: Electron Microscopy photo of Absorbatox™ C35 substance (Laboratory for Electron Microscopy, North-West University, Potchefstroom campus, South Africa, 2520).**

## 4.2 Properties

Zeolites are generally characterised as crystalline hydrated aluminosilicates (a tetrahedron of $SiO_4$ and AlO4) of alkali and alkaline earth cations having an infinite and open three-dimensional structure (Ivkovic *et al.*, 2004; Maeda & Nosé, 1999). Most zeolites in volcanogenic sedimentary rocks were formed by the dissolution of volcanic glass (ash) and later precipitation of micrometer-size crystals. Sedimentary zeolitic tuffs are soft, friable, and lightweight and commonly contain 50 – 95% of a single zeolite; however, several zeolites may coexist, along with unreacted volcanic glass, quartz, K-feldspar, montmorillonite, calcite, gypsum, and cristobalite/tridymite (Mumpton, 1999).

The zeolite group is very diverse; over 100 structural zeolites, both natural and synthetic, have been classified. At least 40 of these zeolites are natural occurring substances, of which clinoptilolite is one of many (Elmore, 2003).

These compounds are able to lose and gain water reversibly and to exchange extra-framework cations, both without change of crystal structure. This property is made possible by the well-defined structure that consists of void spaces within the frameworks (Thom *et al.*, 2003). Clinoptilolite has a system of channels formed by two parallel channels of eight and ten ring windows, parallel to $c$ axis of the structure, that are connected with a third one of eight ring windows (refer to Figure 4.2). The channels of 10 and 8 ring window member tetrahedral rings that have dimensions of 7.2 x 4.4 Å and 5.5 x 4.0 Å respectively, and the size of the third channel is 4.1 x 4.7 Å (Lam *et al.*, 2001).

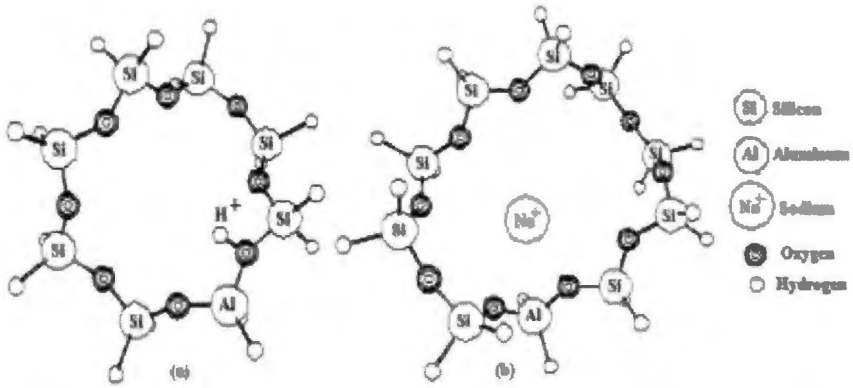



**Figure 4.2: Zeolite models. (a) 8-ring-window model. (b) 10-ring-window model (Lam *et al.*, 2001).**

The large structural cavities and the entry channels leading to them contain water molecules, which form hydration spheres around exchangeable cations. On removal of water by heating to 350 – 400 °C, small molecules can pass through entry channels, but larger are excluded – the so called "molecular sieve" property of crystalline zeolites. Furthermore, zeolites adsorb polar

molecules with high selectivity. The adsorption selectivity for $H_2O$ is greater than for any other molecule (Mumpton, 1999).

## 4.3 Clinoptilolite and the possibilities for its application in medicine

As mentioned before, applications of natural zeolites, including clinoptilolite, make use of one or more of the following properties: (i) cation exchange, (ii) adsorption and related molecular sieving, (iii) catalytic, (iv) dehydration and rehydration, and (v) biological reactivity (Mumpton, 1999).

Zeolites, especially clinoptilolite, differ from other aluminosilicates since they have the ability to adsorb water and gas, and exchange ions (Zarkovic *et al.*, 2003). These substances have been widely exploited in the medical industry. For instance, some zeolites are used as contrast medium in diagnostic methods (Young *et al.*, 1995), as anti-diarhhoeal drugs (Rodríguez-Feuntes *et al.*, 1997) as antacids (Rodríguez-Feuntes *et al.*, 2006), and as support for enzymes and antibodies (Xing *et al.*, 2000). They have also been studied as potential anti-viral (Grce & Pavelić, 2005) and anti-cancer drugs (Pavelić *et al.*, 2001). Further investigation is warranted for human application.

### *4.3.1 Clinoptilolite as immunomodulator and anti-metastatic agent*

It has been postulated that clinoptilolite might have anti-oxidative properties (Zarkovic *et al.*, 2003). Ivkovic and Zabcic (2002a & 2002b) have recently explored this suggestion in two published studies. One of which the tribomechanically activated zeolite (TMAZ), clinoptilolite was assessed for affects on oxidative stress in cancer and diabetes mellitus patients. Blood samples (Randox Total Anti-oxidant status to determine several anti-oxidant parameters and Free Radical Analytical System to determine anti-oxidant effect of TMAZ was used) were taken prior to and after one month of continuous consumption of TMAZ from 114 cancer and 62 diabetes mellitus patients. After one month of treatment both cancer ($p<0.01$) and diabetes (p value not indicated) patients had increased anti-oxidant levels of 42% and 15% respectively. In addition, mean free radical values dropped 26.5% ($p<0.05$) in the cancer group. The investigators reported a global improvement in clinical status and overall quality of life. However, future clinical testing is indicated as this study was not designed as a randomised controlled trial (Ivkovic & Zabcic, 2002a).

Previous results have also found clinoptilolite as a potential agent in cancer therapy. Clinoptilolite treatment of mice and dogs suffering from various tumour types led to improvement of the overall health status, prolongation of life span, and decreased tumour size in some cases. Local application of clinoptilolite to skin cancers of dogs effectively reduced tumour formation and growth. The mechanism of action in animals remains unknown; however, in vitro culture experiments with various cancer cell lines indicated that clinoptilolite treatment modifies intracellular signalling pathways leading to the inhibition of survival signals and induction of tumour suppressor genes (Pavelić *et al.*, 2001).

Zarkovic and colleagues (2003) reported that malignant and normal cells respond differently to the anti-oxidative effects of clinoptilolite, which means that clinoptilolite could have anti-oxidative and anti-tumour effects at the same time. Concomitant therapy with doxorubicin resulted in increased anti-metastatic effects on mammary carcinoma (artificial lung metastasis animal model). It was assumed that clinoptilolite could interfere, through biological and physical modifications, with lipid peroxidation and in particular HNE (4-hydroxynonenal), the toxic aldehyde and end product of lipid peroxidation. Investigators concluded that clinoptilolite could be used as a nutriceutical during chemotherapy of certain cancers (Zarkovic *et al.*, 2003).

### 4.3.2 Enterex™, Neutacid™ and other trademarks by Cuban researchers

An anti-diarrhoeal drug, based on the physical and chemical properties of purified natural clinoptilolite, has been developed suitable for human use. In September 1995, the Cuban Drug Quality Agency approved the use of Enterex™ for diarrhoea in humans. A series of physical, chemical, technological, pharmacological, microbiological, and clinical studies successfully met the requirements set out by the Agency.

Four clinical studies were conducted (it should be noted that not all information is supplied and the studies was not designed as randomised, placebo-controlled trials) (Rodríguez-Feuntes *et al.*, 1997):

- *Effectiveness of Enterex™ in 30 volunteer patients with nonspecific diarrhoea:* Seventy six percent of patients found relief in Enterex™ within 12 hours from the onset of diarrhoea.
- *Etiological study on Enterex™ in the therapy of acute diarrhoea diseases:* Ninety eight percent of the participants (total n = 73) recovered within 24 hours.
- *Enterex™ in the therapy of diarrhoea in diabetes mellitus (neuropathic diarrhoea):* No significant difference between Enterex™ and diphenoxilate were reported.

- *Enterex™ in the therapy of acute diarrhoea from food intoxification:* Large study with 434 participants affected by food intoxicification. Seventy five percent of participants responded to therapy within 24 hours.

No patients dropped out of the study due to side effects and, overall, Enterex™ was well tolerated (Rodríguez-Feuntes *et al.*, 1997). The mechanism of action proposed by the investigators remains unconfirmed.

Neutacid™ is yet another product, developed by the researchers of the University of Havana, Cuba. The drug is based on the chemical and physical properties of the natural zeolite, clinoptilolite extracted from the Tasajera deposit in Cuba. Thirty patients were included in a double-blind study using a placebo. The results showed that 68.6% of patients from the active treatment group reported satisfactory relief and only 14.3% of patients from the placebo group responded with satisfactory relief. Patients did not suffer any side effect during the treatment period. It is thought that Neutacid™ may have a neutralising effect, produced by proton exchange and hydrolysis of species present in the zeolite structure (Rodríguez-Feuntes *et al.*, 2006).

It is hypothesised that the $Fe^{2+}$-containing clinoptilolite (FZ), obtained by hydrothermal transformation of natural purified clinoptilolite could be effective in the treatment of diabetes mellitus. FZ have shown high percentages of glucose capturing *in vitro* (Concepción-Rosabal *et al.*, 1997; Concepción-Rosabal *et al.*, 2000).

These Cuban researchers have determined that phospholipids are rapidly adsorbed on the external surface of a modified $Ca^{2+}$ form of clinoptilolite. The drug molecule, commercially named Colestina™, can produce an interface with a positive charge that can attract bile acids. Pharmacological experiments have shown total cholesterol reduction in normal animals fed high cholesterol diets. Furthermore, the hypocholesterolemic effect of Colestina™ was explored in 20 beagle dogs. The dogs were divided into 4 study groups; 1) control, 2) Ateromixol (hypocholesterolemic agent), 3) Colestina™, and 4) Ateromixol + Colestina™. After 6 weeks of treatment the total cholesterol was reduced with 20% in the Colestina™ group. There was a modest reduction of 14% in the Ateromixol group. However, a 26% reduction was observed in the Ateromixol + Colestina™ group. Dogs were fed with a 1g/kg Colestina dosage and with an Ateromixol dosage of 1mg/kg (Simón Carballo *et al.,* 2001). Although a similar hypocholesterolemic reduction has been observed before with other lipid-lowering drugs the practical utilisation of such a high dosage of Colestina™ remains a problem.

### 4.3.3 Dietary supplementation: Megamin™ and Lycopenomin™

TMAZ® (tribomechanically activated zeolite), a natural zeolite clinoptilolite with enhanced physicochemical properties, is the basis of the dietary supplements Megamin™ and Lycopenomin™, which have demonstrated anti-oxidant activity in humans. A total of 61 patients; with immunodeficiency disorders like rheumatoid arthritis, cancers and other unspecified immunodeficiencies; were administered TMAZ® doses of 1.2 g Lycopenomin™ (n=31) and 3.6 g Megamin™ per day (n=30) for 6 – 8 weeks. Blood and lymphocyte counts were performed at baseline and at the end of study. Blood count parameters were not relevantly affected in either of the two groups. However, Megamin™ administration resulted in significantly increased CD4+, CD19+, and HLA-DR+ lymphocyte counts and a significant decrease in CD56+ cell count. Lycopenomin™ was associated with an increased CD3+ cell count and a decreased CD56+ lympocyte count. The investigators mentioned that no adverse reactions were observed during the treatment period. Clinical relevance of these findings was supported by the improved well-being reported by the patients, who underwent clinoptilolite supplementation therapy. Clearly, these effects must be re-examined in a suitably sized, randomised placebo-controlled trial (Ivkovic *et al.*, 2004).

One critic has condemned the mass-media that claims Megamin™ has strong anticancer properties. It is proposed that the apparent clinical effect may be attributed to higher neuro-cognitive functions like hope and faith that eventually leads to a placebo effect (Momcilović, 1999).

### 4.3.4 Future applications: anti-bacterial, anti-viral, anti-fungus etc.

Although extensive research is still called for, anecdotal information from mining operations in the US indicates that the cuts and scrapes of mine and mill workers exposed to on-the-job zeolite dust healed remarkably quickly. In addition, external application of zeolite powder has been found to be effective in the treatment of athlete's foot (Mumpton, 1999).

Antimicrobial properties of natural synthetic zeolites conditioned in some cation forms, generally silver and zinc, are well constrained and tested in a variety of applications. This includes an active carrier with Zn-erythromycin for anti-acne therapy (Cerri *et al.*, 2004), in dental materials working under anaerobic conditions (Kawahara *et al.*, 2000), as an extractor of pathogens from water (Rivera-Garza *et al.*, 2000), and also as compositions of deodorant with antibacterial activity (Yamamoto *et al.*, 1997). Ag-clinoptilolite is recognized as a low-cost antibacterial material with activity against pathogens like *Pseudomonas aeruginosa* and *Escherichia coli* (Top & Ülkü, 2004). It is speculated that the "*potentials for this antibacterial agent are countless in the medical arena and for everyday household products. In the near future, many medical*

*instruments and artificial devices will be coated with antibacterial zeolite"* (Maeda & Nosé, 1999). Furthermore, it is suggested that antimicrobial agent Ag-zeolite (Zeomic™) could aid in denture plaque control (Nikawa *et al.*, 1997).

Preliminary results (*in vitro*) have shown clinoptilolite, tribomechanically micronized, inhibiting viral proliferation. The micronized zeolite was effective in inhibiting the proliferation of herpes simplex virus 1 (HSV-1), coxsackievirus B5 and echovirus 7. A non-specific mechanism is proposed in which the incorporation of viral particles into pores of MZ takes place rather than any ion exchange properties of the zeolite clinoptilolite. Investigators hypothesised that the possibility of the therapeutical application of micronized clinoptilolite, either locally (skin) against herpesvirus infections or orally in cases of adenovirus or enterovirus infections do exist (Grce & Pavelić, 2005).

## 4.4 Non-medical applications for zeolites

Clinoptilolite is abundantly exploited within the agricultural and industrial sector. The highest benefit of its use is achieved in the field of cat litter, animal bedding and odour absorbents (Armbruster, 2001).

### *4.4.1 Agricultural applications*

Research data reported in the published literature provide evidence of a growth promoting effect when zeolites are used as additives in animal nutrition. Improved weight gain rates have been obtained in fattening pigs and lambs, while a better feed efficiency and egg productivity has been achieved in laying hens by the dietary use of zeolites (Deligiannis *et al.*, 2005; Kyriakis *et al.*, 2002). The apparent ability of clinoptilolite to absorb aflatoxins, i.e. zearalenone, has also been used to protect broiler chickens and other animals from aflatoxicosis (Ortatatli & Oğuz, 2001). Further work is needed to verify and understand the basis of this phenomenon (Mumpton, 1999).

The unique structure allows clinoptilolite to be used as a slow-releasing carrier of agrochemicals of various kinds; fertilisers, pharmaceutically and biochemically effective compounds for veterinary pharmacy and also disinfectants (Reháková *et al.*, 2004).

### *4.4.2 Industrial and Consumer applications*

Natural zeolites have been used and are being used as building stone, as lightweight aggregates in cements and concretes, as filler in paper, in the up take of Cs and Sr from

nuclear waste and fallout, as soil amendments in agronomy and horticulture, in the removal of ammonia from municipal, industrial, and agricultural waste and drinking waters, as energy exchangers in solar refrigerators, as consumer deodorisers, in pet litters, in taking up ammonia from animal manures, and as ammonia filters in kidney-dialysis units (Mumpton, 1999).

## 4.5 Toxicity

According to Ivkovic *et al.* (2004), powdered zeolites are inert and whenever ingested it does not react chemically with food or body fluids or their metabolites. The risk of any associated adverse effects is therefore insignificant (Ivkovic *et al.*, 2004).

According to Rodríguez-Feuntes *et al.* (1997), it was Tillán and colleagues that conducted the first fundamental toxicological study in 1991. Four groups of Wistar rats, male and female, were used in a sub-chronic toxicological experiment for 12 weeks. The control group was fed a normal diet, and 3 groups were fed a diet containing different doses of clinoptilolite (0.1, 0.6, and 1.25 g/kg of weight). Biochemical and biological parameters were evaluated throughout and at the end of the study. Clinoptilolite consumption was not associated with any toxic effects or biological damage (Rodríguez-Feuntes *et al.*, 1997).

A recent study evaluated the long-term effect of clinoptilolite on certain biochemical and haematological parameters of growing and fattening pigs. The clinoptilolite vs. control group did not show any effects on serum K, Na, Ca, P, total protein, albumin and total bilirubin concentrations throughout the study. No profound alteration was noticed in the total hematocrit, leukocyte count and haemoglobin concentration. Although investigators noticed a decrease in the serum urea-N, glucose and cholesterol, they suggested that these alterations are too modest to have any effect on the health status and performance of these animals. The investigators concluded that long-term dietary use of clinoptilolite (inclusion rate of 2%) appears to enhance the performance without causing adverse effects on the health status of growing and fattening pigs (Alexopoulus *et al.* 2007).

Papaioannou and colleagues (2002) revealed that dietary supplementation of clinoptilolite (2% of total feed) during pregnancy and lactation (alone or in combination with antibacterial agent, chlortetracycline) is not associated with any alterations in the uptake and body distribution of vitamins and mineral elements (blood, liver and kidney tissues) in sows.

There is not a lot of data on the systemic effects of zeolites on haematopoiesis. With regards to serum chemistry mixed results have been reported in different animal models (Dwyer *et al.*, 1997; Ward *et al.*, 1999; Ward *et al.*, 1993). In view of this, Martin-Kleiner and colleagues

(2001) investigated whether roughly ground and powdered natural zeolite, mainly clinoptilolite, added to food in a high percentage (12.5 up to 50%, approximately 140 g/kg) would affect serum electrolytes, basic liver and kidney function and body weight of normal laboratory mice and in mice having transplanted mammary carcinoma. Study investigators concluded that powdered clinoptilolite caused a modest (20%) elevation of serum potassium in normal laboratory mice, without changes in other serum electrolytes or in indicators of kidney and liver function. Roughly ground clinoptilolite caused leuko/lymphocytosis, presumably due to intestinal irritation, accompanied by a decline of granylocyte-macrophage progenitors in bone marrow. Similar, but non-significant changes were noted with powdered clinoptilolite. Mice having transplanted mammary carcinoma, in terminal stage, showed increased potassium and decreased sodium and chloride levels, severe anaemia and leukocytes, decreased bone marrow cellularity and diminished content of haematopoietic progenitor cells in the marrow prior to clinoptilolite administration. The clinoptilolite preparations managed to ameliorate the sodium and chloride decline, whereas effects on haematopoiesis were variable (Martin-Kleiner *et al.*, 2001). These results must be seen in the light of others; the latter have used a clinoptilolite inclusion of between 12.5 up to 50 %, whilst other investigators have used lower amounts of clinoptilolite of ± 2% (Alexopoulus *et al.*, 2007; Papaioannou *et al.*, 2002).

Furthermore, toxicological experiments with high dosage oral administration (60–400 mg/rodent) to mice (6 months) and rats (12 months) caused no changes that could be attributed to toxicity of clinoptilolite (Pavelić *et al.*, 2001).

Dietary supplementation with clinoptilolite, commercially called TMAZ®, did not cause any associated adverse effects in immunodeficiency patients during the supplementation period (Refer to "4.3.3 Dietary supplementation: Megamin™ and Lycopenomin™" for more detailed discussion).

# 4.6 Pharmacokinetics and drug-drug interactions

## *4.6.1 Actions after zeolite ingestion*

*"Theoretically, the following actions of ingested zeolite may be expected. First, the ion-exchange properties of the zeolite could alter the pH and the ionic composition (including trace elements) of gastrointestinal fluids, thereby changing the enzymatic activity of gastrointestinal secretions. Second, the zeolite might adsorb small amounts of low molecular weight substances produced by digestive processes (e.g. glucose, amino acids) or by the activity of intestinal bacteria (e.g. ammonia). Inasmuch as any zeolite in the gastrointestinal tract would*

*be expected to be fully hydrated, the low molecular weight substances would probably be absorbed on particle surfaces, but not enter its cavities; ammonia would be exchanged as an ammonium ion. Thirdly, reactive (poly)-silicate anions may be liberated from the zeolite, but the possibility is probably of minor importance in view of the low solubility of clinoptilolite under physiological conditions"* (Martin-Kleiner *et al.*, 2001).

Moreover, clinoptilolite is resistant to degradation by gastric and intestinal juices, and its major constitutive elements are not significantly absorbed from the gut into the systemic circulation. No traces of silicon have been detected in the serum of wistar rats or CBA mice fed with clinoptilolite. Zeolite particles, however, have been found in the first and second layers of duodenal cells (Ivkovic *et al.*, 2004). According to Ivkovic *et al.* (2004) the interaction of orally administered zeolite particles with mucosal associated intestinal lymphoid tissue may trigger an immune response similar to the one observed after the intraperitoneal administration of micronized zeolite. In both cases, the number of peritoneal macrophages, as well as their superoxide anion ($O_2^-$), is increased, while NO production is decreased (Ivkovic *et al.*, 2004).

### 4.6.2 Drug interactions

Farías *et al.* (2003) have studied metronidazole and sulfamethoxazole solutions, before and after contact with clinoptilolite, in a wide range of pH values by UV spectroscopy. Investigators concluded that the structure of the two drugs remained unaltered after interaction with the zeolitic products. There was a slight adsorption with metronidazole in acidic pH, while the zeolitic materials did not adsorb sulfamethoxazole. Farías and colleagues (2003) concluded that zeolitic materials and these drugs could be simultaneously administered to a patient without any loss of the individual pharmaceutical effect of each product.

Although data is unclear, no distinctive adsorption occurred in an *in vitro* suspension with aspirin, metronidazole, sulphametoxazole, theophyline, and propranolol or with phenobarbitone. An *in vivo* diarrhoea study showed no change in chloramphenicol and tetracycline antibiotic activity, when clinoptilolite was used in conjunction with these antimicrobials (Rodriguez-Fuentes *et al.*, 1997).

Clinoptilolite has, however; raised some concerns in agriculture, since it has the potential to adsorb and bind essential nutrients, such as $\beta$-carotene, vitamin A and E or other components that could affect their metabolism and absorption (Katsoulos *et al.*, 2005). On the contrary, recent results have indicated that a clinoptilolite supplementation of 1.25% and 2.5% had no effect on dairy cows' serum concentrations of $\beta$-carotene, vitamin A and E (Katsoulos *et al.*, 2005).

## 4.7 Conclusion

Clinoptilolite, a zeolite of the heulandite group, is the most abundant zeolite in nature (Kyriakis *et al.*, 2002). Based on their unique adsorption, cation-exchange, dehydration-rehydration, and catalytic properties many uses have been explored since the discovery of zeolites in 1756 (Mumpton, 1999). The well-defined structure with a distinctive system of channels within the structural framework are occupied by cations and water molecules, which have freedom of movement that make the cation exchange and reversible dehydration possible (Lam *et al.*, 2001).

Accumulating evidence exists that zeolites, like clinoptilolite, could play an important role in regulation of the immune system, most likely as a non-specific immunomodulator similar to superantigens (Pavelic *et al.*, 2002). If this hypothesis can be confirmed, it is likely that dietary supplementation with natural zeolites, might be promising in the treatment of autoimmune disorders, infectious and malignant diseases (Ivkovic *et al.*, 2004).

Although trademarked products such as the antidiarrhoeal drug Enterex™ (Rodríguez-Feuntes *et al.*, 1997), antacid Neutacid™ (Rodríguez-Feuntes *et al.*, 2006), and the dietary supplement Megamin™ (Ivkovic *et al.*, 2004) are available, little evidence-based data in randomised placebo-controlled fashion exist. Detailed studies, with adequate statistical power and study population, are still awaited before medical application (Martin-Kleiner *et al.*, 2001).

With the latest discovery of immunomodulatory and curative (cuts and wounds) properties, clinoptilolite might be a promising substance for future application in health and nutrition suitable for human (Mumpton, 1999; Pavelić *et al.*, 2001). In addition, the lack of homogeneous mineral phases, chemical compositions and properties of natural zeolites is the main reason to not consider them as raw material for the pharmaceutical industry (Rodríguez-Feuntes *et al.*, 2006). The latter is further complicated by the fact that only a few studies are comparable as the characteristics of the zeolite material depends on the site of origin (Kyriakis *et al.*, 2002).

The methods that were used in this trial in order to assess the efficacy of Absorbatox™ C35 in IBS, are thoroughly discussed in chapter 5.

# CHAPTER 5 PATIENTS AND METHODS

## 5.1 Study Protocol and Ethical considerations

The investigators of this study underwent Good Clinical Practice (GCP) training and received certification for competency. Guidelines set out by the ICH Good Clinical Practice Guidelines and the World Medical Association Declaration of Helsinki (2004) was followed in the preparation of the study protocol. The protocol was authorised by the Ethics Committee of the North-West University, Potchefstroom, South Africa (study number nwu-0001-08-S5). The trial was registered with the South-African Clinical Trial Register (SANCTR) online at www.sanctr.gov.za. All participants gave written informed consent for participation. The **Detailed Study Description and Informed Consent** document, used in this study can be found in **Appendix 1**. [At the time this manuscript was written the Department of Health (DoH) had not yet issued the study with a DoH study number].

## 5.2 Patients

### *5.2.1 Recruitment*

Recruitment of IBS patients was a particular challenge in the present study. Local general medical practitioners and one gynaecologist were asked to recruit IBS patients from their private practices. Doctors were instructed on the process of recruitment and it was apparent that IBS is a common disease; therefore, the required study population would have been easily reached. Furthermore, it is clear from published literature that IBS accounts for approximately 12% of primary care office visits (Birrer, 2002). However, it seems that a lack of interest in the study, a lack of time and overbooked medical practices may have been some of the reasons why doctors responded poorly in IBS patient recruitment.

Eventually other measures needed to be taken in order to attain the proposed study population. Many IBS sufferers remain largely undiagnosed (76.6%) as reported by Hungin and colleagues (2005). Previously, physician referrals and flyers were reported the most effective method of recruiting IBS patients for clinical trials (Chin Feman *et al.*, 2008). For these reasons, it was thought that the North-West University Potchefstroom campus (NWU-P) might have a well

representative study population, consisting of both female and male patients of all ages and ethnicity; thus making it an appropriate resource to recruit patients from. A description of study procedure was compiled in an electronic brochure and mailed to campus students and employees (lecturers and administrative staff) of the NWU, Potchefstroom campus. Flyers, with study information, were distributed mainly by local pharmacies in the Potchefstroom district (refer to **Appendix 2**).

In the end, participants were recruited from a vast number of sites, ranging from medical private practices, local pharmacies by means of flyers, to electronic invitations through mass mailing systems. The recruitment strategy that was used in this study may be considered generalised and transferable to the general public, as participants were not recruited from only one resource (Jones, 1999).

### *5.2.2 Entry criteria*

Due to an absent biological marker (Hadley & Gaarder, 2005) the clinical manifestations remains the primary means in identifying and recruiting patients for IBS clinical research (Hammer & Talley, 1999). Therefore well-defined diagnostic criteria are recommended to diagnose patients for trial participation. The Rome II criteria remain the gold standard and it is widely used (Camilleri *et al.*, 2003; Dunlop *et al.*, 2003; Hawkes *et al.*, 2002; Vahedi *et al.*, 2008; Wang *et al.*, 2006).

However, potential candidates for participation in this trial were screened using the latest diagnostic criteria, the Rome III criteria. It could not be found that this criteria had been used in previous reported IBS trials before. The Rome III criteria were chosen mainly because of its acclaimed superior benefits over the Rome II criteria (Drossman & Dumitrascu, 2006) [refer to chapter 2, section 2.6.1 Diagnostic criteria for a detailed description of the Rome III criteria. This diagnostic tool is attached as **Appendix 3** at the back of this manuscript].

**Patients were included if they met the following requirements (refer to Appendix 3):**

1. Patients between 18 and 65 years of age of any race and/or genders.

2. Patients diagnosed with IBS-D, IBS-A (IBS-M) or IBS-C according to the Rome III criteria. **To avoid any floor effects patients must have had a frequency of at least two IBS episodes (e.g. pain/discomfort) per week (question 1 >4).**

3. In order to fulfil the Rome III criteria patients must have had discomfort for at least 6 months or longer (question 3 = 1). Women should not have experienced IBS symptoms during menstrual bleeding **only** (question 2 = 0, 2). However, women who

experience IBS symptoms **predominantly** during menstruation were considered for participation.

4. Furthermore, two or more of the following according to Rome III principles:

    a) Discomfort gets better or stops with bowel movement (question 4 > 0)

    b) More OR less frequent bowel movements with the start of discomfort/pain (question 5 or 6 > 0)

    c) Looser stool OR harder stools with the onset of discomfort/pain (question 7 or 8 > 0)

5. All participants had to undergo a full blood count and an erythrocyte sedimentation rate test before participation. This screening test was done to exclude any underlying disease entities that may mimic IBS (e.g. infections and cancers).

6. It was further required of patients to be English literate in order to give written informed consent.

**The exclusion criteria as follows:**

1. Patients using the following medicine were excluded[1]:

    Recent antibiotics usage, anticholinergics (including old generation antihistamines), cholestyramine, analgesics, laxatives, antipsychotic medication, anti-diarrhoeals, iron preparations, cisapride, tegaserod, alosetron and other $5HT_3$ receptor antagonists, peppermint oil and iron preparations. Patients using lithium carbonate for the treatment of mood-disorders was excluded from the trial (refer package insert for Absorbatox™Detox capsules).

    **In brief, patients using any medication that affect GI motility, during this study, would have been instructed to discontinue usage. Administration of certain medication may aggravate IBS symptoms. The latter may give a false interpretation that the investigational drug does not improve symptoms. Other**

---

[1] The NWU-P Ethics Committee has granted approval for the abstinence of drugs affecting the gastrointestinal tract during the trial period; however, its usage was not completely prohibited. Participants were encouraged not to use these medications as mentioned, but if it was needed they were instructed to record it in the daily diary, generally referred to in this study as the Participant Booklet.

**drugs, which may improve IBS symptoms, have been prohibited, as these improvements may be interpreted as effects due to the investigational drug.**

Patients that were using chronic medication were not excluded as long as they met the following criteria at onset of study: *1) medication is needed; 2) medication had been used for several weeks; 3) asymptomatic at start of concomitant therapy.*

2. If IBS candidates suffered from conditions like, anaemia, diabetes mellitus (type I & II), lactose intolerance, cholestasis, hyperthyroidism, liver or kidney disease & major psychiatric disorders they were excluded. Some of these conditions may mimic IBS symptoms and therapy may give rise to a type 2 error.

3. Patients with chronic severe constipation with **less than one stool per week**, during the baseline observation period, were excluded.

4. Patients with the following signs were excluded from participation in trial, since these symptoms may be due to diseases other than IBS: **Blood in stools, nocturnal predominant symptoms, palpable abdominal or rectal mass, recent progressive weight loss and rectal bleeding**.

5. Furthermore, patients with co-existing organic gastrointestinal disease were also excluded (e.g. chron's disease, ulcerative colitis and celiac disease).

6. Patients older than 60 years, who have not had a colonoscopy or sigmoidoscopy after onset of IBS symptoms, were excluded. In addition, patients older than 50 years, **with a family history of colorectal cancer**, had to undergo a colonoscopy prior to inclusion.

7. Safety of Absorbatox C35 has not been established in pregnancy, therefore; women that were either pregnant OR currently nursing (lactating) OR both was excluded.

8. Previous trials have excluded patients if they previously received gastrointestinal surgery (refer to references in the following paragraph). In the present trial, if a patient had a history of gastrointestinal surgery and met the Rome III criteria with no confounding alarm symptom the patient was considered for participation, unless the study physician did not find the patient fit for trial participation.

These inclusion and exclusion criteria were based on the latest published recommendations and previously reported utilisation thereof (Bergmann, 1999; Camilleri *et al.*, 2000; Camilleri *et al.*, 2003; Corazziari *et al.*, 2003; Drossman, 2006; Drossman & Dumitrascu, 2006;

Ducrotte *et al.*, 2005; Dunlop *et al.*, 2003; Hatlebakk & Hatlebakk, 2004; Ringel *et al.*, 2001; Tack *et al.*, 2006b).

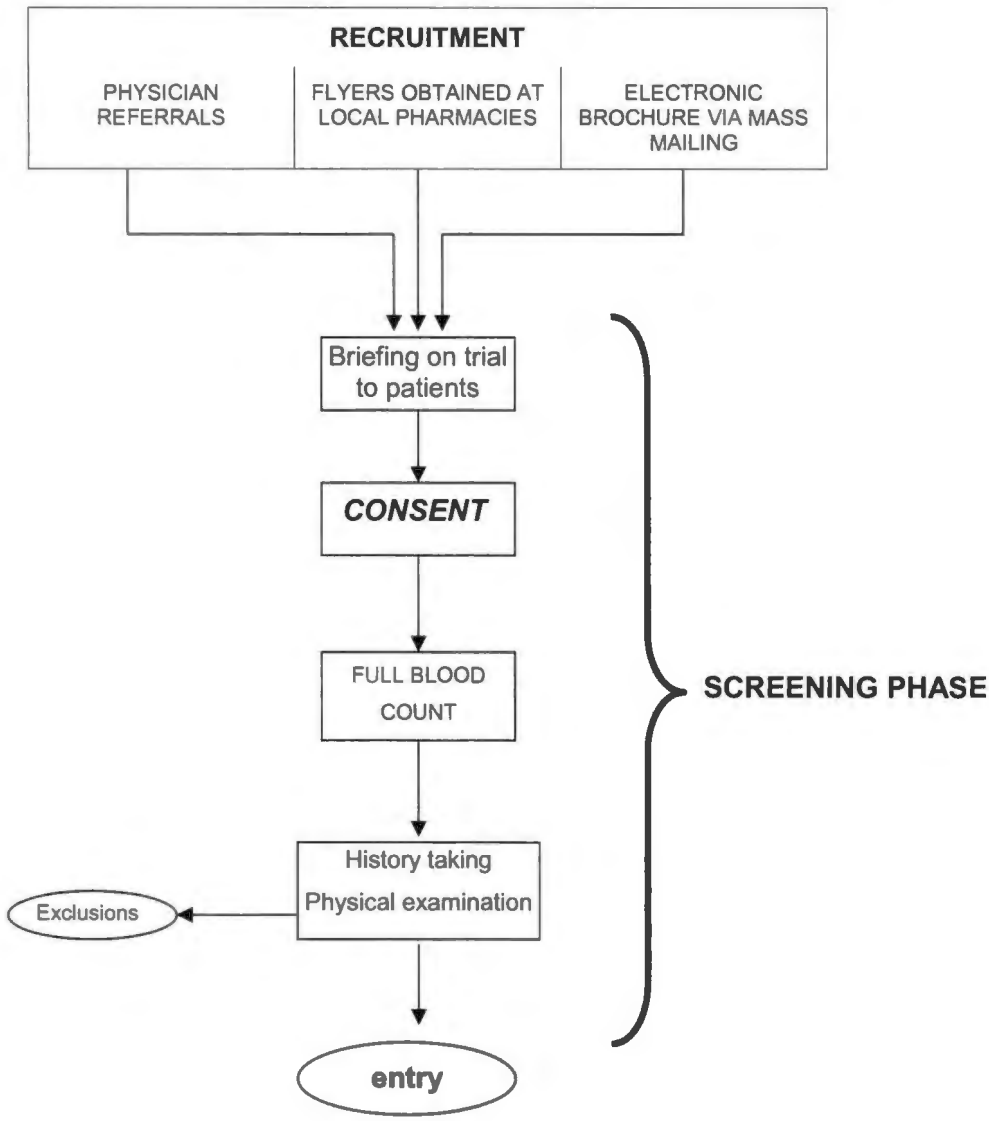



**Figure 5.1: The process potential participants were exposed to before study entry.**

## *5.2.3 Patient screening*

On the successful completion of the full blood count test all potential participants had to be examined by the study physician (JDP). It is quite clear that other gastrointestinal diseases coincide with IBS symptomatology (Hatlebakk & Hatlebakk, 2004) and that further testing was deemed necessary when dealing with alarm symptoms (Zaman, 2002). This study relied on a "red-flag exclusion sheet", compiled by the primary researcher (JRK). It lists all the alarming symptoms that could indicate a differential diagnosis of IBS and it was used by the study physician (JDP) to exclude any alarming symptoms the participants may had suffered from. The list also contains other diseases listed as exclusion criteria (Please refer to **Appendix 4**).

Therefore, if a patient had an abnormal cell count and/or erythrocyte sedimentation rate, experienced one or more of the signs listed as alarm symptoms, fulfilled the exclusion criteria in any way, or the study physician (JDP) did not find the patient fit for participation, then the patient was not entered into the study. Patients were excluded for any reason likely to result in poor compliance with the study protocol.

The flow diagram (Figure 5.1) illustrates the events followed between recruitment and study entry.

## 5.3 Study design and layout

This trial was designed as a double-blind, placebo-controlled, parallel trial with randomisation of treatment. Patients either received a placebo or an active treatment. It must be noted that there were no cross over of treatment, since its use is condemned in IBS clinical trial research (Irvine *et al.*, 2006). Moreover, a follow-up period (e.g. symptom assessments after trial drugs have been discontinued) was not considered due to a time constraint.

As mentioned, neither the investigators nor the patients knew what treatment was given but subsequent to the last visit of the last trial participant, the investigators were "unblinded" to treatment. Patients were later informed on the treatment allocation.

After a 2-week baseline (run-in) period, participants were randomised to receive the respective treatment. The run-in phase served as an important part of the study, as it was compared to the treatment period data to observe treatment effects in the Absorbatox™ C35 and placebo treatment group, respectively. Patients were treated for 4 consecutive weeks (i.e. 30 days). It was expected of participants to complete a study diary throughout the study, referred to as the Participant Booklet. The participant booklet contained questionnaires that enabled the investigator to assess treatment effect. The questionnaires of this study can be viewed as attachment in **Appendix 5**.

During the six weeks of study, participants were expected to attend five office visits. During these sessions, general feedback was obtained, completed questionnaires were collected, and treatment drugs were dispensed. These sessions were also an opportunity for participants to report adverse events. The following table represents the study visits that were divided across the entire duration of the study.

**Table 5.1: Study visits for entire study period.**

| | Run-in period (2 weeks) | | | Randomisation | Treatment period (4 weeks) | | | |
|---|---|---|---|---|---|---|---|---|
| Visit number | V1 | no visit | V2 | | V3 | no visit | V4 | V5 |
| Days or weeks | D1 | + 1W | +1W | | + 1W | + 1W | + 1W | + 1W |

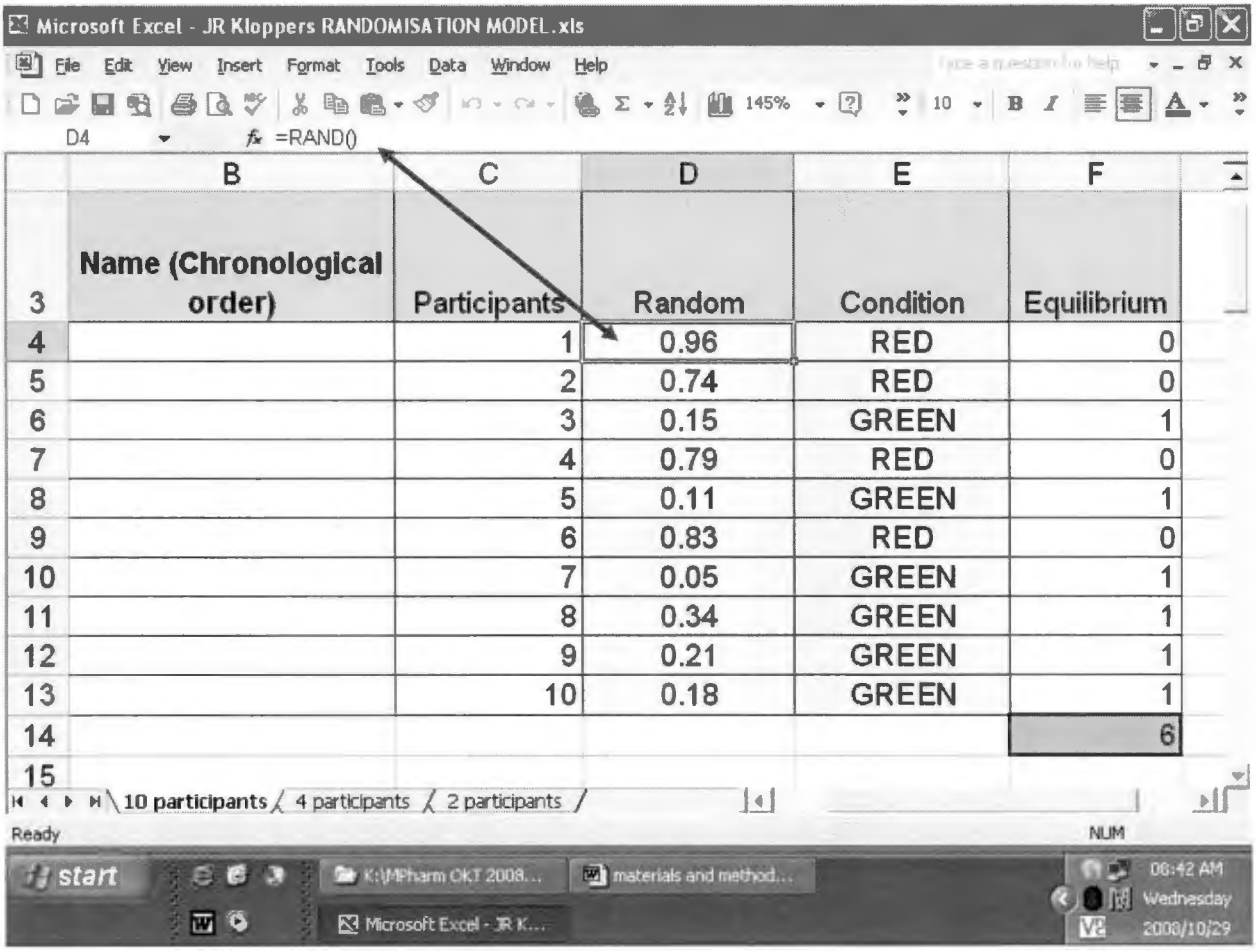
**V=visit, D=day, W=week**

Patients visited the study site two times during the run-in phase and three times during the treatment phase. The visits were scheduled and conducted by the primary researcher (JRK).

## 5.4 Method of randomisation

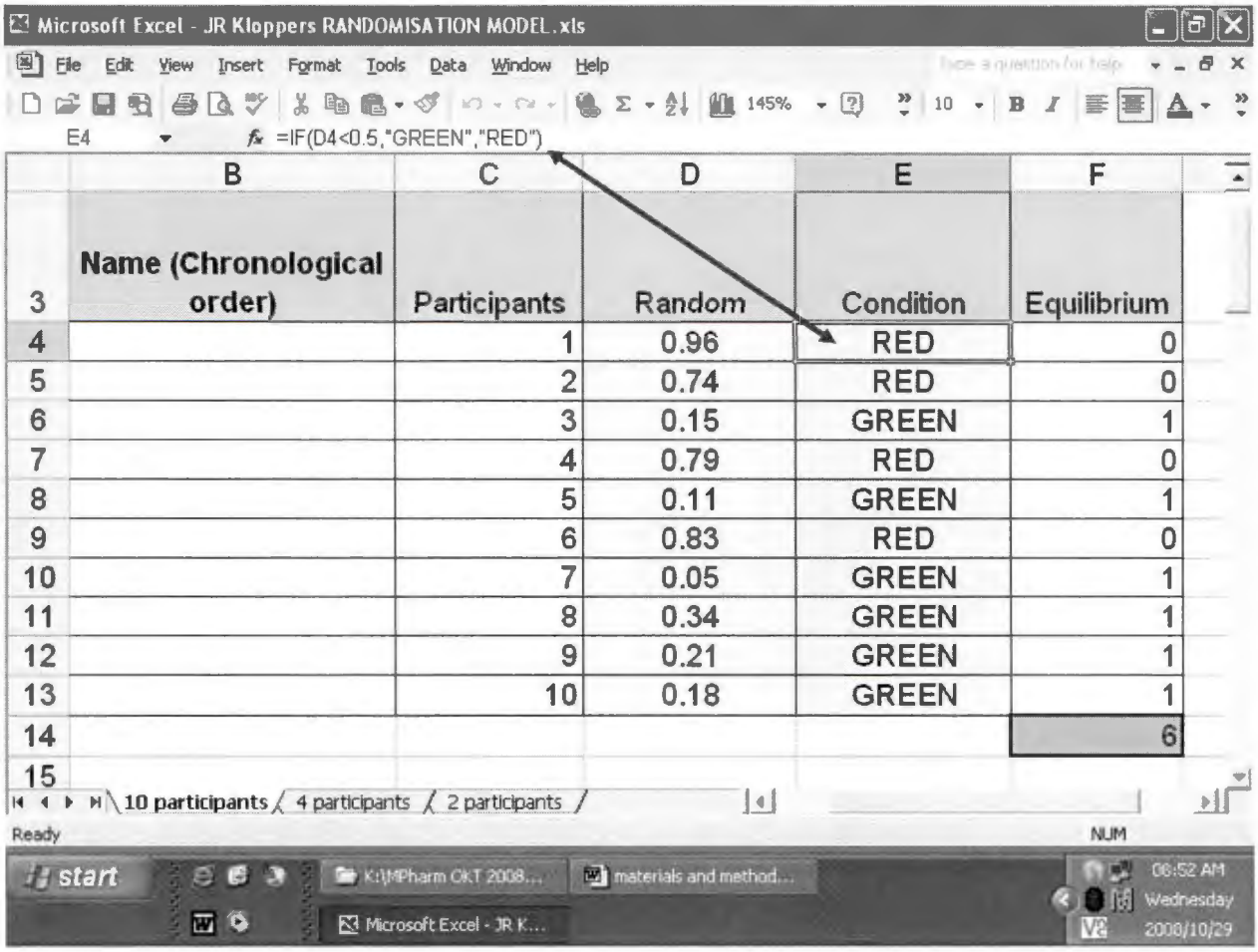
Previous IBS trials have reported uneven distribution of patients between treatment groups when using random number tables, in which odd numbers are used as one treatment group and even numbers as another group (Vejdani *et al.*, 2006). Therefore, the randomisation schedule of this trial was generated by a manually operated computer-generated random codes system. A simple yet effective method was applied using Microsoft Excel (MS-Excel). The study statistician (FS) endorsed the method.

The randomisation model is discussed as if 10 participants are to be randomly allocated into two treatment groups, green and red. In column C the proposed number of participants are listed (in this example 10 participants). The randomisation function of MS-Excel is used and entered in column D [**=RAND()**].



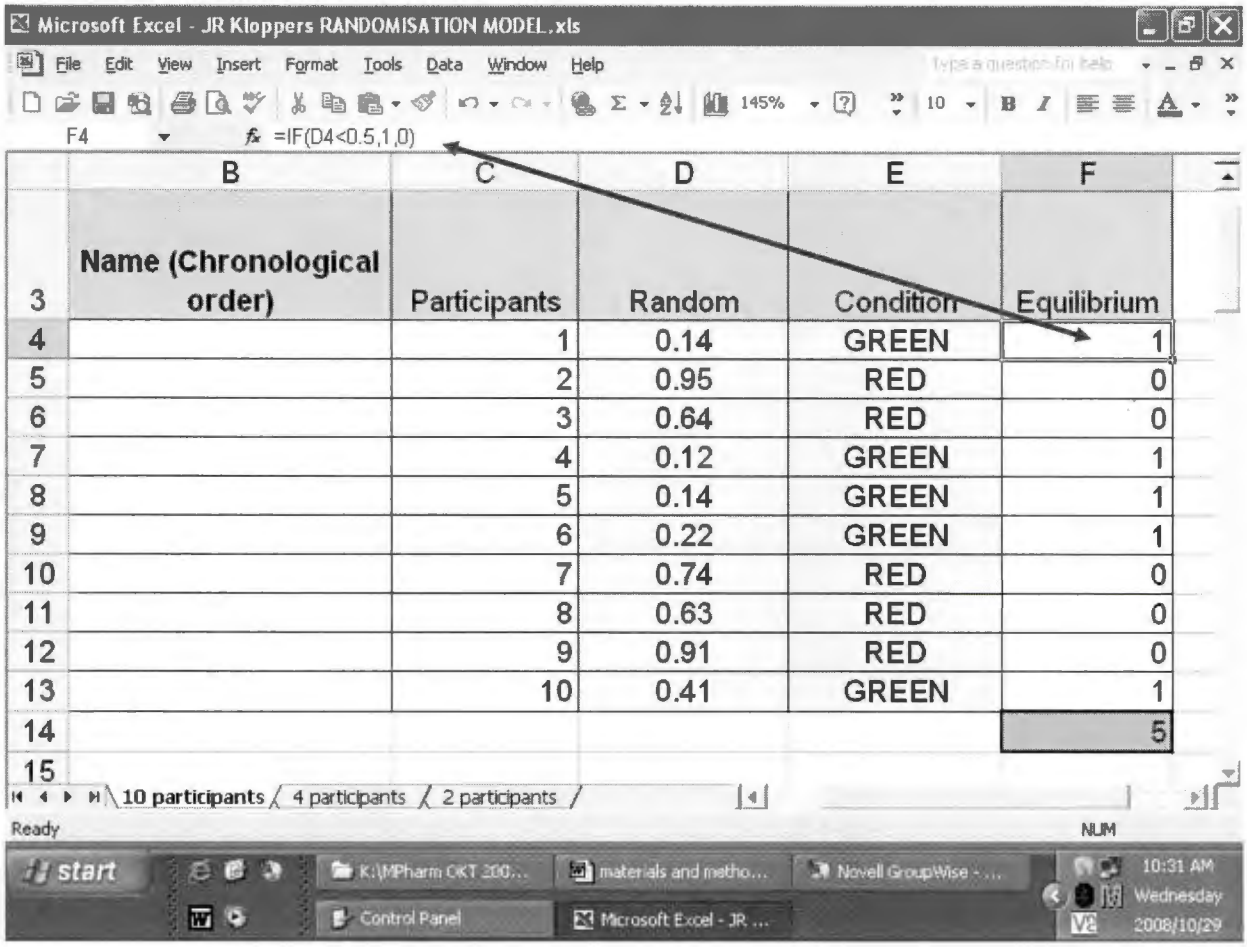

| | B | C | D | E | F |
|---|---|---|---|---|---|
| 3 | Name (Chronological order) | Participants | Random | Condition | Equilibrium |
| 4 | | 1 | 0.96 | RED | 0 |
| 5 | | 2 | 0.74 | RED | 0 |
| 6 | | 3 | 0.15 | GREEN | 1 |
| 7 | | 4 | 0.79 | RED | 0 |
| 8 | | 5 | 0.11 | GREEN | 1 |
| 9 | | 6 | 0.83 | RED | 0 |
| 10 | | 7 | 0.05 | GREEN | 1 |
| 11 | | 8 | 0.34 | GREEN | 1 |
| 12 | | 9 | 0.21 | GREEN | 1 |
| 13 | | 10 | 0.18 | GREEN | 1 |
| 14 | | | | | 6 |
| 15 | | | | | |

In column E the "IF" function is used to allocate a GREEN or RED treatment to the respective participant. If the numeric value in the adjacent column (column D) is smaller than 0.5, a GREEN treatment is allocated to the participant in the same row. In turn, if the numeric value in adjacent cell is larger than 0.5 the RED treatment will be allocated to the participant in that row.




| | B | C | D | E | F |
|---|---|---|---|---|---|
| 3 | Name (Chronological order) | Participants | Random | Condition | Equilibrium |
| 4 | | 1 | 0.96 | RED | 0 |
| 5 | | 2 | 0.74 | RED | 0 |
| 6 | | 3 | 0.15 | GREEN | 1 |
| 7 | | 4 | 0.79 | RED | 0 |
| 8 | | 5 | 0.11 | GREEN | 1 |
| 9 | | 6 | 0.83 | RED | 0 |
| 10 | | 7 | 0.05 | GREEN | 1 |
| 11 | | 8 | 0.34 | GREEN | 1 |
| 12 | | 9 | 0.21 | GREEN | 1 |
| 13 | | 10 | 0.18 | GREEN | 1 |
| 14 | | | | | 6 |
| 15 | | | | | |

This still resulted in uneven distribution of participants between the two treatment groups. To ensure even distribution, an extra column was created with the following function: "**=IF(D4<0.5,1,0)**". The green and red groups are marked with a 1 & 2, respectively, in the adjacent cell. The sum of the data series from F4 to F13 is then calculated in cell F14 [**=SUM(F4:F13**]. When the cursor is placed in an empty cell and the "DEL" button on the keyboard is repeatedly pressed the random allocation list automatically changes every time the button is pressed. The "DEL" button can then be pressed until a sum of 5 is reached in cell F14, i.e. the half of ten. Five patients will then be allocated to the GREEN treatment and 5 will be allocated to the RED treatment.




| | B | C | D | E | F |
|---|---|---|---|---|---|
| 3 | Name (Chronological order) | Participants | Random | Condition | Equilibrium |
| 4 | | 1 | 0.14 | GREEN | 1 |
| 5 | | 2 | 0.95 | RED | 0 |
| 6 | | 3 | 0.64 | RED | 0 |
| 7 | | 4 | 0.12 | GREEN | 1 |
| 8 | | 5 | 0.14 | GREEN | 1 |
| 9 | | 6 | 0.22 | GREEN | 1 |
| 10 | | 7 | 0.74 | RED | 0 |
| 11 | | 8 | 0.63 | RED | 0 |
| 12 | | 9 | 0.91 | RED | 0 |
| 13 | | 10 | 0.41 | GREEN | 1 |
| 14 | | | | | 5 |
| 15 | | | | | |

Treatment assignment was not revealed until the study was completed. To avoid bias, the primary researcher was not responsible for any randomisation procedures. The recruitment administrator (UVK) received training on the process of recruitment, and managed the procedure accordingly. In addition, patients were not identifiable, as a unique study number, consisting of initials and entry number, were used in randomisation.

## 5.5 Drugs and Treatments

### *5.5.1 Absorbatox™ C35 and placebo capsules*

The sponsor company Absorbatox (Pty) Ltd. provided the study drugs (active and placebo drugs).

The contents of the placebo were carefully considered, as some placebos may worsen or even relieve IBS symptoms, as observed by Capello and colleagues (2007). It was decided to use dicalcium phosphate ($CaHPO_4 \cdot 2H_2O$) with the trade name Emcompress®, as placebo. This product is extensively used by the pharmaceutical industry, especially as tablet and capsule

diluents. It is also used in oral pharmaceutical products, food and toothpastes, and is generally regarded as a non-toxic and non-irritant material (Moreton, 2005). For these reasons it was regarded as a suitable placebo in a population that has a sensitive gut.

The placebo capsules were similar in form, colour, taste, size and packaging compared to the Absorbatox™ C35 capsules.

### 5.5.2 Dosage and regimens

Participants in this study received 750 mg Absorbatox™ C35 or placebo three times daily as oral soft gelatine capsules. This dosage were based on an unpublished study by Koot and associates (2006) in which 30 enterotoxin-induced NMRI mice were given different dosages of Absorbatox™ C35 to assess efficacy. The investigators recommended a 30 mg/kg per day dosage. Rodríguez-Feuntes and colleagues (1997) reported positive results with a dosage of 30 mg/kg clinoptilolite of body weight in 2 year-old piglets having dysentery. Furthermore, a recent trial that used clinoptilolite (with similar chemical composition as Absorbatox™ C35) as a dietary supplement in immunodeficiency patients used a 4 956 mg/day and a 1 788 mg/day, respectively (Ivkovic *et al.*, 2004).

Patients were counselled on the use of study medication and were instructed to take one capsule three times daily for 30 consecutive days (treatment phase).

### 5.5.3 Rescue medication

Participants had access to rescue medication during the treatment period. However, patients were instructed to use rescue medication only in times of unbearable symptomatology. All participants, irrespective of treatment group (placebo or active) or bowel subtype (e.g. IBS-D, IBS-C), received a standard rescue pack consisting of loperamide (for diarrhoea), lactulose dry powder (for constipation) and mebeverine (abdominal pain/spasms). Through this method, it was ensured that all participants were exposed to the same interventions. The use of rescue medication between the placebo and Absorbatox™ C35 were compared to evaluate whether the Absorbatox™ C35 group were associated with less rescue medication consumption.

**Table 5.2: Rescue medication, indication and dosage strength.**

| Medication | Indication | Accepted dosage |
|---|---|---|
| Mebeverine 135 mg tablets<br>(Bevispas®) | Abdominal pain/spasms associated with irritable bowel syndrome | 135 mg (one) tablet three times daily |
| Lactulose dry powder 10 g sachet<br>(Laxette Dry®) | Constipation | 10 – 20g daily up to maximum dosage of 40g |
| Loperamide 2mg tablets<br>(Gastron®) | Diarrhoea | 2 to 4 mg, up to four times a day.<br>**Not to exceed 16 mg daily** |

.....

### *5.5.4 Drug compliance*

Each participant had to consume a total of 90 capsules (3 caps. tds over 30 days). At the end of study treatment the returned capsules were counted. The compliance rate was calculated as follows:

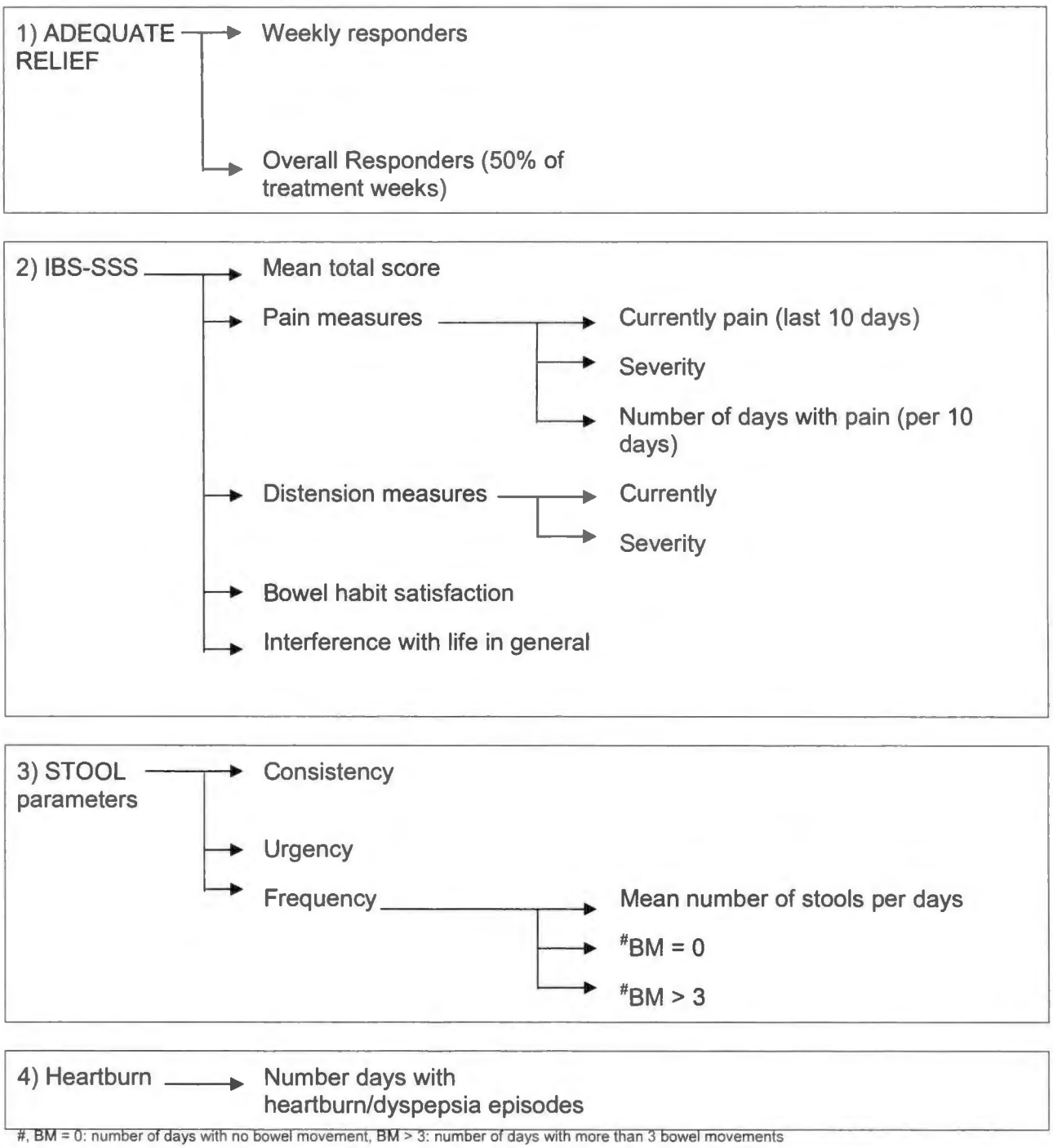
$$\% \text{ Compliance} = \frac{\text{(Actual number of caps. taken)}}{\text{(Number of caps. should have been taken)}} \times 100$$

The actual number of capsules consumed was calculated by subtracting the returned capsules from the number that should have been taken (i.e. 90).

## 5.6 Outcome measures

The outcome measures that will follow were used in consideration with latest recommendations by the Rome III committee (Camilleri *et al.*, 2007; Drossman, 2005, Irvine *et al.*, 2006; Schoenfeld & Talley, 2006), consensus groups (Corazziari *et al.*, 2003) and an independent review panel (Bijkerk *et al.*, 2003). A brief layout of all the assessments is illustrated in Table 5.3. Figure 5.2 is a diagram that illustrates the study layout with the timing of certain assessments.

**Table 5.3: Brief layout of outcome assessments.**




#, BM = 0: number of days with no bowel movement, BM > 3: number of days with more than 3 bowel movements

. . . . .

### *5.6.1 Primary outcome*

The primary outcome was evaluated with the subjective global assessment, *"adequate relief"*. Patients were asked the following: *"In the last 7 days have you had overall adequate relief of your IBS symptoms?"* Participants had two options; they could either answer "yes" or "no". This question was administered every week after treatment had started on **day 21**, **28**, **34** and on **day 42 (i.e. day 7, 14, 21 and 28 of treatment period)**.

A **responder** was classified as someone who responded "yes" to the question in 50% of the treatment weeks. In other words, a participant had to answer "yes" on at least two occasions in order to obtain **responder** status (any 2 weeks out the 4 treatment weeks).

Furthermore, the proportion of patients that answered "yes" (responded to treatment) in each week was evaluated between the treatment groups.

## 5.6.2 Secondary outcome

### 5.6.2.1 Overview of the IBS Severity Scoring System (IBS-SSS)

The severity of participants' symptoms was evaluated using the well explored, Irritable Bowel Syndrome Severity Scoring System (IBS-SSS) developed and validated by Francis *et al.* (1997). A 50-point reduction in severity score, from baseline to the end of therapy, was considered a clinically meaningful effect. It was chosen since it is the only integrative questionnaire, that addresses selected relevant symptoms (Camilleri *et al.*, 2007) and that is responsive to treatment effects (Irvine *et al.*, 2006).

The IBS-SSS uses two sections, part A and B. For the purpose of this study only part A has been used (**Appendix 5:** Questionnaires). It consists of pain, distension, bowel habit satisfaction and global quality of life ("interference with life in general) parameters. Severity measures are scored on a 10 mm visual analogue (VA) scale ranging from 0% to 100% (0% indicates low severity, 100% indicates worse severity).

### 5.6.2.2 Pain, distension, bowel habit satisfaction and interference with life in general measures

Pain is covered in three subsections:

- **currently** suffering from pain (described as *"10 days or so"*) as a simple "yes": or "no" answer,
- **severity** of pain that is scored on visual analogue scale (ranging from 0% - no pain to 100% - very severe),
- **number** of days with pain in previous ten days expressed numerically (max 10, min 0).

Distension (also referred to as bloating or tight tummy) is covered in two subsections:

- **currently** suffering from abdominal distension (described as *"10 days or so"*) as a simple "yes": or "no" answer,

- **severity** of distension that is scored on visual analogue scale (ranging from 0% - no distension to 100% - very severe).

Bowel habit satisfaction as:

- *"How satisfied are you with your bowel habit?"* scored on a visual analogue scale that ranges from 0% - very happy to 100% - very unhappy.

Interference with life in general as:

- *"How much is your IBS affecting or interfering with your life in general?"* also scored on a VA scale ranging from 0% not at all to 100% completely.

#### *5.6.2.3 The calculation of the severity score*

**Total Severity score = visual analogue scale scores for severity pain + (number of days with pain X 10) + severity distension + bowel habit satisfaction + interference with life**

In total the IBS-SSS consists of 4 visual analogue scales (pain, distension, bowel habit satisfaction and interference with life). The severity score is calculated by adding the numeric values, i.e. the values scored on VA scales plus the numeric value scored as the number of days with pain multiplied by ten. A maximum of 500 can be scored and patients can be categorised as having a mild (an overall score between 75 & 175), moderate (175-300), or severe (exceeding 300) disease (Drossman, 2005; Francis *et al.*, 1997).

#### *5.6.2.4 Timing of IBS-SSS assessment during the trial*

The IBS-SSS was used to assess the severity of symptoms on day 10 during the baseline period. The severity of symptoms was also measured during the treatment period, starting on the $10^{th}$ day of treatment and every tenth day thereafter, on day 24, 34 and day 44.

Baseline scores was compared to treatment scores within groups (paired data). Severity scores obtained between two groups (active vs. placebo) was also compared for significance (unpaired data).

## *5.6.3 Stool parameters*

Stool parameters were scored daily from day 1 to day 44 (Figure 5.2). Categorical scales (Likert) were used to measure stool consistency and urgency. Scales were rated 1=very hard, 2=hard, 3=formed (smooth), 4=loose, 5=very loose (watery) for consistency and 1=no urgency at all, 2=not very severe, 3=quite severe, 4=severe, 5=very severe, for urgency.

Stool frequency was numerically rated. It was assumed that a question: *"How many times did you open your bowels today?"* were not appropriate as an answer of 3 may be easily regarded as diarrhoea, when in fact the patient could have suffered from 3 events of constipation. Therefore, the following was asked of patients, and seemed more appropriate: *1) How many times did you open your bowels due to* ***normal, loose or very loose stool,*** *2) How many times did you open your bowels due to* ***hard or very hard stool?***

Number of days with **no bowel movement** (BM=0) and number of days with **more than three bowel movements** were also calculated.

### 5.6.4 Heartburn assessments

The proportion of days with heartburn/dyspepsia episodes was compared between baseline and treatment period in each treatment group, respectively (paired data). In addition, the proportion of days with heartburn between Absorbatox™ C35 and placebo was also compared, during the treatment weeks (unpaired data).

### 5.6.5 End-of-study Marketing Potential (EMP) questionnaire

On the last day of trial, patients were asked whether they would recommend the therapy that was allocated to them to other IBS sufferers and whether they would buy the product in the future. These measures were then evaluated for any differences among treatment groups.

### 5.6.6 Adverse drug events

Adverse drug events were monitored daily. Patients were asked whether they have experienced an adverse event (also described as side effect) from trial medication. It was mandatory that the adverse event (if any) be described in the participant booklet. The intensity of reported adverse event had to be rated as mild (did not affect daily activities), moderate (minor effects on daily activities), and severe (great effect on daily activities) (please refer to **Appendix 5**: Questionnaires).

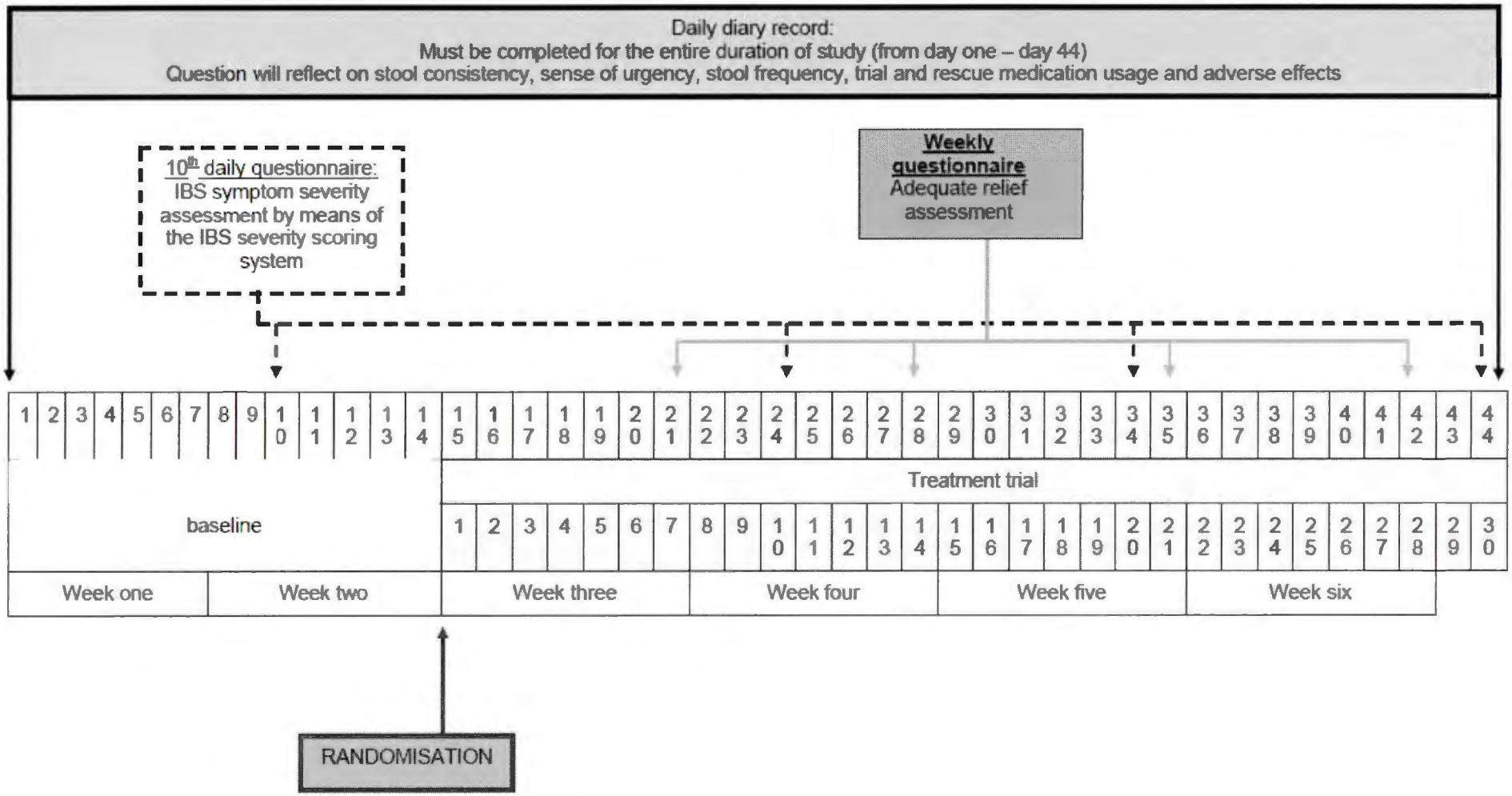



**Figure 5.2: Study layout and timing of outcome measure.**

## 5.7 Statistical Analysis

### 5.7.1 *Power calculation*

A responder rate of approximately 60% compared to a placebo response rate of 45% is considered to be a typical IBS trial scenario (Drossman, 2005). Therefore, it was estimated that to obtain a 75% responder rate in the Absorbatox™ C35 group compared to a responder rate of 40% in the placebo group, 30 IBS patients were needed to reach a statistical power of 80% (two proportions Z-test). The significance level was set at 5% (Irvine *et al.*, 2006).

### 5.7.2 *Data Analysis*

Analysis was mainly done by intention-to-treat, i.e. participants are analysed in the group they were originally randomly allocated to, and not in the way they were actually treated. Some data, however, are presented as per-protocol, in which only participants who complied with the randomised treatment are studied (Banerjee, 2003).

All statistical analysis procedures were done in consultation with the Statistical Consultation Services of the North-West University under guidance of the study statistician (FS). Data were analysed using Statistica Data Analysis Software System (Statsoft, Inc.2007).

#### 5.7.2.1 *Per-protocol analysis*

The nonparametric test, the Friedman analysis of variance (ANOVA) for repeated measures was used to assess the treatment effects across treatment weeks in the placebo and Absorbatox™ C35 group, respectively.

#### 5.7.2.2 *Intention-to-treat analysis*

The Fisher exact (one tailed) test was used for dichotomous data, to assess the difference between the two treatment groups (e.g. **adequate relief**, **currently having distension**, and **currently having pain** parameters).

The Mann-Whitney U test (nonparametric test) was performed to assess treatment differences between groups for quantitative data (**severity of pain, severity of distension, bowel habit satisfaction and interference with life**).

The Wilcoxon matched pairs (signed rank) test was used to assess the treatment effects between end of treatment and baseline observations for placebo and Absorbatox™ C35, respectively (day 10 vs. day 44).

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### 5.7.3 *Level of significance*

The p-value is the probability to reject the null-hypothesis, which states that the two groups being compared are similar, when it is in fact true (Kajee, 2004).

The statistical significance level was pre-set at 0.05, which means that whenever $p < 0.05$, the null-hypothesis is rejected (Steyn *et al.*, 1998).

Chapter 6 elaborates on the results of this study by using descriptive statistics.

# CHAPTER 6 RESULTS

In this chapter Absorbatox™ C35 will be referred to as ABTX.

## 6.1 Recruitment

A total of 67 potential IBS candidates were recruited from the Potchefstroom district. Thirty four candidates were excluded as some have not met inclusion criteria (n = 20) and others did not want to take part (n = 14). Reasons for exclusions are listed in Table 6.1.

**Table 6.1: Reasons for exclusions.**

| Reasons | N | Additional information |
|---|---|---|
| **1. Lack of interest** | **14** | |
| **2. Did not meet inclusion criteria** | **20** | |
| 2.1. Did not meet Rome III criteria | 16 | |
| 2.2. Disease of exclusion | 1 | Diverticular disease of colon |
| 2.3. Prohibited drug | 1 | Lithium carbonate (Camcolit®) |
| 2.4. Surgery | 1 | |
| 2.5. Abnormal FBC/ESR | 1 | |

The remaining 33 were randomly assigned to receive the Absorbatox™ C35 (ABTX) (n = 17) or the Placebo treatment (n = 15). After randomisation two participants from the ABTX group did not receive treatment and were not included in the intention-to-treat analysis (ITT). The one patient did not comply with protocol (did not complete participant booklet throughout baseline period within specified time). It was decided to withdraw this patient. The other patient did not want to return for the treatment phase. Neither of these two patients completed baseline questionnaires and no data was available for ITT.

The 15 participants that received ABTX completed the entire study. However, 2 patients from the Placebo group withdrew due to a course of antibiotic therapy (n = 1) that lead to altered bowel function, and one contracted influenza (according to patient)[2] and stopped taking study

[2] This was not confirmed by the study physician (JDP).

medication (n = 1). Therefore, the reasons for withdrawal were not ascribed to the intervention received. In total, 31 patients were included in the intention-to-treat population of which 15 participants received ABTX and 16 received Placebo treatment.

Only six males were initially recruited of which only one was entered into the study. One male patient had recently undergone surgery, two males did not meet the study entry criteria and two males did not want to take part in the study.

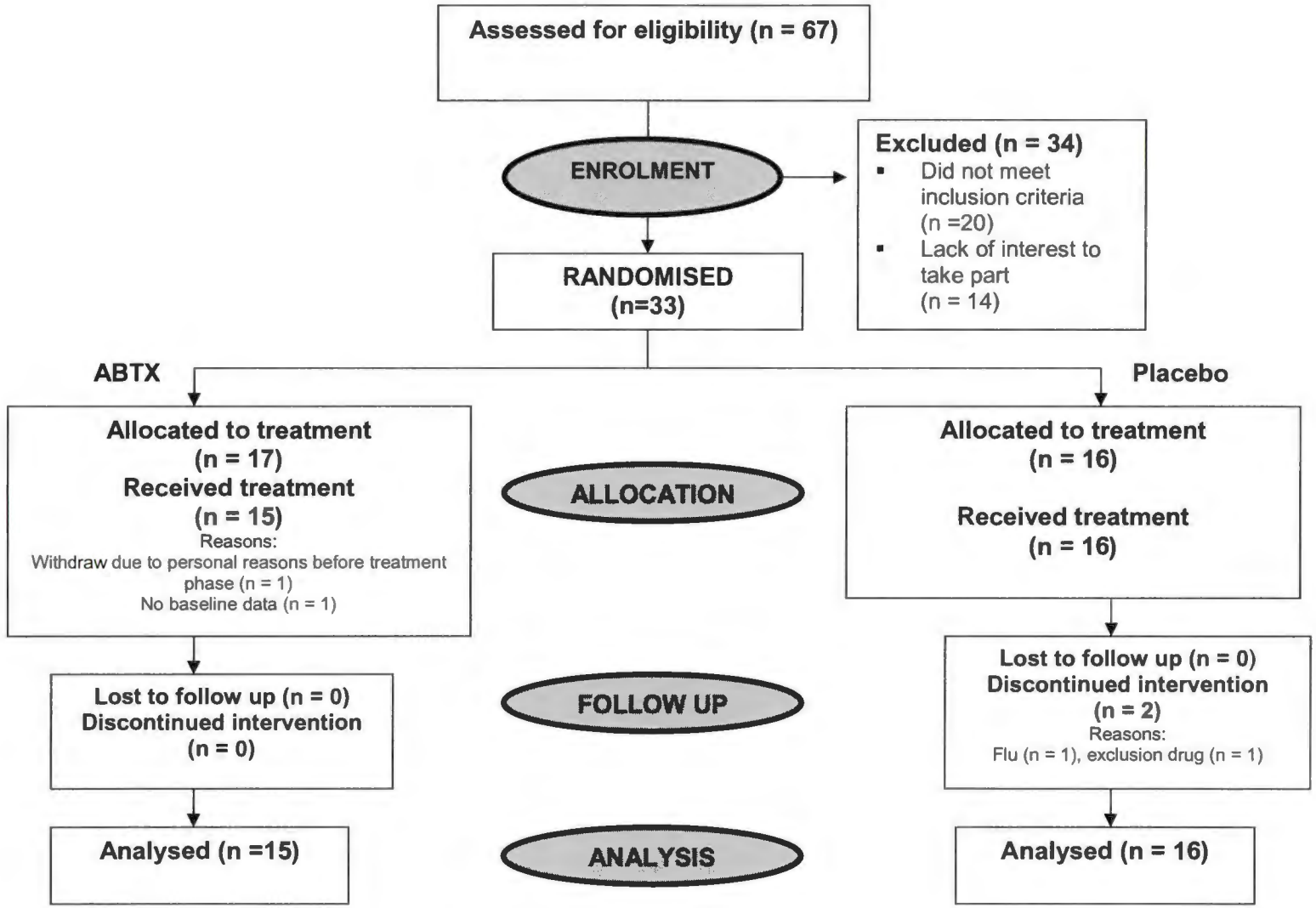



**Figure 6.1: Consort diagram.**

## 6.2 Baseline characteristics

The method of randomisation ensured equal representation of characteristics among treatment groups. Patients from the ABTX group had similar disease characteristics (total severity score, severity of pain, severity of distension, bowel habit satisfaction, and disease interference with life) and mean number of stools, compared to the placebo group. The baseline characteristics are described in Table 6.2.

Although patients in the ABTX group were on average older than patients in the placebo group, no differences were observed in the duration of IBS symptoms (described as the number of years patients are aware of bowel symptoms) and BMI scorings.

In terms of bowel habit subtype (according to Rome III criteria); patients with mixed IBS (IBS-M according to Rome III; IBS-A according to Rome II) were equally represented throughout the treatment groups. Ten IBS-M patients were allocated to the placebo group, and 8 were allocated to the ABTX group. The placebo group had 6 IBS-C patients and the ABTX group had 3. The placebo group consisted of no IBS-D patients, and the ABTX group of 4 IBS-D patients.

**Table 6.2: Summary of demographics and baseline characteristics of study patients.**

| | Absorbatox™ C35 (n = 15) | Placebo (n = 16) |
|---|---|---|
| **Age** (mean ± SD) | 45.93 ± 11.68[&] | 32.63 ± 13.41 |
| **Gender (male:female)** | 1:14 | 0:16 |
| **Race** (n) | | |
| White | 15 | 16 |
| Coloured | 1 | 0 |
| Black | 0 | 0 |
| **BMI** (mean ± SD) | 28.69 ± 6.33 | 26.67 ± 7.38 |
| **Family history of IBS** (%) | 80 | 50 |
| **ROME III bowel classification (n)** | | |
| **IBS-C** | 3 | 6 |
| **IBS-D** | 4 | 0 |
| **IBS-M** | 8 | 10 |
| **Symptoms** (mean ± SD) | | |
| Duration of symptoms (years) | 15.98 ± 11.47[ns] | 14.06 ± 11.92 |
| IBS TOTAL Severity score (max 500) | 271.89 ± 70.73[ns] | 298.00 ± 79.04 |
| Pain/discomfort[#] | 42.13 ± 22.92[ns] | 43.43 ± 27.64 |
| Distension[#] | 48.16 ± 22.64[ns] | 63.63 ± 27.75 |
| Satisfaction with bowel habit[#$] | 64.01 ± 27.66[ns] | 71.47 ± 17.22 |
| IBS interference with life[#*] | 71.59 ± 16.69[ns] | 68.84 ± 22.73 |
| Mean number of stools per day | 1.43 ± 0.86[ns] | 1.42 ± 0.99 |
| **Medication usage** (%) | | |
| Antacids/PPI | 20 | 18.75 |
| CAM users | 46.67 | 25 |
| Zelnorm® (previously used) | 26.67 | 0 |
| Laxatives | 20 | 25 |
| Antidiarrhoeals | 6.67 | 0 |
| Antispasmodics | 66.67 | 50 |

**&: p > 0.05**
**#: maximum = 100, scored on visual analogue scales**
**$: 0 = completely satisfied; 100 = completely unsatisfied**
***: 0 = no interference at all; 100 = completely interfering**
**ns: no statistically significant differences were noted between treatment groups (p > 0.05).**

## 6.3 Primary outcome: Adequate Relief

### 6.3.1 *Patients classified as overall responders*

At the end of the 30-day treatment 50% (8/16) of patients from the placebo and 80% (12/15) of patients from the ABTX group were classified as **overall responders** (patients responded to treatment in 50% of treatment weeks). The Fisher exact (one tailed) test revealed no significance (p = 0.085).

### 6.3.2 *Weekly response to treatment*

After the first week of treatment the proportion of responders (participants that answered "yes" to the adequate relief questionnaire) were similar in both groups, with 43.75% (7/16) from the placebo group and 46.67% (7/15) from the ABTX group (p = 0.578, Fisher exact test). Sixty percent (9/15) of patients from the ABTX group and 50% (8/16) of patients from the placebo group responded to treatment during the second week of treatment (p = 0.422).

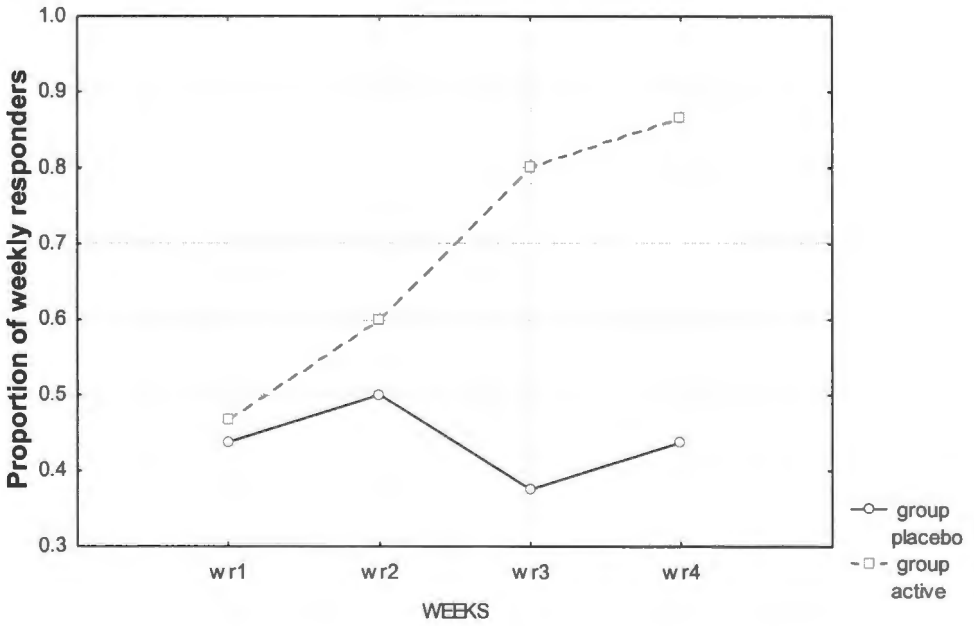



**Figure 6.2: Proportion of participants responding to treatment during week 1 (wr1), week 2 (wr2), week 3 (wr3) and week 4 (wr4) of treatment weeks (Fisher-exact).**

The ABTX group had a significantly better performance over the placebo group during the third week of treatment (wr3, Figure 6.2), as 12 patients responded to treatment in comparison to the 6 patients that responded in the placebo group, i.e. 80% from the ABTX vs. 37.50% from the placebo (p = 0.02) group. At the end of treatment, during week four (wr4, Figure 6.2), 86.67% (13/15) in the ABTX group responded significantly

to treatment in comparison with the 43.75% (7/16) of patients in the placebo group (p = 0.016).

It must be noted that missing data of the previous assessment was replaced by "last-observation-carried-forward".

## 6.4 Secondary outcome: the IBS-SSS

Data was not distributed normally; it was therefore decided to make use of non-parametric tests throughout the analysis of the IBS-SSS and its sub-components.

### *6.4.1 Total IBS Severity score according to IBS-SSS*

In the per-protocol analysis the non-parametric test for repeated measures, the Friedman ANOVA test, was performed to analyse data. Patients in the placebo group showed a statistical significant improvement (i.e. decrease in score) in the total severity score across treatment weeks (p = 0.005). Similarly, the total severity of symptoms in the patients from the ABTX was also significantly decreased across treatment weeks (p < 0.001). Refer to Figure 6.3.

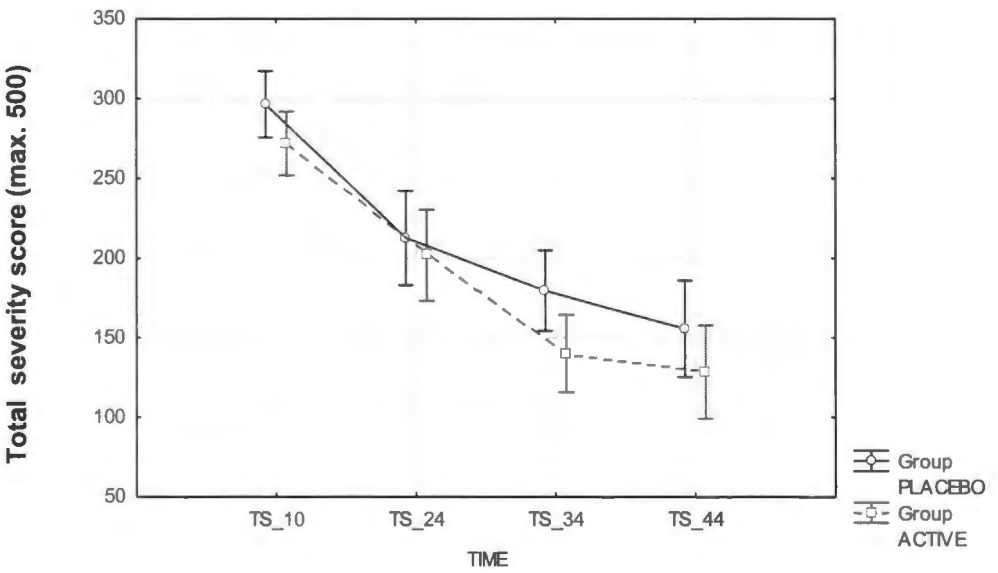



**Figure 6.3: Total IBS Severity Score (mean ±SEM) before treatment (TS_10), after 10 days of treatment (TS_24), after 20 days of treatment (TS_34) and after 30 days of treatment (TS_44) for placebo and Absorbatox™ C35 (Friedman ANOVA).**

The non-parametric Wilcoxon matched pairs (signed rank) test was performed to assess the difference in total severity scores between day 10 and day 44 for the placebo and ABTX groups, respectively (ITT). The mean score for **placebo group dropped significantly** from 298.00 (day 10, baseline) to 155.6 (day 44, end of treatment) ($p = 0.005$). In the **ABTX** group, the mean severity score **dropped** from 271.9 (day 10) to 128.6 (day 44), revealing statistical **significance** ($p < 0.001$, Wilcoxon matched pairs test). Refer to figure 6.4

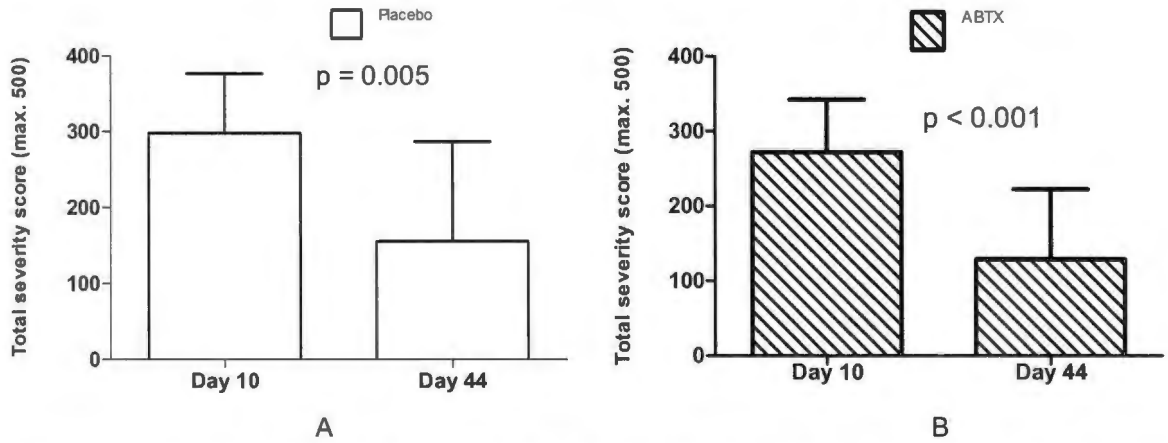



**Figure 6.4(A): Mean ± SD total severity scores on day 10 (baseline) and on day 44 (end of treatment) for placebo (Wilcoxon matched pairs test) Figure 6.4(B): Mean ± SD total severity scores on day 10 (baseline) and on day 44 (end of treatment) for ABTX (Wilcoxon matched pairs test).**

The non-parametric Mann-Whitney U test was performed to assess the difference between treatment groups on day 10, day 24, day 34 and day 44, respectively. The baseline severity scores were similar between the treatment groups, (298.0 vs. 271.9 for placebo and ABTX, respectively, $p = 0.247$) and **no significant differences were noted** during treatment on **day 24** ($p = 0.953$), **day 34** ($p = 0.371$) and on **day 44** ($p = 0.777$). Refer to Table 6.3.

**Table 6.3: Mean total severity scores on day 10, 24, 34 and 44 for placebo and Absorbatox™ C35 (Mann-Whitney U test).**

| Assessment Interval (day) | Group | Mean total severity scores | SD | p-value |
|---|---|---|---|---|
| **10** | **placebo** | 298.00 | 79.04 | 0.247 |
| | **ABTX** | 271.9 | 70.73 | |
| **24** | **placebo** | 205.3 | 115.7 | 0.953 |
| | **ABTX** | 201.7 | 98.74 | |
| **34** | **placebo** | 179.6 | 106.3 | 0.371 |
| | **ABTX** | 140.2 | 82.08 | |
| **44** | **placebo** | 155.6 | 131.20 | 0.777 |
| | **ABTX** | 128.6 | 93.87 | |

## 6.4.2 Pain measures

### 6.4.2.1 Current pain

The patients from the placebo group did not show significant decreases across the treatment weeks according to the Friedman ANOVA analysis (p = 0.070) (per-protocol analysis). However, the ABTX patients' current pain situation decreased significantly across the treatment weeks (p = 0.025).

The Fisher exact (one tailed) test was performed to assess the difference between the treatment groups (placebo vs. ABTX) on day 10, 24, 34 and 44, respectively. At **baseline** (measured on day 10) the percentage of patients that "currently" (referred to as having pain in the last 10 days) suffered from pain were **similar between the treatment groups** (93.75% and 93.33% for placebo and ABTX respectively, p = 0.742, Fisher exact test). There was **no significant difference** observed **between the two treatment** groups in treatment on **day 24 (p = 0.641), 34 (p = 0.600)** and **44 (p = 0.587)** (Fisher exact test). Refer to Table 6.4.

**Table 6.4: Percentage of participants currently suffering from abdominal pain on day 10, 24, 34, 44 [Fisher exact (one tailed) test].**

| Days | Absorbatox™ C35 | | Placebo | | p-value (Fisher-exact, one tailed) |
|---|---|---|---|---|---|
| | % (n) | n | % (n) | n | |
| **10** | 93.33 | 15 | 93.75 | 16 | 0.742 |
| **24** | 80.00 | 15 | 81.25 | 16 | 0.641 |
| **34** | 66.67 | 15 | 64.29 | 14 | 0.600 |
| **44** | 60.00 | 15 | 57.14 | 14 | 0.587 |

### 6.4.2.2 Severity of pain

Before treatment had started the severity scores for pain was similar between the two groups (p = 0.874, Mann Whitney U test).

The severity of pain was significantly decreased across the treatment weeks, in the placebo and in the ABTX treatment groups according to the Friedman ANOVA analysis (p = 0.033 and 0.001 for placebo and ABTX respectively). Refer to figure 6.5.

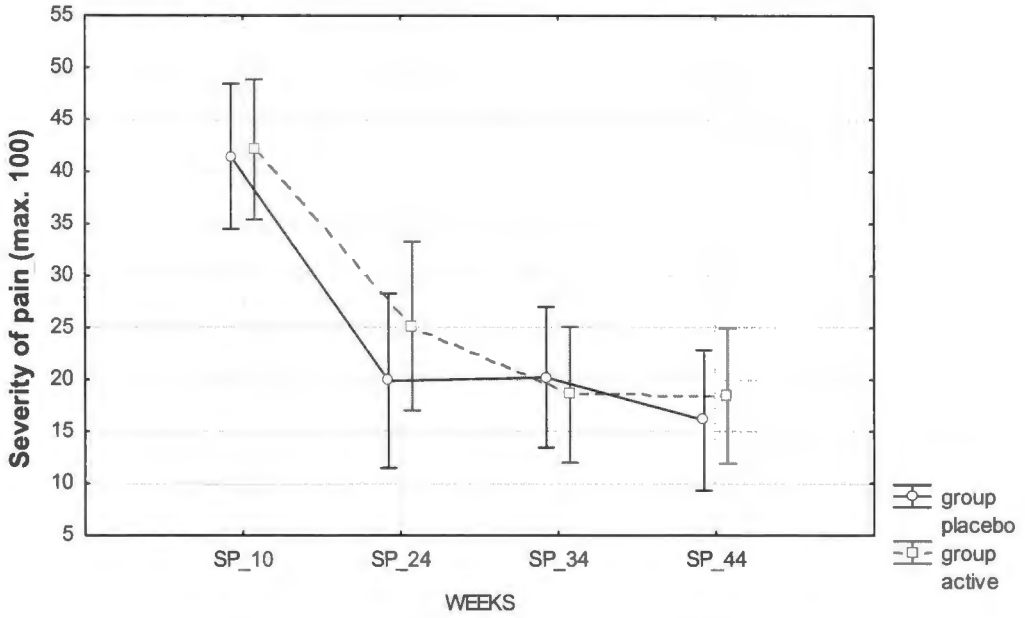



**Figure 6.5: Severity of pain scores (mean ± SEM) before treatment (SP_10), after 10 days of treatment (SP_24), after 20 days of treatment (SP_34) and after 30 days of (SP_44) treatment for placebo and ABTX (Friedman ANOVA).**

The severity of pain **dropped significantly** in the **placebo** group, from 43.43 (day 10, baseline) to 16.09 on day 44 (Wilcoxon signed rank test, p = 0.426, Figure 6.6). Likewise according to Figure 6.6, the **ABTX** group had a **significant difference** in the severity of pain between **day 10** and **day 44** (Wilcoxon signed rank, p = 0.003).

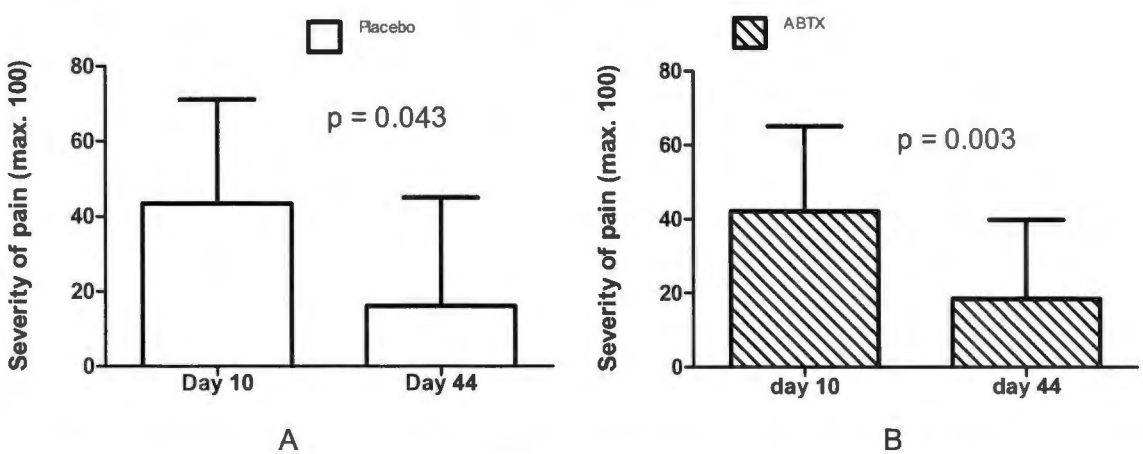



**Figure 6.6(A): Mean ± SD pain severity scores on day 10 (baseline) and on day 44 for placebo (Wilcoxon matched pairs test) Figure 6.6(B): Mean ± SD pain severity scores on day 10 (baseline) and on day 44 for ABTX (Wilcoxon matched pairs test).**

During treatment, on **day 24, 34 and 44 no significance** in pain severity between treatment groups (placebo vs. ABTX) was observed through the Mann-Whitney analysis (refer to Table 6.5).

**Table 6.5: Mean Severity of pain scores on day 10, 24, 34 and 44 for placebo and Absorbatox™ C35 (Mann-Whitney U test).**

| Assessment Interval (day) | Group | Mean pain severity scores | SD | p-value |
|---|---|---|---|---|
| 10 | placebo | 43.43 | 27.64 | 0.890 |
| | ABTX | 42.13 | 22.92 | |
| 24 | placebo | 18.30 | 32.20 | 0.126 |
| | ABTX | 25.17 | 28.72 | |
| 34 | placebo | 20.24 | 28.98 | 0.809 |
| | ABTX | 18.55 | 21.45 | |
| 44 | placebo | 16.09 | 28.99 | 0.244 |
| | ABTX | 18.45 | 21.42 | |

#### *6.4.2.3 Number of days with pain*

Both treatment groups showed significant statistical decreases in the number of days with pain (scored as number of days with pain during previous 10 days) across the treatment weeks (Friedman ANOVA, $p = 0.005$ for placebo, $p < 0.001$ for ABTX). Figure 6.7 shows the number of days decrease for the placebo and ABTX groups in a parallel manner.

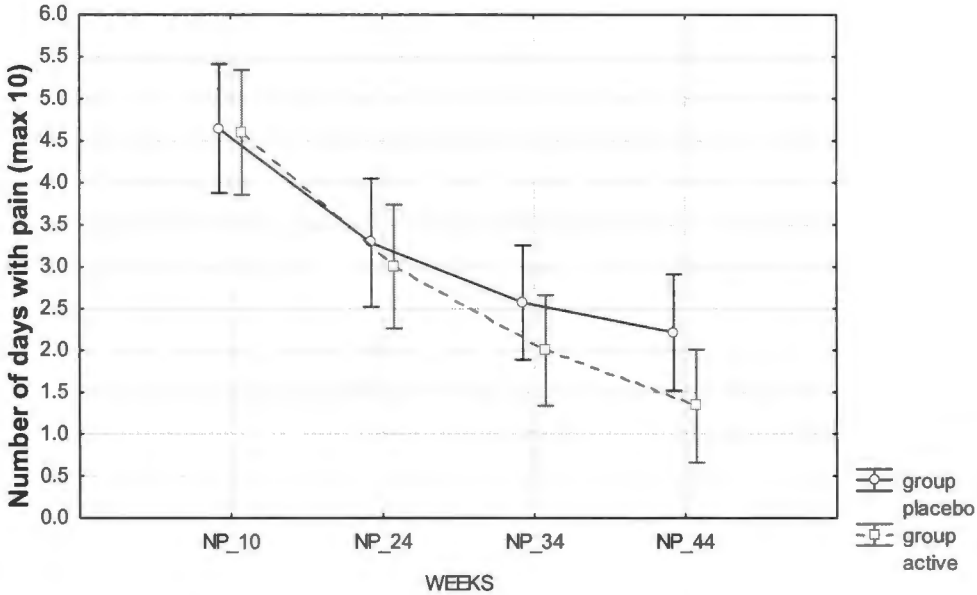
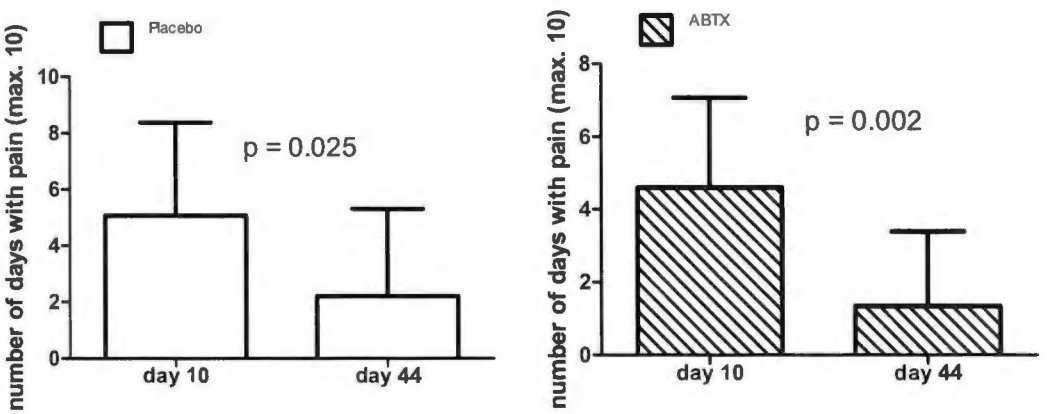



**Figure 6.7: Mean days with pain before treatment (NP_10), after 10 days of treatment (NP_24), after 20 days of treatment (NP_34) and after 30 days of treatment (NP_44) for placebo and ABTX (Friedman ANOVA).**

According to Figure 6.8 the number of days with pain were significantly lower at day 44 compared to the baseline score, on day 10 in the placebo group (p = 0.025) and in the ABTX group (p = 0.002) (Wilcoxon matched pairs test).




A B

**Figure 6.8(A): Mean ± SD number of days with pain on day 10 (baseline) and on day 44 (end of treatment) for Placebo (Wilcoxon matched pairs test) Figure 6.8(B): Mean ± SD number of days with pain on day 10 (baseline) and on day 44 for ABTX (Wilcoxon matched pairs test).**

The baseline (day 10) mean days with pain were similar between the two groups (5.60 and 4.60 for the placebo and ABTX groups respectively, p = 0.720, Mann-Whitney U test). The mean number of days with pain **did not differ significantly between the treatment groups** on any of days 24, 34 and 44 according to Mann-Whitney U test (refer to Table 6.6).

**Table 6.6: Mean Number of days with pain (max. 10, rated as number of days with pain in past 10 days) on day 10, 24, 34 and 44 for placebo and Absorbatox™ C35 (Mann-Whitney U test).**

| Assessment Interval (day) | Group | Mean number of days with pain | SD | p-value |
|---|---|---|---|---|
| 10 | placebo | 5.06 | 3.32 | 0.735 |
| | ABTX | 4.60 | 2.47 | |
| 24 | placebo | 3.44 | 2.988 | 0.811 |
| | ABTX | 3.00 | 2.54 | |
| 34 | placebo | 2.57 | 2.93 | 0.771 |
| | ABTX | 2.00 | 2.17 | |
| 44 | placebo | 2.21 | 3.09 | 0.648 |
| | ABTX | 1.33 | 2.06 | |

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## *6.4.3 Distension*

### *6.4.3.1 Current distension*

**The baseline** measures for "currently having distension" (referred to as having distension/bloating/swollen/tight tummy in the last 10 days) were **similar between the treatment groups** (Table 6.7).

Patients "current distension" situation **decreased significantly** across the treatment weeks in both the placebo (p = 0.025) and ABTX (p = 0.011) groups (Friedman ANOVA, PP).

The Fisher exact (one tailed) test was performed to assess the difference between the treatment groups during treatment weeks on day 24, 34 and day 44 (placebo vs. ABTX). **No significance** was observed on day 24 (62.50% placebo vs. 66.67% ABTX, p = 0.533), day 34 (64.33% placebo vs. 53.33% ABTX, p = 0.413) and day 44 (42.86% placebo vs. 53.33% ABTX, p = 0.424).

**Table 6.7: Percentage of participants currently suffering from abdominal distension on day 10, 24, 34 and 44 [Fisher exact (one tailed) test].**

| Days | Absorbatox™ C35 | | Placebo | | p-value (Fisher exact, one tailed) |
|---|---|---|---|---|---|
| | % (n) | n | % | n | |
| **10** | 93.33 | 15 | 93.75 | 16 | 0.742 |
| **24** | 66.67 | 15 | 62.50 | 16 | 0.553 |
| **34** | 53.33 | 15 | 64.33 | 14 | 0.413 |
| **44** | 53.33 | 15 | 42.86 | 14 | 0.424 |

### *6.4.3.2 Severity of distension*

The mean severity score for distension was **significantly decreased in the placebo** as well as in the **ABTX** groups across the treatment weeks. The Friedman ANOVA test for repeated measures revealed statistical significant decreases in severity for the placebo ($p = 0.042$) and ABTX ($p < 0.001$) groups. Refer to Figure 6.9.

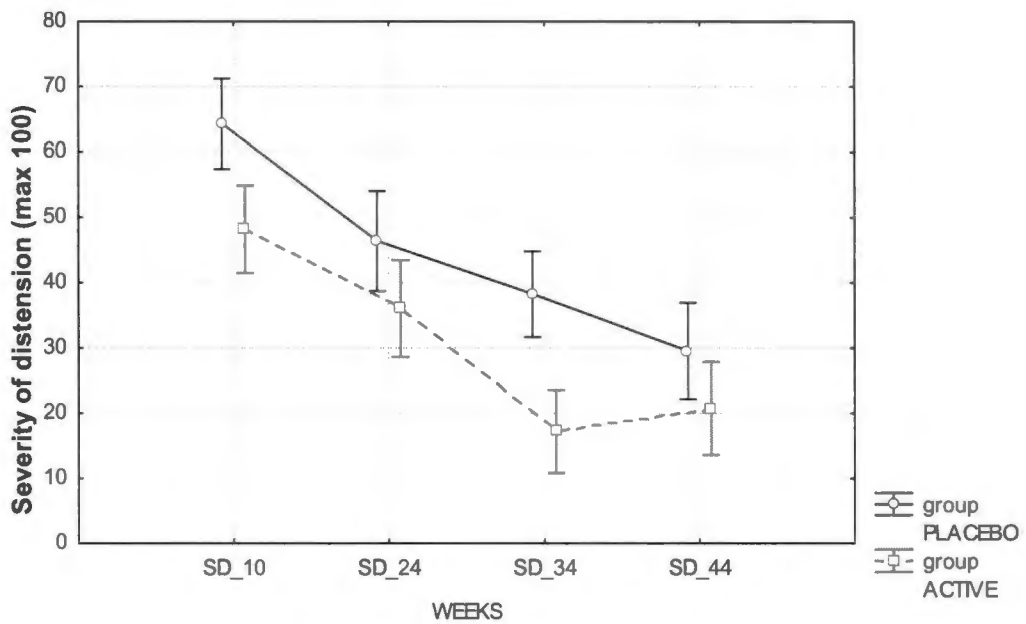


**Figure 6.9: Mean Severity of distension scores before treatment (SD_10), after 10 days of treatment (SD_24), after 20 days of treatment (SD_34) and after 30 days of treatment (SD_44) for placebo and ABTX (Friedman ANOVA).**

The Wilcoxon matched pairs test, performed to assess differences in mean severity scores before (day 10) and after treatment (day 44), revealed **significant decreases in the placebo** (day 10: 63.63 vs. day 44: 29.55, p = 0.022) and in the **ABTX** (day 10: 48.16 vs. day 44: 20.72, p = 0.006) groups.

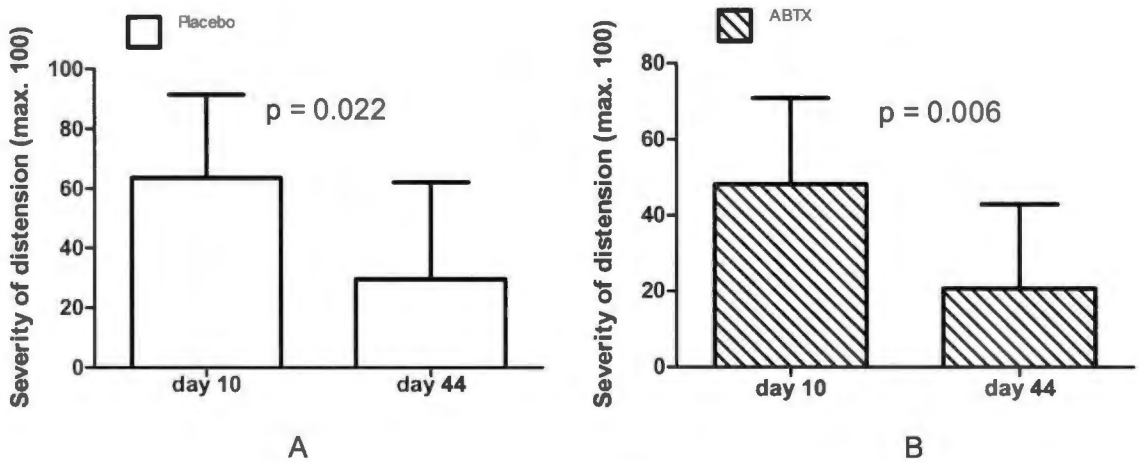


**Figure 6.10(A): Mean ± SD severity of distension scores on day 10 (baseline) and on day 44 (end of treatment) for placebo (Wilcoxon matched pairs test) Figure 6.10(B): Mean ± SD severity of distension scores on day 10 (baseline) and on day 44 for ABTX (Wilcoxon matched pairs test).**

The Mann-Whitney U test was performed to assess the difference in severity between the treatment groups (placebo vs. Absorbatox™ C35) on day 10, 24, 34 and 44 respectively. During baseline no significant diferences were observed between the treatment groups (day 10, p = 0.082). At day 24 **no significance** was observed (p = 0.692). However, on **day 34** the **ABTX** group's severity score was **significantly lower** compared to the **placebo** (38.25 vs. 17.20, p = **0.024**) group. Subsequently, on **day 44 no significant difference** in severity scores was noted (29.55 vs. 20.72, p = 0.553).

**Table 6.8: Mean Severity of distension scores on day 10, 24, 34 and 44 for placebo and Absorbatox™ C35 (Mann-Whitney U test).**

| Assessment Interval (day) | Group | Mean severity of distension scores | SD | p-value |
|---|---|---|---|---|
| **10** | **placebo** | 63.63 | 27.75 | 0.082 |
| | **ABTX** | 48.16 | 22.64 | |
| **24** | **placebo** | 40.85 | 33.44 | 0.692 |
| | **ABTX** | 36.04 | 25.17 | |
| **34** | **placebo** | **38.25** | 29.66 | **0.024** |
| | **ABTX** | **17.20** | 18.71 | |
| **44** | **placebo** | 29.55 | 32.55 | 0.553 |
| | **ABTX** | 20.72 | 22.17 | |

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### 6.4.4 *Bowel habit satisfaction*

At baseline participants had a bowel habit satisfaction score of 73.29 and 64.00 (VAS: 100 = very unhappy, 0 = very happy) for the placebo and ABTX groups, respectively (p = 0.540, Mann-Whitney U test). Refer to Table 6.9

To assess the treatment effect across the treatment weeks, the Friedman ANOVA test for repeated measures was performed within the placebo and ABTX group, respectively (Figure 6.11). Analysis has shown **significant improvement** across treatment weeks in the **placebo** group (p = 0.003). A similar trend was observed in the **ABTX** group, as satisfaction scores dropped significantly across treatment weeks (p < 0.001).

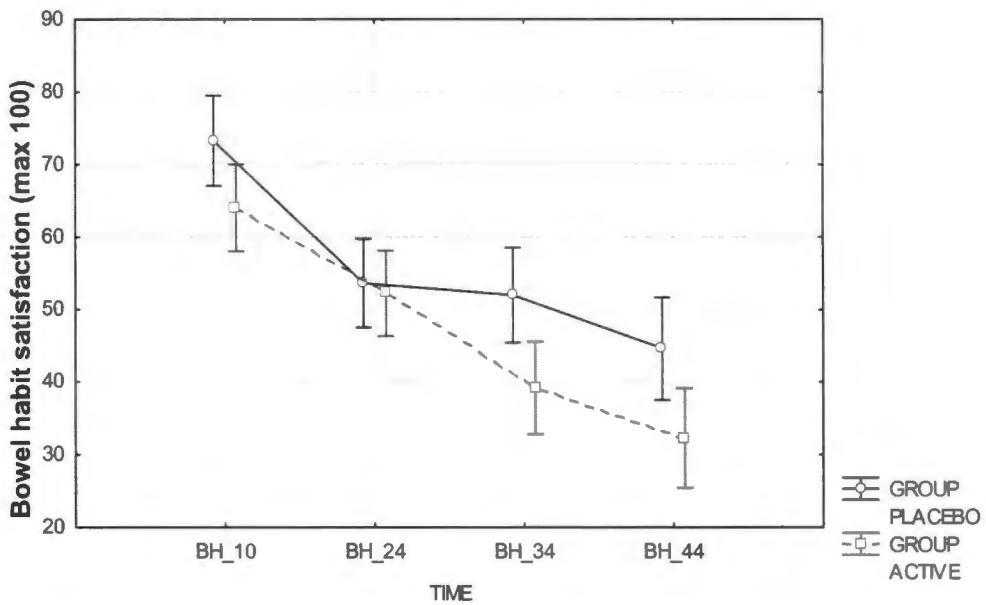


**Figure 6.11: Mean Bowel habit satisfaction scores before treatment (BH_10), after 10 days of treatment (BH_24), after 20 days of treatment (BH_34) and after 30 days of treatment (BH_44) for placebo and ABTX (Friedman ANOVA).**

The Wilcoxon matched pairs test revealed **significant differences between day 10** and **day 44** for the **placebo** (p = 0.005) and **ABTX** (p = 0.006) groups, respectively.

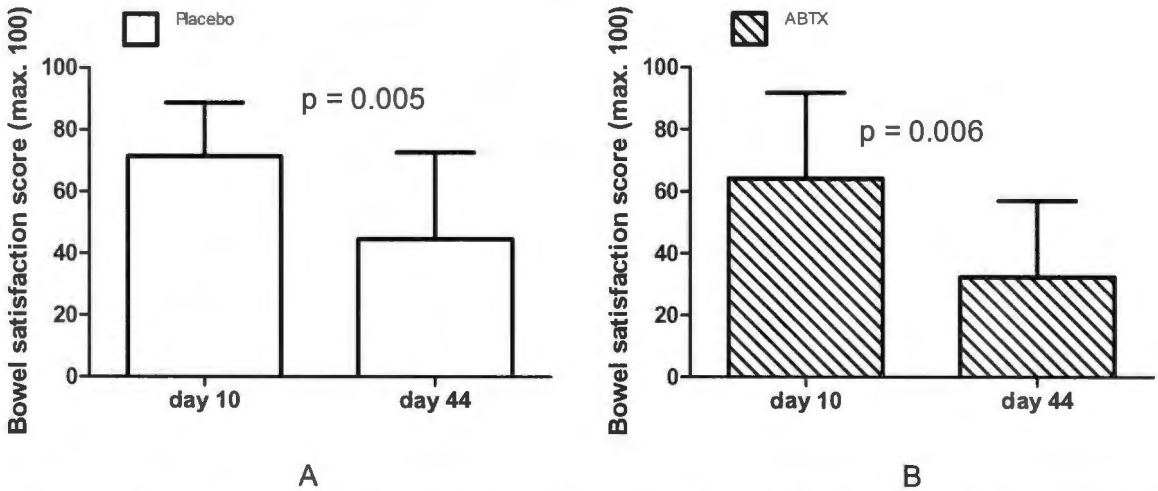


**Figure 6.12(A): Mean ± SD bowel habit satisfaction scores on day 10 (baseline) and on day 44 (end of treatment) for placebo (Wilcoxon matched pairs test) Figure 6.12(B): Mean ± SD bowel habit satisfaction scores on day 10 (baseline) and on day 44 for ABTX (Wilcoxon matched pairs test).**

The Mann-Whitney U test was performed to assess the treatment effects between treatment groups (placebo vs. ABTX) on specific intervals (on day 24, 34, 44 respectively). **No significance was observed** on day 24 (p = 0.812), day 34 (p = 0.176) or on day 44 (p = 0.176). Refer to Table 6.9.

**Table 6.9: Mean Bowel habit satisfaction scores on day 10, 24, 34 and 44 for placebo and Absorbatox™ C35 (Mann-Whitney U test).**

| Assessment Interval (day) | Group | Mean Bowel habit satisfaction scores | SD | p-value |
|---|---|---|---|---|
| 10 | placebo | 71.47 | 17.22 | 0.540 |
| | ABTX | 64.01 | 27.66 | |
| 24 | placebo | 52.31 | 21.46 | 0.812 |
| | ABTX | 52.22 | 23.20 | |
| 34 | placebo | 51.97 | 20.46 | 0.176 |
| | ABTX | 39.17 | 27.91 | |
| 44 | placebo | 44.62 | 28.13 | 0.176 |
| | ABTX | 32.27 | 24.70 | |

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### 6.4.5 *Interference with life in general*

The Friedman ANOVA (PP) revealed significant decreases across the treatment weeks to the extent that IBS interfered with patients' lives in both the placebo ($p < 0.001$) and ABTX groups ($p < 0.001$).

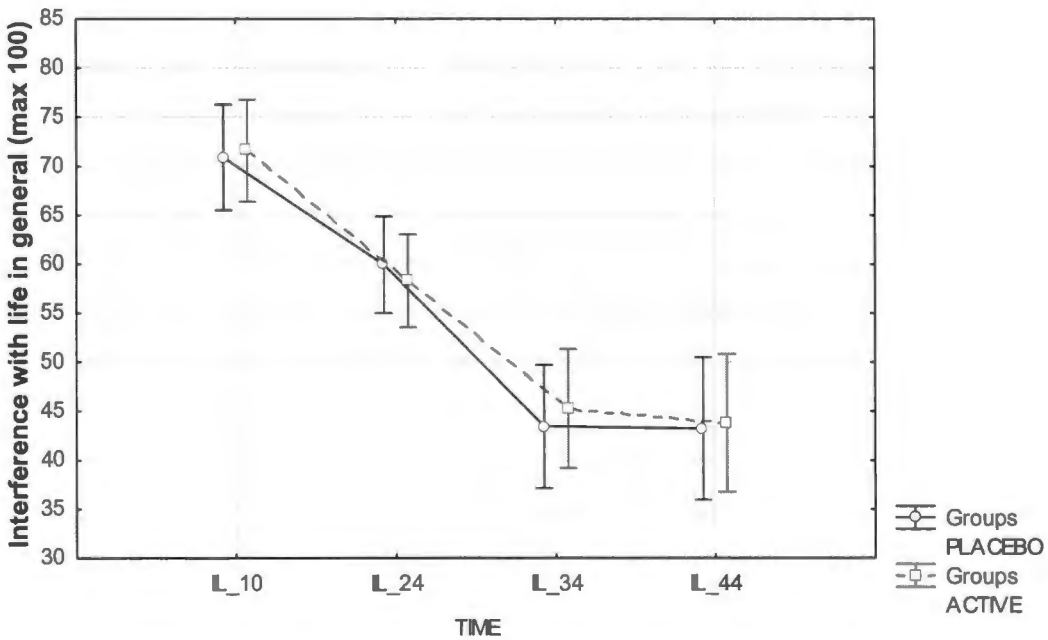


**Figure 6.13: Mean interference with life scores before treatment (IL_10), after 10 days of treatment (IL_24), after 20 days of treatment (IL_34) and after 30 days of treatment (IL_44) for placebo and ABTX (Friedman ANOVA).**

At the end of treatment (**day 44**), the interference with life score for the **placebo was significantly decreased**, compared with the **baseline** (day 10) score ($p = 0.002$, Wilcoxon). With regards to the **ABTX** group, the interference with life score was also **significantly decreased** between day 10 and day 44 ($p < 0.001$, Wilcoxon).

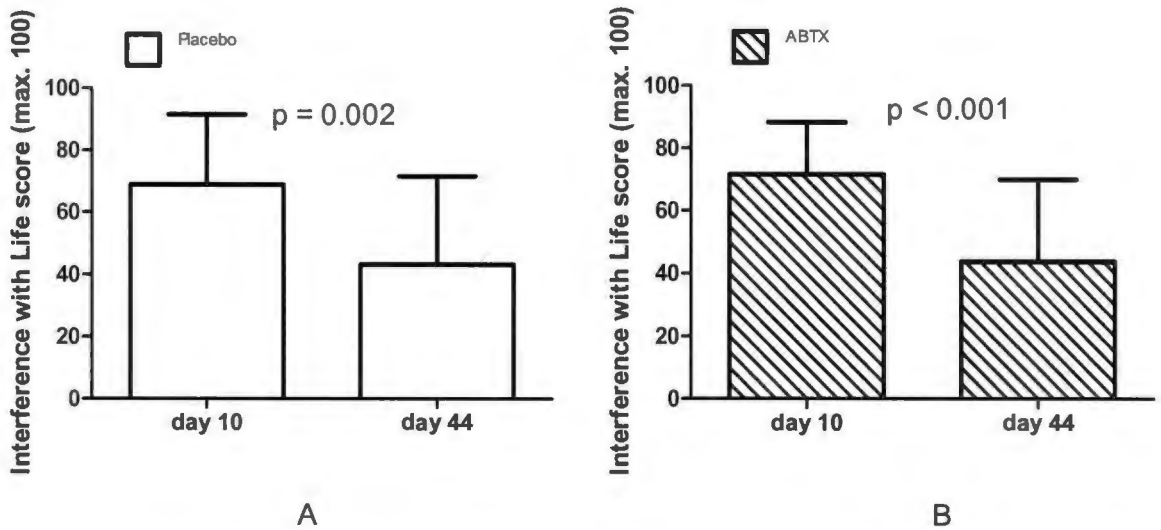


**Figure 6.14(A): Mean ± SD interference with life scores on day 10 (baseline) and on day 44 (end of treatment) for placebo (Wilcoxon matched pairs test) Figure 6.14(B): Mean ± SD inference with life scores on day 10 (baseline) and on day 44 for ABTX (Wilcoxon matched pairs test).**

The Mann-Whitney U test analysis between treatment groups (**placebo vs. ABTX**) revealed **no significance** on day 24, 34 and 44, respectively. Refer to Table 6.10.

**Table 6.10: Mean Interference with Life scores on day 10, 24, 34 and 44 for placebo and Absorbatox™ C35 (Mann-Whitney U test).**

| Assessment Interval (day) | Group | Mean interference scores | SD | p-value |
|---|---|---|---|---|
| 10 | placebo | 68.84 | 22.73 | 0.857 |
| | ABTX | 71.59 | 16.69 | |
| 24 | placebo | 59.51 | 18.70 | 0.857 |
| | ABTX | 58.31 | 18.55 | |
| 34 | placebo | 43.43 | 24.38 | 0.896 |
| | ABTX | 45.27 | 22.61 | |
| 44 | placebo | 43.22 | 28.33 | 0.983 |
| | ABTX | 43.82 | 26.15 | |

## 6.5 Stool parameters

### 6.5.1 Stool consistency

#### 6.5.1.1 Mean stool consistency

The mean stool consistency scores (scored on the categorical scale: 1 = very hard, 2 = hard, 3 = smooth, 4 = loose, 5 = very loose) between the placebo and ABTX groups differed from each other at week one, baseline. The Mann-Whitney U test has showed significant differences at week one (2.75 vs. 1.96, placebo and ABTX, respectively, p = 0.011). During week two (baseline) participants from both groups had similar ratings (2.77 vs. 2.05, placebo and ABTX respectively, p = 0.060). In addition, during the treatment weeks (on week 3, 4, 5 and 6 respectively), no significant differences were observed between the treatment groups (Mann-Whitney U test).

**Table 6.11: Mean stool consistency scores for week 1 – 6 [scored on categorical scale: 0 – very hard to 5 – very loose (watery)] (Mann-Whitney test u test).**

| | Week | Absorbatox™ C35 | | Placebo | | p-value |
|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | Mann-Whitney U test |
| Baseline | 1 | 2.75 | 0.89 | 1.96 | 0.76 | 0.011 |
| | 2 | 2.77 | 1.02 | 2.07 | 0.58 | 0.060 |
| Treatment | 3 | 2.63 | 0.75 | 2.22 | 0.61 | 0.127 |
| | 4 | 2.59 | 0.66 | 2.35 | 0.78 | 0.428 |
| | 5 | 2.68 | 0.54 | 2.38 | 0.88 | 0.176 |
| | 6 | 2.86 | 0.39 | 2.53 | 0.75 | 0.196 |

#### 6.5.1.2 Proportion smooth stool consistency

The number of smooth stool incident cases was scored over the total cases of bowel movements, to get the proportion of smooth stool consistency. The Wilcoxon matched pairs (signed rank) test was performed to compare the difference in the proportion of smooth stool during baseline phase and treatment phase (paired data: placebo-baseline vs. placebo-treatment and ABTX-baseline vs. ABTX-treatment). The values are expressed in mean ± SD.

In **the placebo** group a proportion of 0.18 ± 0.19 was reported for baseline. This proportion **increased significantly** to 0.35 ± 0.22 (p = 0.011, Wilcoxon matched pairs test) during treatment. As for the **ABTX** group, **significance** was achieved as the proportion of smooth stool **increased** from 0.27 ± 0.27 during baseline to 0.54 ± 0.23 during treatment phase (p = 0.003, Wilcoxon matched pairs test).

Patients in the placebo and ABTX group had similar ratings in terms of proportion smooth stool (0.18 ± 0.19 vs. 0.27 ± 0.27 for placebo and ABTX respectively, p = 0.264, Mann-Whitney U test). During the treatment period, the Mann-Whitney U test revealed **significant differences** in the proportion of smooth stool form **between the placebo and ABTX (p = 0.049)** groups.

### *6.5.2 Stool urgency*

At baseline patients from the placebo and ABTX groups had similar urgency scores (1.87 vs. 2.10, p = 0.513; Mann-Whitney U test). No significant changes were observed during week 3, 4, 5, or 6 (treatment weeks) between the placebo and ABTX groups (Mann-Whitney U test). Refer to Table 6.12.

**Table 6.12: Mean urgency scores between treatment groups for week 1 – 6 [rated on categorical scale; 0 – no urgency at all; 5 – very severe urgency] (Mann-Whitney U test).**

| | Week | Absorbatox™ C35 | | Placebo | | p-value |
|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | Mann-Whitney U test |
| Baseline | 1 | 2.10 | 0.70 | 1.87 | 0.68 | 0.513 |
| | 2 | 2.31 | 1.00 | 2.10 | 0.60 | 0.634 |
| Treatment | 3 | 1.99 | 0.72 | 1.98 | 0.47 | 0.827 |
| | 4 | 2.02 | 0.57 | 1.93 | 0.54 | 0.677 |
| | 5 | 2.05 | 0.61 | 2.19 | 0.76 | 0.467 |
| | 6 | 1.94 | 0.60 | 2.33 | 0.67 | 0.156 |

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## 6.5.3 Stool frequency

### 6.5.3.1 Mean number of bowel movements per day

At baseline (week one and week two) a similar mean number of bowel movements (stools) was observed between the placebo (week one: 1.44, week two: 1.40) and ABTX (week one: 1.46, week two: 1.40) groups. The Mann-Whitney U test revealed no significance between the treatment groups at baseline (week one and two).

During **week 3** ($p = 0.648$), **week 4** ($p = 0.937$), **week 5** ($p = 0.252$) and **week 6** ($p = 0.228$) **no significant differences between the placebo and the ABTX** groups was observed. Refer to Table 6.13.

**Table 6.13: Mean number of bowel movements per day between treatment groups (Mann-Whitney U test).**

| | Week | Absorbatox™ C35 | | Placebo | | p-value |
|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | Mann-Whitney U test |
| Baseline | 1 | 1.46 | 0.78 | 1.44 | 1.10 | 0.6779 |
| | 2 | 1.40 | 0.94 | 1.40 | 0.87 | 0.858 |
| Treatment | 3 | 1.50 | 0.83 | 1.38 | 0.90 | 0.648 |
| | 4 | 1.36 | 0.74 | 1.34 | 0.66 | 0.937 |
| | 5 | 1.45 | 0.59 | 1.16 | 0.77 | 0.252 |
| | 6 | 1.52 | 0.59 | 1.23 | 0.60 | 0.228 |

### 6.5.3.2 Mean proportion of days with no bowel movement (BM = 0)

During week 1 ($p = 0.515$) and 2 ($p = 0.584$), the BM = 0 scores were similar between the treatment groups (Mann-Whitney U test). A mean score of 0.21 and 0.22 was scored during week one and two, for the ABTX group. For the placebo group, 0.31 and 0.27 were scored during week one and two, respectively.

During the treatment weeks no significance was observed between the placebo and ABTX during week 3, 4, 5 or 6 (Mann-Whitney U test).

**Table 6.14: Mean proportion of days with no bowel movement (no stool; BM = 0) of placebo and Absorbatox™ C35 (Mann-Whitney).**

| | Week | Absorbatox™ C35 | | Placebo | | p-value |
|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | Mann-Whitney u test |
| Baseline | 1 | 0.21 | 0.19 | 0.31 | 0.32 | 0.515 |
| | 2 | 0.22 | 0.58 | 0.27 | 0.27 | 0.584 |
| Treatment | 3 | 0.18 | 0.44 | 0.25 | 0.22 | 0.351 |
| | 4 | 0.22 | 0.94 | 0.22 | 0.22 | 0.984 |
| | 5 | 0.13 | 0.13 | 0.28 | 0.33 | 0.378 |
| | 6 | 0.09 | 0.24 | 0.17 | 0.22 | 0.502 |

#### *6.5.3.3 Mean proportion of days with more than 3 bowel movements (BM > 3)*

Baseline scores for BM > 3 were similar between the two treatment groups (p = 0.818; 0.931 for week 1 & 2, respectively; Mann-Whitney U test).

No significant difference was observed between treatment groups during week 3 (p = 0.710), week 4 (p = 0.309), week 5 (p = 0.292) and week 6 (p = 0.620).

**Table 6.15: Mean proportion of days with more than 3 bowel movement (BM > 3) of placebo and Absorbatox™ C35 (Mann-Whitney U test).**

| | Week | Absorbatox™ C35 | | Placebo | | p-value |
|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | Mann-Whitney U test |
| Baseline | 1 | 0.06 | 0.13 | 0.03 | 0.06 | 0.818 |
| | 2 | 0.06 | 0.15 | 0.04 | 0.08 | 0.931 |
| Treatment | 3 | 0.02 | 0.05 | 0.03 | 0.06 | 0.710 |
| | 4 | 0.03 | 0.06 | 0.01 | 0.04 | 0.309 |
| | 5 | 0.03 | 0.06 | 0.01 | 0.04 | 0.292 |
| | 6 | 0.01 | 0.04 | 0.01 | 0.03 | 0.620 |

## 6.6 Heartburn episodes

The heartburn episodes data were normally distributed, therefore; the Unpaired t-test (t-test for independent samples) was used instead of the Mann-Whitney U test, and the Paired t-test instead of the Wilcoxon matched pairs (signed rank) test. During baseline the mean proportion of days with heartburn/dyspepsia [Question: *Did you experience any episodes of heartburn or indigestion (dyspepsia) today?*] was 0.37 for the placebo and 0.19 for the ABTX groups, no statistical significance was observed (p = 0.129, Unpaired t-test).

**Table 6.16: Mean proportion of days with heartburn/dyspepsia at baseline and treatment phase for placebo (paired t test).**

| | Mean | SD | p-value |
|---|---|---|---|
| **Baseline** | 0.37 | 0.34 | **0.030** |
| **Treatment** | 0.21 | 0.22 | |

Patients in the **placebo** group showed a **significant** decrease in proportion of days with heartburn/dyspepsia episodes between baseline and treatment period (0.37 vs. 0.21, p = 0.035, Paired t-test). The T-test analysis revealed **no significant** decrease in mean proportion of days

with heartburn episodes between baseline and treatment period in the **ABTX** group (0.189 vs. 0.128, p = 0.393, Paired t-test).

**Table 6.17: Mean proportion of days with heartburn/dyspepsia at baseline and treatment phase for Absorbatox™ C35 (paired t-test).**

| | Mean | SD | p-value |
|---|---|---|---|
| **Baseline** | 0.19 | 0.29 | **0.393** |
| **Treatment** | 0.13 | 0.17 | |

In addition, the Unpaired t-test analysis revealed **no significant difference between the placebo and ABTX** groups during the treatment period (p = 0.223).

## 6.7 Drug compliance

In the intention-to-treat population, the compliance was 98.30% in the placebo group, and 94% in the ABTX group. The Per-protocol analysis revealed a compliance of 94.52% and 94.00% for the placebo and ABTX groups, respectively.

Two patients form the placebo group withdrew; one stopped taking medication on the 16$^{th}$ day (case#1) of treatment and the other on the 13$^{th}$ day (case#2) of treatment. It was calculated that case#1 had to return 42 capsules. In fact, 33 capsules were returned. Case#2 returned 39 capsules, whilst 51 capsules had to be returned.

## 6.8 Rescue medication consumption

The number of times patients have used *any* rescue medication was scored over the number of treatment days (30). Data was analysed using the Mann-Whitney U non-parametric test to compare differences in the placebo and ABTX groups. A proportion of 0.09 ± 0.21 (mean ± SD) and 0.06 ± 0.08 were scored for the ABTX and placebo groups, respectively (p = 0.516).

**Table 6.18: The distribution on number of times participants used a particular Rescue Medication in placebo and Absorbatox™ C35.**

| | Placebo | Absorbatox™ C35 |
|---|---|---|
| Loperamide 2 mg tablets | 0 | 0 |
| Lactulose Dry 20 g | 15 | 34 |
| Mebeverine 135 mg | 13 | 7 |

## 6.9 EMP questionnaire

At the end of the treatment period, 64.29% (9/14) of the patients in the placebo group said that they would recommend the treatment for other sufferers compared to 86.67% (13/15) in the ABTX group ($p = 0.166$, Fisher exact (one tailed) test). In addition, 57.14% (8/14) of the patients in the placebo group said that they would use the product in the future compared to 86.67% (13/15) of patients in the ABTX group ($p = 0.086$, Fisher exact test, one tailed).

## 6.10 Adverse drug events

Patients that experienced ***at least*** one adverse event (AE) due to the trial medication were 56.25% (9/16) and 40% (6/15) for the placebo and ABTX group, respectively. The Fisher exact test (one tailed) revealed no significant difference between the treatment groups ($p = 0.293$).

The number of days patients had experienced an adverse event was scored over the number of treatment days and expressed as the proportion of days with adverse events. The Mann-Whitney U test revealed no significant difference between the placebo and ABTX groups ($p = 0.259$).

Participants had to rate the severity of adverse events, as mild, moderate or severe. A total of 38 AE were reported in the placebo group, of which 13 were rated as mild, 20 as moderate and 5 as severe. Eleven cases of AE were reported in the ABTX group of which 3 were rated as mild, 7 as moderate and 1 as severe. Table 6.19 is a summary of reported adverse events.

Table 6.19: Summary of adverse events.

| | Placebo (n = 16) | Absorbatox™ C35 (n = 15) |
|---|---|---|
| **Mean proportion of days with adverse events (Mean ± SD)** | 0.09 ± 0.15 | 0.02 ± 0.04 |
| **Maximum proportion of days with adverse event reported** | **0.53** | **0.13** |
| **Adverse events that were reported (at least once):** | | |
| **Gastrointestinal disorders (%):** | | |
| Abdominal distension | 3 (18.75) | 0 |
| Abdominal discomfort | 1 (6.25) | 0 |
| Abdominal pain (spasm/cramps) | 3 (18.75) | 2 (12.25) |
| Belching | 1 (6.25) | 0 |
| Constipation | 0 | 0 |
| Diarrhoea | 0 | 0 |
| Dry mouth | 0 | 1 (6.25) |
| Flatulence | 1 (6.25) | 1 (6.25) |
| Heartburn | 1 (6.25) | 0 |
| Loose stools | 0 | 2 |
| Nausea | 2 (12.25) | 2 (12.25) |
| Vomiting | 1 (6.25) | 0 |
| **Urinary tract disorders:** | | |
| Polyuria | 1 (6.25) | 0 |
| **Nervous system disorders:** | | |
| Dizziness | 0 | 1 (6.25) |
| Headache | 1 (6.25) | 1 (6.25) |
| **Other:** | | |
| Fever | 1 (6.25) | 0 |
| Flu-like symptoms | 0 | 2 (12.25) |

## 6.11 Summary of results

Absorbatox™ C35 participants had a greater trend towards adequate relief compared to placebo. The Wilcoxon matched pairs tests have shown reductions of symptoms scores in both the active and placebo groups. The Mann Whitney U test analysis could not reveal any difference between the placebo and ABTX groups, except for one parameter (severity of distension after 20 days of treatment). With regards to stool parameters, no differences were noted between ABTX and placebo, except for the proportion of smooth stool. Absorbatox™ C35 participants had a greater proportion of smooth stool cases, compared to the placebo participants. The following table summarise the results of the present trial.

**Table 6.20: Summary of primary outcome, secondary outcome and stool parameters results in the ITT population (ns: not significant).**

| Measurement | p – values (results) | Statistical significance/ clinical meaning full effect | Data comparison | Method of analysis |
|---|---|---|---|---|
| ***Primary Outcome:*** | | | | |
| 1) Overall responders | 0.085 | ns | ABTX vs. placebo (unpaired) | Fisher exact test (one-tailed) |
| 2) Weekly response to treatment: | | | | |
| Week one: | 0.578 | ns | | |
| Week two: | 0.422 | ns | | |
| Week three: | **0.02** | **significant** | | |
| Week four: | **0.016** | **significant** | | |
| ***Secondary outcome:*** | | | | |
| 1) Total severity score (IBS-SSS) | | | Day 10 vs. day 44 (paired data) | Wilcoxon signed rank |
| ▪ **placebo** | **0.005** | **significant** | | |
| ▪ **ABTX** | **<0.001** | **significant** | | |
| **50 points reduction in IBS-SSS from day 10 to day 44?** | | | | According to Francis *et al.*, 1997 |
| ▪ **placebo** | **↓ 142.4 (298.0 – 155.6)** | **YES** | | |
| ▪ **ABTX** | **↓143.3 (271.9 – 128.6)** | **YES** | | |
| ***Stool parameters:*** | | | | |
| 1) Stool consistency | | | ABTX vs. placebo (unpaired data) | Mann-Whitney U test |
| ▪ Mean stool consistency | | | | |
| *Week one* (baseline) | **0.011** | **Significant** | | |
| *Week two* (baseline) | 0.060 | ns | | |
| *Week three* | 0.127 | ns | | |
| *Week four* | 0.428 | ns | | |
| *Week five* | 0.176 | ns | | |
| *Week six* | 0.196 | ns | | |
| ▪ Proportion smooth stool ABTX vs. placebo | **0.049** | **significant** | ABTX vs. placebo (unpaired data) | Mann-Whitney U test |
| 2) Urgency | | | | |
| ▪ Mean stool urgency | | | ABTX vs. placebo (unpaired data) | Mann-Whitney U test |
| *Week one* (baseline) | 0.513 | ns | | |
| *Week two* (baseline) | 0.634 | ns | | |
| *Week three* | 0.827 | ns | | |
| *Week four* | 0.677 | ns | | |
| *Week five* | 0.467 | ns | | |
| *Week six* | 0.156 | ns | | |

| | | | | |
|---|---|---|---|---|
| 3) Frequency | | | | |
| ▪ Mean number of BM/day | | | placebo vs. | Mann- |
| *Week one* (baseline) | 0.678 | ns | ABTX | Whitney U |
| *Week two* (baseline) | 0.858 | ns | (unpaired | test |
| *Week three* | 0.648 | ns | data) | |
| *Week four* | 0.937 | ns | | |
| *Week five* | 0.252 | ns | | |
| *Week six* | 0.228 | ns | | |
| ▪ Mean proportion of days with BM = 0 | | | placebo vs. ABTX | Mann- Whitney U |
| *Week one* (baseline) | 0.515 | ns | (unpaired | test |
| *Week two* (baseline) | 0.584 | ns | data) | |
| *Week three* | 0.351 | ns | | |
| *Week four* | 0.984 | ns | | |
| *Week five* | 0.378 | ns | | |
| *Week six* | 0.502 | ns | | |
| ▪ Mean proportion of days with BM > 3 | | | | |
| *Week one* (baseline) | 0.818 | ns | placebo vs. | Mann- |
| *Week two* (baseline) | 0931 | ns | ABTX | Whitney U |
| *Week three* | 0.710 | ns | (unpaired | test |
| *Week four* | 0.309 | ns | data) | |
| *Week five* | 0.292 | ns | | |
| *Week six* | 0.620 | ns | | |

The findings of this trial are discussed in Chapter 7 and are put into context with relevant literature.

# CHAPTER 7 DISCUSSION

## 7.1 Introduction

In the current trial a natural zeolite, Absorbatox™ C35 were proposed and tested as a new CAM agent in IBS. This is the first clinical study in which the natural zeolite is assessed for efficacy in IBS patients. Due its unique structure (Zarkovic *et al.*, 2003), ion and gas adsorbing properties and beneficial effects on the GI tract (Ackley *et al.*, 2003; Mumpton, 1999; Rodríguez-Feuntes *et al.*, 1997; Simón Carballo *et al.*, 2001; Varel *et al.*, 1987; Ward *et al.*, 1993), Absorbatox™ C35 was worthy of investigation in a randomised, double-blind, placebo-controlled trial in IBS patients.

Since IBS is a chronic disorder, safety and tolerability are valid factors to consider when a certain drug is used for one or more symptoms, as sufferers are likely to consume medication over long periods of time (Heading *et al.*, 2006). The active form of Absorbatox™ C35 has not shown side effects in various animal (Pavelić *et al.*, 2001) and human studies (Ivkovic *et al.*, 2004), and has lacked significant side effects in the present trial. Absorbatox™ C35 might be effective as an adjuvant treatment in the management of IBS in patients with similar characteristics as were demonstrated in this trial. Although largely unavoidable, the placebo effect and the small sample size may be labelled as study limitations. A larger clinical trial over an extended period of time is needed to prove the exact efficacy of Absorbatox™ C35 in IBS.

## 7.2 Methodology and Study limitations

The American College of Gastroenterology (ACG) has developed a grading tool in which the quality of trial methodology is evaluated and quantitatively scored on a 14-point scale. The Quantitative Assessment of Trial Methodology Scale (QATMS) uses 14 different criteria. For each separate criterion a value of one is awarded if the specific trial met the requirement stated in the criterion. Therefore, a maximum of 14 can be scored, and quality score of > 10 is considered as "high quality", quality score between 6 and 10 as "intermediate", while a trial scoring lower than 5 is labelled as low quality trial methodology. The criteria are as follows (American College of Gastroenterology, 2002):

1 Rome criteria used to identify patients with IBS;

2 randomised;

3 parallel-trial design (no cross-over);

4 double-blinded;

5 account of patient disposition (discontinuations, withdrawals, etc.);

6 no placebo run-in;

7 baseline assessment of symptoms;

8 minimum treatment duration of 8 – 12 weeks;

9 follow up of symptoms after treatment is stopped;

10 compliance with treatment is measured;

11 sample size calculation is provided and adequate sample size enrolled;

12 primary outcome measure is improvement of global IBS symptoms;

13 primary outcome measure is based on patient assessment; and

14 primary outcome measure uses a validated scale to assess IBS symptoms.

The methodology used in this particular trial was of high quality, as a QATMS score of 11 would be achieved according to the ACG. All criteria is met except, 8 (minimum treatment duration of 8 – 12 weeks), 9 (follow up of symptoms after treatment is stopped) and 11 (sample size calculation is provided and adequate sample size enrolled).

It should be emphasised that this was a pilot study in which treatment duration of four weeks is considered more than adequate (Corazziari *et al.*, 2003). In fact, previous IBS pilot trials have also used short treatment durations of 4 weeks (Camilleri *et al.*, 2003; Capello *et al.*, 2007) and even 3 weeks (Dunlop *et al.*, 2003; Wang *et al.*, 2006). According to Hahn *et al.*, (1997) IBS is characterised by frequent episodes of symptomatic periods lasting 4 weeks on average (Camilleri *et al.*, 2003). Furthermore, abdominal pain/discomfort and distension occur between 28% - 33% of days over a 12-week period, lasting an average of five days per episode (Hahn *et al.*, 1998). In addition, IBS patients were only considered for the current trial if they had at least report two episodes of pain/discomfort per week before the study (Refer to Chapter 5, section 5.2.2). A four week treatment was therefore considered valid as patients were likely to experience at least two episodes of discomfort per week. With regards to the small sample size

of 31 patients (ITT) analysed, it must be noted that the recruitment was a great challenge, as it was mentioned and discussed in chapter 5. However, from the results it was clear that a trend towards improvement, although not significant in all parameters, was observed. Likewise, other IBS pilot trials have previously been successfully conducted with 44 (Camilleri *et al.*, 2003), 34 (Barker *et al.*, 2003), 32 (Vejdani *et al.*, 2006), 28 (Hawkes *et al.*, 2002), 25 (O'Sullivan & O'Morain, 2000), and even 17 (Tack *et al.*, 2006b) IBS patients. A follow-up period after treatment was not conducted in the present trial and it is surely recommended. The chapter that follows will discuss certain recommendations for future research.

This trial has recruited patients from various resources using inclusion/exclusion criteria in concordance with latest literature recommendations (Bergmann, 1999; Corazziari *et al.*, 2003; Irvine *et al.*, 2006). It must be mentioned that various IBS trials have ruled out organic cause by standard laboratory and radiological tests, and rectosigmoidoscopy (Johanson *et al.*, 2008; Vahedi *et al.*, 2008; Vejdani *et al.*, 2006). However, due to a tight budget and with the intention to keep this trial a non-invasive one, laboratory and radiological test was not performed on IBS candidates. This may easily be labelled as a limitation of this study. Rigorous diagnostic testing in typical IBS cases has been criticised before, and it is often thought that symptoms should lead the diagnosis rather than extensive and expensive diagnostic testing (Holten, 2003). According to Cash & Chey (2004), in a typical IBS patient with no alarm symptoms current best evidence does not support the routine use of blood test, stool studies, breathe tests, abdominal imaging or lower endoscopy in order to exclude organic gastrointestinal disease. Alarm symptoms, referred to as red-flags, remains the key indicators of possible diseases other than IBS (Hatlebakk & Hatlebakk, 2004). Alarm symptoms were incorporated into the Red-flag exclusion sheet as used in the current trial (**Appendix 4**). If an IBS candidate had an alarm symptom, the patient was not considered for participation. Thus, it may be assumed that a full blood count with ESR in conjunction with the use of red-flag exclusion sheet and Rome III symptom based criteria would have effectively exclude patients with gastrointestinal diseases other than IBS. In addition, prevalence of celiac diseases has been found to be higher in a symptom based IBS population compared to the general population (Cash *et al.*, 2002). Therefore it might be necessary to do a serum screening for tissue transglutaminase in IBS-D patients to rule out any celiac disease cases (Cash *et al.*, 2002). In the current trial and in others (Camilleri *et al.*, 2000; Camilleri *et al.*, 2003; Wang *et al.*, 2006), these cases might have been missed. The randomisation method used in this study should have effectively reduced or even neutralised the cases of celiac disease that might have been missed by the study physician (JDP). A FBC, as was used in this trial, might have been effective in excluding celiac disease cases (Holten, 2003).

## 7.3 Discussion of results

### 7.3.1 Baseline characteristics

Participants from the Absorbatox™ C35 group's mean age were significantly higher than their placebo counterparts (**Table 6.2**). However, the duration of symptoms, the severity, frequency and impact of symptoms were similar between the two treatment groups. Therefore, all patients had similar disease characteristics before treatment. It could, therefore, be safely assumed that a difference in treatment can be ascribed to a true actual difference and not due to dissimilarities in the degree of symptoms. The current study was mainly comprised of women (only one male in the Absorbatox™ C35 group). It is known that women are more likely to suffer from IBS than men (Payne, 2004). During the recruitment phase, a small number of male patients were available to enrol. Those who were available were eventually excluded because they did not meet entry criteria (**Section 6.1**). The small male representation seen in this trial might be ascribed to the low healthcare seeking behaviour that is known to be characteristic of male IBS patients (Heitkemper & Jarrett, 2008), as the study largely relied on the response of potential IBS candidates from advertisements (i.e. flyers and e-mail).

Before treatment, both treatment groups were classified as having a "moderate" degree of symptoms (**Table 6.3**), according to the IBS-SSS (Francis *et al.*, 1997).

### 7.3.2 Primary outcome

As previously discussed (**Section 5.6.1**), the global assessment question, **"In the last 7 days, have you had overall adequate relief of your IBS symptoms?"** was used to classify participants as responders or non-responders. Participants reporting "yes" in 50% of treatment weeks (i.e. any two weeks of the 4 week treatment period) were regarded as *overall* responders.

It is still unclear what constitutes as a responder or a clinical benefit in IBS trials (Camilleri *et al.*, 2007). Recently there have been various consensus reports on the primary (Camilleri *et al.*, 2007) and secondary outcomes (Drossman, 2005) of IBS trials. It is generally recommended to use a binary (*yes/no*) measure as a primary endpoint (Schoenfeld & Talley, 2006) and the FDA has approved this as a primary endpoint in IBS trials (Camilleri *et al.*, 2007).

In the current trial, the binary primary outcome, satisfactory relief (i.e. in the last 7 days have you had satisfactory relief of your IBS symptoms) was first considered in the development of the protocol (Schoenfeld & Talley, 2006). During the consulting of newer literature (Camilleri *et al.*,

2007), it was replaced by the more accepted "Adequate relief" (It must be noted that this amendment was made before the protocol was submitted for ethical approval).

**OVERALL RESPONDERS:** With regards to the findings; the Absorbatox™ C35 group yielded a greater proportion of *overall* responders than the placebo group. Although without statistical significance, the improvement trend has favoured the Absorbatox™ C35 group. Drossman (2005) has described a typical IBS trial as one in which a responder rate of 60% compared to a placebo response rate of 45% is achieved. It is unlikely that a statistically significant difference would be reached in such a small sample size as was used in the present trial. In addition, the disorder is characterised by a well-known placebo effect during drug trials (Spiller, 1999). Therefore, these results must be viewed in the light of others. According to Camilleri (2004) it is worth noting that even the best results from large multi-centre studies with approved therapeutic agents (e.g. tegaserod or alosetron) achieved primary clinical endpoints in less than 70% of patients. Furthermore, it is generally accepted that a 10-15% improvement of the global outcome measures over placebo could be considered as a clinically significant therapeutic gain (Corazziari *et al.*, 2003). In the current trial Absorbatox™ C35, according to abovementioned statements, has effectively managed to yield overall responders of more than 70%, and the effect over placebo was more than 10 - 15% (i.e. 30% gain over placebo; **Section 6.3.1**).

**WEEKLY RESPONDERS:** The number of weekly responders (proportion of patients that answered *"yes"* to the adequate relief questionnaire in each week on day 21 - after one week of treatment, on day 28 - after two weeks of treatment, on day 35 – after three weeks of treatment, and on day 42 – after four weeks of treatment) were similar at week one and week two, between the treatment groups. However, the proportion of weekly responders in the Absorbatox™ C35 group were significantly greater compared to the placebo, after three weeks (**Figure 6.2**). This trend was maintained through week 4 as the proportion of responders in the Absorbatox™ C35 group was significantly greater than the placebo group. From these results it was clear that Absorbatox™ C35 should be used for a minimum period of three weeks before a statistically significant gain over placebo, in global symptoms, is obtained.

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### *7.3.3 Secondary outcome*

An integrative symptom assessment was done through the use of the IBS-SSS (Irritable Bowel Syndrome Severity Scoring System). This questionnaire has been proven to be responsive to treatment effects (Irvine *et al.*, 2006), and is widely recommended by clinicians and researchers (Bijkerk *et al.*, 2003, Camilleri *et al.*, 2007; Drossman, 2005).

Francis and co-workers (1997), developers of the IBS-SSS, has stated that a 50-point score change is a reliable indication of symptom improvement. In view of this, a 50-point reduction in

symptom scores between baseline (measured on day 10) and at the end of therapy (measured on day 44) was considered a clinical meaningful difference, in this particular trial.

#### *7.3.3.1 Total severity score*

Both the placebo and the Absorbatox™ C35 group, showed statistically significant changes across treatment weeks, in the total severity score [**Figure 6.3**, per-protocol analysis (PP)]. In the ITT (intention-to-treat) population, the end of treatment total score (day 44) were significantly decreased compared to baseline (day 10) in both the placebo and Absorbatox™ C35 groups [**Figure 6.4(A) & (B)**]. Finally, both the placebo and the Absorbatox™ C35 group had more than 50 point reductions at the end of treatment compared to baseline (**Table 6.3**) with a 50% reduction in total score observed in both groups (day 44 vs. day 10). It was therefore unlikely to observe treatment differences between the two groups (**Table 6.3**), as both groups' total severity score decreased gradually throughout the treatment weeks. It must be further noted, that both treatment groups changed from having "moderate" symptoms at baseline to "mild" symptoms at the end of treatment, according to the IBS-SSS (Francis *et al.*, 1997).

#### *7.3.3.2 Pain, distension, "bowel habit satisfaction" and "interference with life in general" findings*

**CURRENT PAIN:** In the PP population the placebo group was not able to show significant reductions in their current pain situation while the Absorbatox™ C35 group's current pain situation decreased significantly across treatment weeks. However, comparisons between the two groups did not reveal any significance on any of the measurement days (day 24, 34, 44; **Table 6.4**).

**SEVERITY OF PAIN:** The severity of pain was significantly decreased across treatment weeks in the placebo as well as in the Absorbatox™ C35 group (**Figure 6.5**, PP). The ITT has revealed that at the end of treatment both the Absorbatox™ C35 and the placebo groups' pain severity decreased significantly compared to baseline scores (**Figure 6.6**, ITT). Likewise, as with "current pain", no significance was observed between treatment groups in the severity of pain, on any of the measurement days (**Table 6.5**).

**NUMBER OF DAYS WITH PAIN:** A similar trend was observed in the mean number of days with pain, as both groups showed fewer days with pain across treatment weeks (**Figure 6.7**, PP) and significant changes at the end of treatment compared to baseline (**Figure 6.8**, ITT). Once again, no treatment differences between the placebo and Absorbatox™ C35 groups were observed in the mean number of days on any of the assessment intervals (**Table 6.6**).

**CURRENT DISTENSION:** With regards to distension, a rather similar pattern was observed as with the pain findings; both groups showed significant reduction in "currently having distension" across treatment weeks (PP). During treatment, there was no difference in values between placebo and Absorbatox™ C35 groups on any of the days (**Table 6.7**).

**SEVERITY OF DISTENSION:** The PP has shown significant decreases across treatment weeks in both the placebo and Absorbatox™ C35 groups (**Figure 6.9**, PP). Although both groups have shown severity of distension reductions from baseline (**Figure 6.10,** ITT), Absorbatox™ C35 had a significant less severity mean on day 34 compared to placebo. Unfortunately, this effect was not sustained, as a subsequent measurement (day 44, after 30 days of treatment) revealed no significance between placebo and Absorbatox™ C35 (**Table 6.8**).

**BOWEL HABIT SATISFACTION:** The current trial showed that this IBS population were largely unsatisfied with their **bowel habit** prior to treatment (**Table 6.9**). The baseline scores dropped significantly across the treatment weeks in the PP population (**Figure 6.11**). Furthermore, ITT analysis revealed significant differences between day 10 and day 44, in both the placebo and Absorbatox™ C35 group (**Figure 6.12**). At the end of treatment patients from both groups were much more satisfied with their bowel habits. Again, no significant difference was observed between placebo and Absorbatox™ C35 on any of the assessment intervals (**Table 6.9**, ITT).

**INTERFERENCE WITH LIFE IN GENERAL:** The extent to which IBS interfere with patients' lives were particularly high in this IBS population (**Table 6.10**). However, the impact of IBS on participants' lives gradually decreased across the 4 weeks of treatment, in both the placebo and Absorbatox™ C35 groups (**Figure 6.13**, PP). Compared to baseline (day 10) the end of treatment "interference" was significantly lower in both treatment groups (**Figure 6.14**, ITT). For this reason no significant differences were observed between treatment groups on any of the assessment days (**Table 6.10**).

## *7.3.4 Bowel function (stool parameters) findings*

### *7.3.4.1 Stool consistency*

**MEAN STOOL CONSISTENCY:** It was found that the mean stool consistency was not a reliable parameter [scored on categorical scale from 0 - very hard to 5 - very loose, scoring 3 – smooth (normal) stool, was regarded as the optimal score; the mean for each week was calculated] in the current trial, since the placebo and Absorbatox™ C35 groups showed significant differences during week one (baseline) (**Table 6.11**). No significant differences was however, observed in week two (baseline). The differences in week one may be attributed to the small sample size that led to an unequal distribution of bowel sub typing in each treatment group. The placebo group had more constipation-predominant patients than the Absorbatox™ C35 group.

In addition; the placebo group had no diarrhoea-predominant patients (**Table 6.2**). It is therefore likely that the placebo group could have had a lower mean stool consistency compared to Absorbatox™ C35 at week one (baseline). As the patients entered the treatment weeks, the differences subsided as both groups' mean stool consistency increased gradually. The outcome was that no differences were observed between treatment groups in any of the 4 treatment weeks.

**PROPORTION OF SMOOTH STOOL CASES:** The proportion of smooth stool consistency was also evaluated (number of smooth stool incident cases over total bowel movement incident cases). During treatment both treatment groups had a greater proportion compared to baseline. However, Absorbatox™ C35 had a significantly greater proportion of smooth stool compared to placebo during the treatment weeks.

#### *7.3.4.2 Urgency*

In terms of urgency, similar findings during baseline were observed between the treatment groups. No significant difference was observed between the Absorbatox™ C35 and placebo on any of the 4 weeks of treatment.

#### *7.3.4.3 Frequency*

**MEAN NUMBER OF BOWEL MOVEMENTS:** The mean number of bowel movements per day did not change from baseline in either of the treatment weeks. Moreover, there was no significant difference observed between the Absorbatox™ C35 and placebo group during any of the treatment weeks.

**PROPORTION OF DAYS WITH BM = 0 AND BM > 3:** The proportion of days with no bowel movement did not differ significantly between treatment groups on any of the treatment weeks (**Table 6.14**). In the same way, the proportion of days with more than 3 bowel movements also did not differ between the Absorbatox™ C35 and placebo on any of the treatment weeks (**Table 6.15**).

### *7.3.5 Heartburn episodes findings*

Heartburn and other gut symptoms have been reported in 25% to 50% of IBS patients (Birrer, 2002) and it is further documented that IBS patients are more likely to suffer from GORD than healthy controls (Hungin *et al.*, 2003). Neutacid™ is a product that consists of the natural zeolite, clinoptilolite, and is registered in Cuba as an antacid (Rodríguez-Feuntes *et al.*, 2006). It was, therefore; proposed that Absorbatox™ C35 (with similar properties as Neutacid™) may reduce the incidence of heartburn episodes.

**MEAN PROPORTION OF HEARTBURN DAYS:** The mean proportion of heartburn days during treatment decreased significantly compared to baseline, in the placebo group (**Table 6.16**). On the contrary, different from what has initially been anticipated, no significant difference was observed from baseline to treatment, in the ABTX group (**Table 6.17**). Furthermore, no significant difference was seen between placebo and Absorbatox™ C35, during treatment. At baseline, the mean proportion of days with heartburn was relatively low in the Absorbatox™ C35 group. Although not significantly lower than the placebo group, it might have happened that a floor effect had occurred in the active treatment group, in which mild symptoms leave little room for detecting an improvement.

Although it is not possible to confirm it, the question that was used to assess these symptoms ["*Did you experience any episodes of heartburn or indigestion (dyspepsia) today*"], may not have been interpreted correctly by the participants.

In addition, these findings must be viewed with caution, as heartburn and dyspepsia are not the same conditions, in fact a consensus states that if gastro-oesophageal reflux symptoms (e.g. heartburn and acid regurgitation) is present concomitantly with dyspepsia the differential diagnosis should rather favour the GORD (Corazziari, 2004). In contrast, there is evidence that symptoms (listed in parenthesis) of dyspepsia (early satiety, vomiting, nausea, pain, bloating), GORD (heartburn, bloating, regurgitation), chronic constipation (gas, bloating) and IBS (abdominal pain, bloating, constipation, diarrhoea) may all coincide with one another (Cremonini & Talley, 2004).

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### *7.3.6 EMP findings*

The End-of-study marketing potential (EMP) questionnaire is a novel questionnaire that was used to address the global impression of the treatment received, in which participants were asked whether they would recommend the treatment to other IBS sufferers and whether they will use the product in the near future. The questionnaire was administered at the end of treatment and relied solely on the participant's perception of treatment. According to the published literature, available to the primary researcher (JRK), this type of questionnaire has never been utilised before in IBS pilot trials (Camilleri *et al.*, 2003; Cappello *et al.*, 2007; Talley *et al.*, 2008; Vahedi *et al.*, 2008; Vejdani *et al.*, 2006; Wang *et al.*, 2006) and it has never been recommended before (Bijkerk *et al.*, 2003; Corazziari *et al.*, 2003; Drossman, 2005; Irvine *et al.*, 2006; Veldhuyzen van Zanten *et al.*, 1999).

Although without statistical significance, a greater proportion of Absorbatox™ C35 participants compared to placebo participants reported that they would recommend the treatment to other IBS sufferers and would use the product in the near future.

### 7.3.7 Rescue medication consumption

Both treatment groups have used mebeverine and lactulose, however; no loperamide was used during trial treatment. No significant difference between the Absorbatox™ C35 and placebo groups with regards to rescue medication consumption was observed. Therefore, Absorbatox™ C35 was not characterised with less rescue medication consumption. Although not statistically analysed, the Absorbatox™ C35 group were characterised with more lactulose consumption compared to placebo. In addition, participants from the placebo group consumed more mebeverine during the treatment weeks (refer to **Table 6.18**).

### 7.3.8 Safety and tolerability

Although there were no statistical significant differences in the proportion of patients experiencing at least one adverse event (AE), it must be noted that participants in the placebo group were 10% more likely to experience an AE compared to Absorbatox™ C35. Furthermore, the patient with the highest score for the maximum proportion of days with AE came from the placebo group (**section 6.10**).

### 7.3.9 Dropouts

This study managed to keep the dropouts relatively low, i.e. 12.12% (4/33). This drop-out rate was in concordance with literature recommendations (Veldhuyzen van Zanten *et al.*, 1999).

## 7.4 Summary

Participants were equally distributed, in terms of duration and severity of symptoms, between the two treatment groups. Participants from the Absorbatox™ C35 group reported better global response to treatment compared to the placebo (i.e. more overall responders came from the Absorbatox™ C35 group), but did not reach statistical significance. During week 3 and 4 of treatment a statistical significant proportion of Absorbatox™ C35 participants reported having *"adequate relief"* compared to the placebo participants. With regards to separate symptom parameters (pain, distension, bowel habit, interference with life in general), both Absorbatox™ C35 and placebo groups showed significant improvements compared to baseline observations. After 20 days of treatment Absorbatox™ C35 participants had less severity of distension compared to placebo participants, however; the subsequent assessment revealed that this effect was not sustained after day 34 to the end of study. Stool parameter (consistency, urgency, frequency) analysis did not reveal any differences between the Absorbatox™ C35 and the placebo groups, but the active group was associated with a greater proportion of smooth

stool passage than the placebo group. In addition, heartburn was left untreated within the Absorbatox™ C35 group, while participants from placebo had less heartburn episodes when compared to baseline. A greater proportion of patients from the Absorbatox™ C35 group considered the use of allocated treatment in future (statistically not significant). Likewise, a greater proportion of patients from the Absorbatox™ C35 group reported that they will recommend the treatment to other IBS sufferers (statistically not significant). Absorbatox™ C35 was not associated with less frequent rescue medication consumption. However, it appeared that participants from the ABTX group consumed more Lactulose than their placebo counterparts. Drug compliance and adverse events were similar between the two groups.

The **placebo effect** was constantly present for the most part of the trial. This phenomenon is widely discussed in subjective disorders, like depression (Khan *et al.*, 2008) and pain (Quessy & Rowbotham, 2008), but also in neurodegenerative disorders like Parkinson's disease (Colloca *et al.*, 2005). IBS is no exception as previous trials have reported high response to placebos in 40% to 70% of patients (Patel *et al.*, 2005). The reasons for the placebo effect is rarely known (Thompson, 2000), but higher cognitive functions such as the endogenous endorphins may be a role player in the evolution of this common phenomenon (Vahedi *et al.*, 2008).

In the current trial, guidelines were followed to keep the placebo response as small as possible (Patel *et al.*, 2005; Pitz *et al.*, 2005), however it was largely unavoidable as a crude form of treatment is given (e.g. counselling, reassurance and cognitive behavioural treatment) in each and every trial, regardless of the treatment that is received (Spiller, 1999). In addition, if this kind of reassurance had not provided, it might have led to a larger dropout rate (Spiller, 1999). According to Pitz and colleagues (2005), many predictors of high placebo response exist in IBS trials. The type of diagnostic criteria has been shown to be a predictor of high placebo response; Manning criteria have shown larger placebo responses compared to Rome criteria (Patel *et al.*, 2005). IBS candidates in this trial were diagnosed according to the Rome criteria and therefore this problem should have been reduced. A greater number of office visits were found to decrease the placebo response (Patel *et al.*, 2005). However, on the contrary Dorn and colleagues (2007) have reported the opposite; i.e. placebo response increases as office visits increase.

According to German researchers, women in IBS trials are more prone to placebo response than men (Enck *et al.*, 2005), this may further explain the high placebo response seen in this particular study, since this trial only successfully enrolled one male patient.

Participants in this trial were instructed to use Absorbatox™ C35 or placebo, one capsule three times daily; this might have had a significant contribution to the placebo response, as the placebo response increases with frequency of intervention (Pitz *et al.*, 2005). Furthermore, an

adequate run-in phase was used and laboratory testing was done before randomisation, all in concordance with guidelines to minimise the placebo response (Patel *et al.*, 2005; Veldhuyzen van Zanten *et al.*, 1999). Some authors' recommend that IBS trials should be designed over long periods (longer than 3 months) to allow the placebo response to recede (Spiller, 1999); interestingly enough, the duration of treatment had no effect on the placebo response as reported recently by Patel *et al.* (2005).

This clinical trial pilot study has shown that IBS patients receiving Absorbatox™ C35 have an insignificant but greater tendency towards improvement in terms of global symptom endpoints, compared to the placebo-receiving patients. Although, the placebo effect was present throughout the trial and little (if any) significance were observed between the two groups in terms of separate symptom ratings (pain, distension, bowel habit, interference), difference was however, seen in the severity of distension after 20 days (**Table 6.8**) of treatment. Furthermore, the proportion of patients reporting adequate relief in the Absorbatox™ C35 group reached significance after 3 weeks (i.e. 21 days) of treatment. However, these benefits were short lived and were not sustained up to the end of the study.

Chapter 8 contains the final synopsis. Conclusions are outlined and the implications and recommendations for future IBS trials with Absorbatox™ C35 are briefly discussed

# CHAPTER 8 SYNOPSIS

## 8.1 Research background

IBS is a chronic or recurrent gastrointestinal disorder without any structural abnormalities, infection, or metabolic markers (Ringel *et al.*, 2001). This functional gastrointestinal disorder is associated with significant burdens on patients' wellbeing (Gralnek *et al.*, 2000) and has a enormous economic impact through direct costs in health care utilization and indirect costs through absenteeism from work (Camilleri *et al.*, 2002).

The basic symptoms of IBS are abdominal discomfort, bloating and altered defecation (Cash & Chey, 2004), but IBS is also characterised with co-morbid disorders, including GORD, functional dyspepsia and fibromyalgia (Whitehead *et al.*, 2002). Psychiatric disorders, especially major depression and anxiety occur in up to 94% of IBS patients (Whitehead *et al.*, 2002).

With regards to the treatment of IBS; the serotonin modifying agents, alosetron and tegaserod for IBS-D and IBS-C respectively, have been considered to be promising therapeutic agents (Johanson, 2004). Controversially, after substantial number of years of marketing from the pharmaceutical industry, the FDA has called for withdrawal of both drugs mainly because of serious post-marketing side effects (Parischa, 2007; Spiller, 2008). This has left many IBS sufferers with limited treatment options. Meanwhile, the FDA registered lubiprostone as a new agent for IBS-C patients (Aschenbrenner, 2008), but it is not clear whether this drug will reach South Africa soon.

Some IBS patients have resorted to complimentary and alternative medicine, probably due to ineffective conventional western medicine (Koloski *et al.*, 2003) but little robust data is available on the efficacy of CAM in IBS (Drossman *et al.*, 2002). A recent meta-analysis has shown probiotics effective in some circumstances (Nikfar *et al.*, 2008) and a Traditional Chinese Herbal compound has proved efficacious in global scores (Bensoussan *et al.*, 1998).

The lack of pathophysiological understanding is the main reason for poor IBS management (Barbara *et al.*, 2004). Several hypotheses have been proposed, including abnormal motility, visceral hypersensitivity, inflammation and infection (i.e. PI-IBS), neurotransmitter imbalance (e.g. serotonin pathways), and psychological factors (e.g. stress) (Gilkin, 2005). Physiologically, primary afferent neurons (involved with visceral sensations) react to intestinal distension, mechanical distortion and chemical changes (Ducrotte *et al.*, 2005). In addition, IBS patients

are often considered to be visceral hypersensitive to luminal factors and intestinal gas (Camilleri *et al.*, 2002).

Absorbatox™ C35 is a non-toxic and natural zeolite with a well-defined tetrahedron structure of $AlO_4$ and $SiO_4$ (Pavelić *et al.*, 2002). The substance has some well-explored ion exchange, water and gas adsorbing properties (Grce & Pavelić, 2005). It was thought that this agent may play a role as an ameliorating agent in the adsorption of certain endogenous chemicals and gasses which may cause some of the IBS symptoms like diarrhoea, bloating, and abdominal discomfort.

The current pilot study was designed as a randomised, double-blind, placebo-controlled trial with a parallel group assignment, in which 31 IBS patients were treated with Absorbatox™ C35 or placebo for 4 weeks (i.e. 30 days).

## 8.2 Study aims and objectives

The aim of this pilot study was to explore the efficacy of a natural zeolite, Absorbatox™ C35, as a complimentary treatment in the management of IBS.

The objectives of this trial were outlined in chapter one.

## 8.3 Conclusion

Although a small sample size was used and a high placebo response was observed throughout the study, the following pivotal conclusions were made with respect to the objectives:

- From the global symptom endpoint it was evident that Absorbatox™ C35 was associated with a greater trend towards better improvement compared to placebo. Thus, Absorbatox™ C35 was superior to placebo in the management of global IBS symptoms.
- With regards to separate symptom ratings, (i.e. abdominal pain and distension/bloating, bowel habit satisfaction and disease interference with life) Absorbatox™ C35 has failed to show continuous significant better improvements over placebo.

- Absorbatox™ C35 was not superior in improving stool urgency and frequency compared to placebo. However, the incidence of smooth stool was significantly higher in the Absorbatox™ C35 group compared to placebo group.
- The Absorbatox™ C35 was not associated with fewer rescue medication consumption compared to the placebo group. In fact, participants from the placebo group showed less tendency towards rescue medication consumption.
- During the treatment phase Absorbatox™ C35 was not capable of reducing the incidence of heartburn episodes compared to baseline observations. There was no treatment differences observed between the Absorbatox™ C35 and placebo group, with regards to heartburn episodes on any of the treatment weeks.

## 8.4 Recommendations for future research applications

Investigators that wish to further assess the efficacy of Absorbatox™ C35 in IBS might gain valuable insights from this pilot study. In addition, the primary researcher has made some recommendations with regards to recruitment, statistical power, the duration of treatment, post treatment period, health related quality of life measurements, combination therapy and dose titration. The recommendations that follow were based on the experiences gained from the findings of this pilot study. Under certain conditions, these recommendations were verified with appropriate literature recommendations.

**RECRUITMENT:** Recruitment is a vital part of IBS trials with immense challenges (Chin Feman *et al.*, 2008), and in this trial it was no exception. Future trials should allow sufficient time for recruitment. The recruitment source needs to be carefully considered as IBS incident cases from primary care are infrequent and patients have intermediate symptom severity (Jones, 1999). Primary care patients may be more generalised and transferable with the general population compared to secondary care (Jones, 1999). In addition, tertiary care patients have severe symptoms and are anxious (Longstreth *et al.*, 2001). IBS patients recruited via the Internet have a poorer quality of life compared to patients from clinics (Jones *et al.*, 2007). The current trial has recruited patients mainly by some form of advertisement and they were moderate in symptom severity.

**SUFFICIENT STATISTICAL POWER:** From the current findings, a larger trial with sufficient statistical power is warranted. In consultation with the study statistician (FS), it was estimated that if the current results were to be repeated in a larger trial, achieving a 80% and a 50% response rate in Absorbatox™ C35 and placebo respectively, one would need 45 IBS patients per treatment

group to obtain statistical significance (A two proportions Z test was used with a statistical power of 80% and a significance level of 5%). One should further consider the dropout rate of between 10%-20% in IBS trials (Veldhuyzen van Zanten *et al.*, 1999). This study recruited 67 IBS candidates, but only entered 33. Therefore to enter 90 patients, under the same conditions, it is estimated that approximately 200 IBS candidates should be recruited.

**HEALTH RELATED QUALITY OF LIFE (HRQOL) MEASUREMENT:** This study did not evaluate quality of life measures. However, the IBS-SSS tool that was used has incorporated a "global" quality of life measure by assessing the disease interference with life in general (**Appendix 5**). In future, to obtain a complete quality of life assessment, a validated measure might be useful in order to assess whether Absorbatox™ C35 affects quality of patients' lives after a duration of treatment. The HRQoL measurement can be done prior to treatment (baseline) and after the treatment is stopped (end of treatment). The baseline data can then be compared to the end-of-treatment data to evaluate any treatment related improvements. In turn, the active group can be compared to the control, to evaluate if there were any superior benefits from receiving the active drug. Bijkerk and colleagues (2003) and Drossman (2005) recommended the IBS-QoL by Patrick *et al.* (1998).

**STUDY DURATION:** This pilot study has made use of a 2-week run-in period and a 4-week treatment period in concordance with literature recommendations (Corazziari *et al.*, 2003). It might also be necessary to evaluate whether Absorbatox™ C35 has any long-term benefits. Previously, the Rome Committee has recommended an 8- to 12-week treatment period (Drossman, 2005). Controversy still remains with regards to the time-period it takes for the placebo response to recede. Spiller (1999) has noted a decline of placebo response after 12 weeks; however Patel and colleagues (2005) have indicated that the placebo response is not associated with the study duration. In view of these findings an extended treatment period might be conducted over 8 to 12 weeks.

**POST-TREATMENT PERIOD:** A post-treatment period may give insight on whether Absorbatox™ C35 has extended benefits beyond drug withdrawal. A 4-week post-treatment period, in which participants are followed-up without receiving any treatment, seems adequate (Capello *et al.*, 2007; Wang *et al.*, 2006).

**COMBINATION THERAPY:** The current trial did not prove Absorbatox™ C35 to have significant benefits over placebo with regards to bowel function (stool parameters). For this reason, it can be considered to treat the participants with Absorbatox™ C35 or placebo in combination with a laxative or anti-diarrhoeal, depending on the patients bowel sub-typing (IBS-C, IBS-D or IBS-A). Vejdani *et al.* (2006) effectively applied this method that used the study drug (Carmint) in combination with loperamide and psyllium.

**DOSE TITRATION:** Future trials can make use of dose titration (Spiller, 1999), in which the participant is allowed to increase or decrease the dose according to individual response. Through this, the therapy can indeed be individualised and if the placebo group increased their dose more than the active group, it can be owed to a lack of effectiveness. Changing doses can be used as a measure of success of treatment. Dose titration can be successfully implemented in an Absorbatox™ C35 trial, as the drug is well tolerated.

## 8.5 Contextualisation

IBS patients' symptoms fluctuate from time to time and the large placebo response is usually anticipated. The persistence of the placebo effect remains, however unexplained (Talley, 2003). Non-specific therapy, like reassurance, is inevitable in IBS trials and may eventually contribute to the placebo response (Spiller, 1999). Dorn (2007) and colleagues have not found CAM more prone to a placebo response compared to conventional therapies. When conducting IBS clinical drug trials the high placebo response should not be neglected. If not adequately controlled, it might overshadow any benefit of a specific drug (Spiller, 1999). The current trial has taken the necessary measures to minimise this effect, but still the findings have showed a high response to the placebo.

Furthermore, it should be emphasised that seeking one single therapy to treat such a diverse syndrome consisting of multiple symptoms, is merely impossible (Farthing, 1998). However, this study has yielded an 80% and 50% responder rate in global symptom endpoints in the active and control groups, respectively. Although these findings did not reach statistical significance, a European body has considered a 10%-15% gain over placebo a clinical meaningful difference (Corazziari *et al.*, 2003).

IBS remains a common disorder with some uncommon aetiology. Many IBS sufferers seek relief in CAM. Their place in the IBS treatment armamentarium is still not clear. Probiotics, peppermint oil and certain forms of herbal remedies have been proven efficacious before and much research is still awaited. Whether Absorbatox™ C35 is a promising CAM agent needs to be elucidated.

Meanwhile, clinicians should try and understand CAM therapies, but most importantly evaluate them against best current evidence. Researchers however should explore their efficacy and exposed these agents or practices to randomised controlled trials with adequate statistical power using robust methodology.

# APPENDIX 1

# INFORMED CONSENT DOCUMENT

**DETAILED PATIENT INFORMATION LEAFLET AND**

**INFORMED CONSENT DOCUMENT**

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**Patient's name:** _____

**Patient's number:** _____

**Study title:** A randomised, controlled trial of Absorbatox C35 in Irritable Bowel Syndrome: A pilot study

**Sponsor:** Absorbatox (Pty) Ltd, 154 Edward Street, Hennopspark, Centurion.

**Recruitment doctor:** _____

| | |
|---|---|
| **Study supervisor:** | **Dr Johan C Lamprecht (Registered Pharmacist)** |
| **Co-supervisor:** | **Prof Jacques R Snyman (Registered physician)** |
| **Study Physician:** | **Dr Jesslee M du Plessis (Medical physician)** |
| **Primary Researcher:** | **Mr J. Rial Kloppers (Masters Degree student)** |
| **Institution:** | Northwest-University, Potchefstroom campus, South-Africa |

**Daytime hours telephone numbers:**

Dr JM du Plessis (018) 299 2204

Dr JC Lamprecht (018) 299 2254 **OR**

Mr JR Kloppers 083 389 3021

**After hours:**

Mr JR Kloppers 083 389 3021

**Introduction:**

You are invited to participate in a research study. Your participation is entirely voluntary. It is important not to rush into a decision. This document is compiled to make sure that you understand all the benefits, risks, discomfort as well as the purpose of this study. It also states that you may withdraw from the study at any time and it explains the alternative procedures that are available to you. This information will help you to decide whether you want to take part in this research study or not. You should not, however, agree to take part unless you are satisfied with all the procedures involved.

This document may contain words that you do not understand. Please ask the researcher to explain any words or information that you do not clearly understand. You may take home an unsigned copy of this consent form to consider or discuss with family or friends before making your decision. No guarantee or assurance can be made as to the results of the study.

Unfortunately, you may not take part in another investigational medicine research study during the course of this study. If you have a personal/family doctor, please discuss or inform him about your possible participation in this study.

It is very important that you are honest to the research staff about your health status, and about your present and past usage of any other medication. Non-disclosure of other medicines you consume should inflict harm to yourself by participating in this study.

If you agree to participate in this study you will be asked to sign this document. You will be given a copy to keep for your own personal records.

The sponsoring company of the study is Absorbatox (Pty) Ltd, and will work in association with the School of Pharmacy, North-West University, Potchefstroom campus in collaboration with the Department of Pharmacology, Faculty of Health Sciences, University of Pretoria. In this document, this company will be referred to as "the Sponsor".

**Nature and purpose of the study:**

You have been diagnosed as suffering from Irritable Bowel Syndrome and we would like you to consider taking part in the research of the efficacy of a new medicine, referred to as Absorbatox C35. It is a new product with the potential to improve your symptoms. This medication is new in its class and belongs to the range of chemical compounds, known as zeolites. This medication is registered in South Africa as a complimentary medicinal product (Absorbatox detox®); however, it is not currently specifically indicated for use in Irritable Bowel Syndrome.

The primary purpose of this study will be to evaluate the efficacy of Absorbatox C35 in patients with Irritable Bowel Syndrome treated over four consecutive weeks (30 days). The study will compare the efficacy of Absorbatox C35 with placebo. A placebo is an inactive substance and it does not contain any medicine. You will be allocated either to the active or to the placebo treatment (i.e. like spinning a coin, and you don't know what side will land on top). Neither you nor the research staff will know which treatment you are receiving during your participation in the study. This procedure is called blinding; it will ensure that the information gathered during the study is accurate. In case of an emergency, it will however be possible to determine which treatment you have been receiving. Every participant will be granted access to, what researchers call "rescue medication", during the entire course of the study. In other words, you will be able to obtain symptom relief in cases when the study medication does not control your symptoms. The rescue medication is thoroughly discussed on a later stage in this document.

**Description of the Study:**

Approximately 60 participants will participate in this study. Participants will be recruited from local doctors' practices and thereafter be referred to the School of Pharmacy, North-West University, Potchefstroom Campus. Participants will be between the ages of 18 and 65 years. The duration of study will be six (6) weeks and entails five (5) study visits. This study will only be conducted in South Africa at the mentioned institution only.

**Study Procedures:**

*Briefly:*

If you agree to take part in this study, you will first be asked questions and examined by your doctor, to see if you qualify for this study. Before receiving your first dose of study medicine, you will have to take part in a run-in (baseline or clean-out), period. This period will continue for 2 successive weeks (14 days), in which you will be asked **not** to use any medication for your symptoms. During this 2-week period you will be asked to record your symptoms on a daily diary scoring card. After the 2-week period, you will be designated to receive either the placebo or the active treatment, (keep in mind that neither you nor we will know what treatment is given).

You will then receive medicine over four (4) consecutive weeks (30 days) in which you will be asked to complete certain questionnaires. The questionnaires completed, will give the researchers an indication of your health status at that specific interval. Furthermore, it will also help us to see if there is an improvement of your symptoms. In other words, these questionnaires will reflect on your symptoms and the researchers will be able to see whether the treatment is successful or not. It is very important that you complete all the questionnaires

regularly and accurately; otherwise, the researchers will not be able to interpret your symptoms correctly.

*Study visits:*

At each visit your general health status will be establish. You will be asked questions on your health, about your symptoms experienced any side effects you are developing as well as on any other medications you are taking.

| | Run-in period (2 weeks) | | | Randomisation | Treatment period (4 weeks) | | | |
|---|---|---|---|---|---|---|---|---|
| Visit number | V1 | no visits | V2 | | V3 | no visit | V4 | V5 |
| Days or weeks | D1 | + 1W | +1W | | + 1W | + 1W | + 1W | + 1W |

**V=visit, D=day, W=week**

On day 1, a blood sample will be drawn to establish your total blood count. (This is a safety preventative measure). Through this procedure the researchers will make sure that you do not suffer from underlying diseases (e.g. infections, cancer etc.), other than Irritable Bowel Syndrome. On visit one, you will receive your diary card in which you will record your symptoms on a daily basis for the entire 6 weeks.

After this 2 week run-in (clean-out) period, you will be asked to come back for your next visit (V2). During this visit, you will receive your study medication, as well as rescue medication and you will be asked for your completed daily recordings. After one week has past you will be asked to return for visit 3 (V3). During this session your study medication will be counted, therefore; **it is important to bring your study medication with you every time you visit the site**. During this session, you will be consulted by a health care professional, to ensure good health status.

At the last two visits (V4 and V5) similar procedures as V3 will be followed. At the last visit (V5) all study medication and questionnaires will be recollected from you.

Each visit will not consume more than 30 minutes of your time. The contact sessions relates to five (5) visits of 30 minutes each at the most.

*Questionnaires and timing*

The daily diary card needs to be completed daily during the entire study. You will also be asked to complete two (2) other questionnaires at specific intervals.

The first questionnaire (the adequate relief questionnaire) consists of one question only. It will reflect upon your global well-being. You will be expected to complete this questionnaire once weekly after you have received your study medication.

The second questionnaire (The Severity Scoring System) consists of four questions. It will reflect upon your severity of symptoms. You will be asked to complete it on the tenth day from your first visit and on every tenth day after you have received your study medication.

| **Questionnaire** | **When?** | **Where?** | **How many times, in total do I have to complete it?** |
|---|---|---|---|
| 1 | Every week after you have received your study medication | At home | 4 |
| 2 | On the tenth day from your first visit and on every tenth day after you have received your study medication. | At home | 4 |
| Daily diary card | On every day of the study (All the days between your first and last visit) | At home | During the whole duration of the study |

It is recommended that you complete your questionnaires every day before bedtime, in that way the whole day will be taken into consideration.

*Study medication:*

You will receive either 750 mg Absorbatox C35 capsules, or the placebo capsules. During the course of the study, you will take one capsule three times daily, preferably in the morning, afternoon and in the evening. Your study medication should be taken orally with a glass of water. You will also have certain medication (rescue medication), other than the study medication, to your exposal. The researchers will refer to this medication as "rescue medication". You will be asked to use this medication only in times of unbearable symptoms. In other words you will be able to obtain relief even if your symptoms are not controlled by the study medication. If rescue medication is taken you are to record it on the daily diary card.

The rescue medication is as follows:

| Medication | Indication | Accepted dosage |
|---|---|---|
| Mebeverine 135 mg tablets<br>(Bevispas®) | Pain and discomfort associated with irritable bowel syndrome | 135 mg (one) tablet three times daily |
| Lactulose dry powder 10 g sachet<br>(Laxette®) | Constipation | 10 – 20g daily up to maximum dosage of 40g |
| Loperamide 2mg tablets<br>(Gastron®) | Diarrhoea | 2 to 4 mg, up to four times a day. |

If any medical emergency occurs during the study, the sponsor will make your study medication available to the investigators. Otherwise, you will be informed about the study medication received, after all data has been analysed and the study is completed.

During this study you should not take other medication that is indicated for IBS (e.g. tegaserod (Zelnorm®), cisapride (Prepulcid®), alosetron (Lotronex®), laxatives and anti-diarrhoeal agents), except medication provided by the investigators of this study. The product Zelnorm® (tegaserod) is recently discontinued for treatment in South Africa. If you are using lithium carbonate (Camcolit®) you will be excluded from the study, since it is speculated that the investigational drug of this study may lower the therapeutic effect of lithium carbonate. You will influence your study results negatively, by using other medication that is prohibited. If you fulfil specific criteria, certain medication will be allowed (e.g. chronic medication like antidepressants). It is important that you provide the investigators with the correct information concerning your medication usage (prescription and over-the-counter medication) before and during the study.

**Foreseeable risks or discomforts:**

No significant side effects have been reported after Absorbatox C35 has been administered to other patients. Safety in pregnancy has not been established yet. The procedure required for taking a full blood count may be associated with minor discomfort. The researchers responsible for this procedure are registered professionals and will do everything to ensure your safety. They will work under sterile conditions to minimise risks at all times.

**Unforeseen risks:**

In previous studies Absorbatox 35C showed insignificant side effects. However, if any side effect may occur during this trial the researchers request you to report any such an experience at your earliest convenience, or during the scheduled visits throughout the study.

**Benefits:**

The study medication may improve your symptoms, but there is also a possibility that you may experience no benefit from the medication during the clinical trial period. However, your participation in this study will contribute to medical knowledge that may, in future help other patients with IBS including yourself.

**Alternative treatment:**

A range of medicines is currently used to treat Irritable Bowel Syndrome. If you decide not to take part in this study, you may still receive the best current medicinal treatment regimen from your usual doctor. However, current scientific evidence indicates that all standard medicines currently available may or may not improve your IBS symptoms.

**Special precautions:**

The study medication's content should not be inhaled into the lungs. It is indicated that study medication should be administered 2 hours before, or after, other medication. It may be taken with food or on an empty stomach.

**Participants' responsibilities:**

The safety of Absorbatox C35 in pregnancy has not been establish yet, therefore if you decide to become pregnant or if you are currently pregnant you will not be considered to participate in this study. If you wish to stop taking the study medication at any point in time, you must inform the researchers as soon as possible.

**Financial arrangements:**

The sponsor company and the North-West University will cover some study related costs, including the study drugs. Rescue medication and medical procedures related to the study will also be sponsored by different pharmaceutical companies as well as funds made available for research by the North West University.

Neither you nor your medical scheme will be expected to pay for any study medication, study related procedures and/or consultations.

**Reimbursement of study participation:**

You will not receive any payment, for participation in this study. However, at the end of the study the investigators will compensate you with a minimum of R50 per study visit for your transport fees.

**Voluntary participation and withdrawal:**

Your participation in this study is entirely voluntary and you may refuse participation before and during the study, without stating a reason. Your participation in this study will not affect your future access to medical care. Participants can also be withdrawn if they fail to comply to study treatment and/or required procedures.

**Ethical approval:**

The following study will be conducted in accordance with the ICH Good Clinical Practice Guidelines and the World Medical Association Declaration of Helsinki (last updated 2004). This study will be approved by the North-West University Ethics Committee, prior to the commencement of the clinical trial.

**Insurance and guidelines:**

The North-West University has insurance for you and the researchers in the event of a study related injury or illness. Such injury or illness is one that occurs as a direct result of the administration of the study medicine or of the study specific procedures. The investigators, sponsor and the North-West University will follow guidelines laid down by the Association of the British Pharmaceutical Industry (ABPI guidelines), and the Guidelines for Good Practice in the Conduct of Clinical trials in Human Participants in South-Africa.

Further detailed information regarding payment of medical treatment and compensation due to injury can be obtained from the researchers, if required.

The North-West University will not be held liable for the following conditions:

- Medical treatment of other injuries or illnesses
- Injury caused by negligence
- Injury caused by deviation from protocol guidelines

**Data handling and confidentiality:**

All the records obtained in this study will be kept strictly confidential. Data that identify you as a participant will be kept confidential. Study data will be analysed and will be published in scientific journals, however, these results will not identify you as a participant. The sponsor, the researchers and the North-West University Ethics Committee will review this information. Therefore, by signing inform consent you authorise the researchers to release your records to the above-mentioned authorities. These records and information will be utilised by them in order to carry out certain obligations related to this clinical study.

Any Information regarding your health will be kept in strict confidence and any important results that are gathered during the course of the study that may affect your health will be discussed with you immediately.

All participants will receive a unique study number consisting of your initials and participant number. You will be allowed to access your data after the study has been completed.

**The 24-hour telephone number through which you can reach one of the investigators or authorised personnel is: _____**

If you would like more information regarding your rights as a participant in a research study, you may contact Prof Christiaan Brink, Head of Ethics Committee, North-West University at (018) 299 2234. In the event of you consulting the North-West University Ethics Committee and not receive answers to your satisfaction you may furthermore write to the South African Medicines Control Council at:

**The Registrar**

**Medicines Control Council SA**

**Department of Health**

**Private Bag X828**

**PRETORIA**

**Personal/Family doctor and or specialist notification option:**

Please indicate whether you want the researchers to notify your personal/family doctor and/or specialist of your participation in this study:

☐ **Yes**, I want my personal doctor or specialist informed on my participation in this study

☐ **No**, I do not want my personal doctor specialist informed on my participation in this study.

☐ **I do not have** a personal doctor or specialist.

**Informed consent:**

1. I hereby confirm that I have been informed, by the researcher, regarding the nature, procedure, benefits and risks of this clinical research study.
2. I understand that I will receive signed copies of this document.
3. I understand that my unwillingness to provide sufficient information regarding my health and medication usage may affect my health and study results.
4. I have read and understood the patient information leaflet regarding this research study.
5. I am aware that the results of the study, including personal details regarding my sex, age, date of birth, initials and diagnosis will be kept strictly confidential when processing a study report. By signing this consent, I am authorising that my records may be utilised by authorised bodies (e.g. North-West University Ethics Committee and the SA Medicines Control Council).
6. I agree that the North-West University as well as the sponsor can process the data collected from this study in a computerised system.
7. I understand that my participation in this study is voluntary and that I may refuse to participate or withdraw from this study, without stating a reason.
8. I understand that my personal details will remain confidential.
9. I have had sufficient opportunity to ask questions and declare myself prepared to participate in the study.

**To be completed by the Patient:**

Print name _____

Signature _____ Date _____

**To be completed by the doctor:**

The following mentioned doctor has given oral and written information regarding the research study.

Print name _____

Signature _____ Date _____

**To be completed by a witness:**

Print name _____

Signature _____ Date _____

**To be completed by a researcher:**

Print name _____

Signature _____ Date _____

# APPENDIX 2

# RECRUITMENT FLYER

## (AFRIKAANS & ENGLISH)

# Abdominale ongemak? Versteurde gastroïntestinale funksie?.....

# .....Prikkelbare dermsindroom (PDS)?

## *("Irritable Bowel Syndrome?")*

**.....KONTAK ONS VANDAG of voor 15 Augustus 2008!**

U WORD UITGENOOI OM DEEL TE NEEM AAN 'n KLINIESE NAVORSINGSPROGRAM goedgekeur deur die Noordwes Universiteit Etiek Komitee (NWU-0001-08-S5)

Onder die leiding van die vakgroep Kliniese Farmasie, Noordwes Universiteit word die volgende KLINIESE program geloods:

**'n Komplementêre produk, Absorbatox™ C35 word getoets vir effektiwiteit in pasiënte met spastiese kolon/prikkelbare dermsindroom (PDS)**

**Wat behels dit?**

- Ses weke program met vyf kontaksessies van tussen 10 – 15 min elk.
- Vier weke behandeling.
- Aktiewe bestanddeel in Absorbatox™ C35, gelys as GRAS (Generally Recognised as Safe) verbinding in VSA. Absorbatox™ C35 by Medisyne Beheerraad van SA geregistreer.
- Alle prikkelbare dermsindroom lyers tussen 18 en 65 jaar welkom om deel te neem!
- Alle intervensies is gratis.
- Deelnemers ontvang 'n fooi vir reiskostes aangegaan.
- Aantal PDS pasiënte reeds deel van program.

**Wil u deel wees?**

Indien u aan 'n spastiese kolon ly met versteurde gastroïntestinale funksie SMS slegs die woorde: **'STEL BELANG'** na 083 389 3021 en een van die studie personeel sal u terug skakel. Indien u meer inligting benodig, kan u die volgende personeel raadpleeg:


Rial Kloppers (M-studie navorser) Sel: 083 389 3021 (e-pos:12795836@nwu.ac.za)

Dr Jesslee du Plessis (Mediese dokter) Tel: 018 299 2204

Dr Johan C Lamprecht (Studieleier) Tel: 018 299 2254

# Abdominal discomfort? Disturbed bowel function?.....

# .....Spastic Colon/Irritable Bowel Syndrome *(IBS)?*

**....CONTACT US TODAY or before the 15th August 2008!**

YOU ARE INVITED TO TAKE PART IN A CLINICAL RESEARCH TRIAL authorised by the Ethics Committee of the North-West University (NWU-0001-08-S5).

The following clinical trial will be launched under the supervision of the Subject Group Clinical Pharmacy, North-West University:

**Absorbatox™ C35, a complimentary product, will be assessed for efficacy in the treatment of Spastic Colon (also known as Irritable Bowel Syndrome).**

**What does it entail?**

- A six week study with five contact sessions (approx. 10 -15 min each).
- Four weeks of treatment.
- The active ingredient in Absorbatox™ C35 is listed as a GRAS (Generally Recognised as Safe) compound in USA. Absorbatox™ C35 is registered with the Medicine Control Council of South-Africa.
- IBS sufferers between the ages of 18 - 65 are invited to take part.
- Study procedures and interventions are free of charge.
- Participants will receive a small fee for travelling costs.
- Several IBS patients are already taking part.

**Do you want to take part?**

If you suffer from Irritable Bowel Syndrome (spastic colon) and want to know more SMS **'INTERESTED'** to 083 389 3021 and one of the study personnel will contact you.

Or, you may contact one of the following personnel:


Rial Kloppers (Primary Investigator) Cell: 083 389 3021 (e-mail: 12795836@nwu.ac.za)

Dr Jesslee du Plessis (Medical doctor) Tel: 018 299 2204

Dr Johan C Lamprecht (Study leader) Tel: 018 299 2254



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# APPENDIX 3

# ROME III DIAGNOSTIC CRITERIA

## *Rome III criteria:*

| *Write the number, that fits the best to the patient's description of symptoms, in the far right-hand column:* | | |
|---|---|---|
| **Questions to be asked from patients** | **Number scale that describes patient's symptomatology** | |
| 1. In the last 3 months, how often did you have discomfort or pain anywhere in your abdomen? | 0. Never →<br><br>1. Less than one day a month<br>2. One day a month<br>3. Two to three days a month<br>4. One day a week<br>5. More than one day a week<br>6. Every day | Skip remaining Questions |
| **2. For Women:** Did this discomfort or pain occur only during your menstrual bleeding and not at other times? | 0. No<br>1. Yes<br>2. Does not apply because I have had the change in life (menopause) or I am a male | |
| 3. Have you had this discomfort or pain 6 months or longer? | 1. No<br>2. Yes | |
| 4. How often did this discomfort or pain get better or stop after you had a bowel movement? | 0. Never or rarely<br>1. Sometimes<br>2. Often<br>3. Most of the time<br>4. Always | |
| 5. When this discomfort or pain started, did you have **more** frequent bowel movements? | 0. Never or rarely<br>1. Sometimes<br>2. Often<br>3. Most of the time<br>4. Always | |
| 6. When this discomfort or pain started, did you have **less** frequent bowel movements? | 0. Never or rarely<br>1. Sometimes<br>2. Often<br>3. Most of the time<br>4. Always | |
| 7. When this discomfort or pain started, were your (bowel movements) stools looser? | 0. Never or rarely<br>1. Sometimes<br>2. Often<br>3. Most of the time<br>4. Always | |
| 8. When this discomfort or pain started, how often did you have harder stools? | 0. Never or rarely<br>1. Sometimes<br>2. Often<br>3. Most of the time<br>4. Always | |
| 9. In the last 3 months, how often did you have hard or lumpy stools? | 0. Never<br>1. Sometimes<br>2. Often<br>3. Most of the time<br>4. Always | |
| 10. In the last 3 months, how often did you have loose, mushy or watery stools? | 0. Never<br>1. Sometimes<br>2. Often<br>3. Most of the time<br>4. Always | |

## Diagnostic Criteria*

Recurrent abdominal pain or discomfort** at least 3 days/month in last 3 months associated with two or more of criteria #1 to #3 below:

***Pain or discomfort at least 2-3 days/month (question 1 > 2)***

***For women, does pain occur only during menstrual bleeding?***

***(question 2 = 0 or 2)***

#1. Improvement with defecation

***Pain or discomfort gets better after bowl movement (BM) at least sometimes (question 4 > 0)***

#2. Onset associated with a change in frequency of stool

***Onset of pain or discomfort associated with more stools at least sometimes (question 5 > 0), OR***

***Onset of pain or discomfort associated with fewer stools at least sometimes (question 6 > 0)***

#3. Onset associated with a change in form (appearance) of stool

***Onset of pain or discomfort associated with looser stools at least sometimes (question 7 > 0), OR***

***Onset of pain or discomfort associated wit harder stools at least sometimes (question 8 > 0)***

* Criteria fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis:

***Yes. (Question 3=1)***

*[**"Discomfort" means an uncomfortable sensation not described as pain.]*

***In pathophysiology research and clinical trials, a pain/discomfort frequency of at least two days a week is recommended for subject eligibility:***

***Pain or discomfort more than one day per week (question 1 > 4)***

**IBS subtypes:**

**Criteria for IBS-C (IBS with constipation):**

***(question 9 > 0) and (question 10 = 0)***

**Criteria for IBS-D (IBS with diarrhoea):**

***(question 9 = 0) and (question 10 > 0)***

**Criteria for IBS-M (mixed IBS):**

***(question 9 > 0) and (question 10 > 0)***

**Criteria for IBS-U (un-subtyped):**

***(question 9 = 0) and (question 10 = 0)***

# APPENDIX 4: RED-FLAG EXCLUSION SHEET

## *Red-flag exclusion sheet:*

*Study title:*
A randomised, controlled trial of Absorbatox C35 in Irritable Bowel Syndrome: A pilot study

*Investigators:*


| | |
|---|---|
| Dr Johan Lamprecht | (018) 299 2254 |
| Dr Jesslee du Plessis | (018) 299 2204 |
| Mr Rial Kloppers | 083 389 3021 |



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**Name of medical doctor**_____

**Contact details of doctor** _____

_____

## GENERAL PATIENT INFORMATION

**Name:**_____

**Contact details:**

Cell phone: _____ work: _____ home: _____

## INFORMATION REGARDING IBS DIAGNOSIS

Indicate, with a cross, whether patient has one or more of the following conditions and/or signs. {Please note that, for any reason a condition or sign is at the time of form completion not known a question mark (?) may be indicated in the appropriate column}

| Conditions | x |
|---|---|
| Anaemia | |
| Diverticular disease of colon | |
| Diabetes Mellitus (type 1 & 2) | |
| Cholestasis | |
| Liver or kidney disease | |
| Chron's Disease | |
| Ulcerative colitis | |
| Celiac disease | |
| Major psychiatric disorders | |
| Lactose (milk) intolerance | |
| Uncontrolled Thyroid disorders | |
| Infection | |
| Colorectal carcinoma | |

| Signs | x |
|---|---|
| Blood in stools | |
| Nocturnal predominant symptoms | |
| Palpable abdominal or rectal mass | |
| Lymphadenopathy | |
| Hepatosplenomegaly | |
| Rectal bleeding | |
| Recent progressive weight loss | |

Signature (doctor): _____

# APPENDIX 5

# STUDY QUESTIONNAIRES

**Daily diary record**

Study title:
***A randomised, controlled trial of Absorbatox C35 in Irritable Bowel Syndrome: A pilot study***

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Name:_____ Date: _____

## Instructions:

Please follow the instructions carefully
Complete this questionnaire before **bedtime each day**
Remember to put down your name (surname as well) and the date.
Make sure that you answer **question 1 – question 7**
Answers must reflect your symptoms of the specific day.
If there are any difficulties in interpreting questions the following investigating personnel may be contact at any time of the day:
Mr. JR Kloppers 083 389 3021
Dr JC Lamprecht 082 412 4866

### PART A: Stool consistency

**Question 1:** How was your stool form/consistency today? Rate your stool form, by crossing the block that describes it the best.
*(EXAMPLE: if your stool form was "very hard" today the right answer would be:*

| | |
|---|---|
| X 1 | **Very hard** |

Now please rate yours

| | |
|---|---|
| **1** | **Very hard** |
| **2** | **Hard** |
| **3** | **Formed/Smooth** |
| **4** | **Loose** |
| **5** | **Very loose** |

**OPTIONAL:** Any other information you feel you would like to mention, with regards to your stool consistency_____

_____

**PART B: Sense of urgency**

**Question 2:** Rate your sense of urgency (when you have to rush to the bathroom to open your bowels) that you experienced throughout today.
*(EXAMPLE: if your sense of urgency were "quite severe" throughout the day, the correct answer would be the following*

| | |
|---|---|
| ~~3~~ | **Quite severe** |

Now please rate yours:

| | |
|---|---|
| **1** | **No urgency at all** |
| **2** | **Not very severe** |
| **3** | **Quite severe** |
| **4** | **Severe** |
| **5** | **Very severe** |

## PART C: Stool frequency

**Question 3:** How many times did you open your bowels today, due to normal, loose or very loose stool? ☐

**Question 4:** How many times did you open your bowels today, due to hard or very hard stool? ☐

**PART D: Heartburn (acid reflux)**

Did you experience any episodes of heartburn or indigestion (dyspepsia) today?
Yes ☐ No ☐

## PART E: Medication usage

**Question 5:** Did you take your study (trial) medication correctly, throughout the day?
Yes ☐
No ☐

**Question 6:** Did you make use of your rescue medication today?
Yes ☐
No ☐

**If yes**, please specify which rescue medication did you take?

Loperamide (for diarrhoea) ☐

Lactulose (for constipation) ☐

Mebeverine (for pain/cramps) ☐

## PART F: Adverse events

**Question 7:** Did you experience any adverse event/unwanted effect today, from the trial medication *(NOTE: This does not include your symptoms associated with your bowel problems/IBS)*.

Yes ☐ No ☐

**If yes**, please specify (describe) the adverse event(s) you experienced throughout the day?

_____

_____

**If yes**, please classify the severity of your adverse event(s) you experienced today.

**Mild** (did not effect your usual daily activities) ☐

OR

**Moderate** (had some minor effects on daily activities) ☐

OR

**Severe** (had a great effect on daily activities) ☐

## PART G: Additional information

Any other comments regarding this day. _____

_____

**Primary outcome: adequate relief**

**Study title:**
A randomised, controlled trial of Absorbatox C35 in Irritable Bowel Syndrome: A pilot study
**Investigating personnel:**
Mr JR Kloppers 083 389 3021
Dr JC Lamprecht 018 299 2254

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Name: _____
Date: _____

## Instructions:

**This questionnaire only consist of a single question**
**Your answer should reflect on how you felt through the past 7 days**

(Key: The phrase "***overall*** adequate relief" means "overall (in general) relief of symptoms experienced by the patient to the patient's personal satisfaction").

**Question 1:**
In the last 7 days have you had ***overall*** adequate relief of your IBS symptoms?

| YES | NO |
|---|---|

**Secondary outcome measure: the IBS-SSS**

**Study title:**
A randomised, controlled trial of Absorbatox C35 in Irritable Bowel Syndrome: A pilot study
**Investigating personnel:**
Mr JR Kloppers 083 389 3021
Dr JC Lamprecht 018 299 2254

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Name : _____

Date: _____

# Irritable Bowel Syndrome Severity Scoring System (by Francis *et al.* 1997)

**INSTRUCTIONS:**

This form is designed to enable us to record and monitor the severity of your IBS. It is to be expected that your symptoms might vary over time, so please try and answer the questions based on how you currently feel (in your situation, **the past 10 or so days**). All information will be kept in strict confidence.

1. For questions where a number of different responses are a possibility please circle the response appropriate to you.
2. Some questions will require you to write in an appropriate response.
3. Some Questions require you to put a cross on a line which enables us to judge the severity of a particular problem.
   For example:
   ***How severe was your pain?***

   ***Please place your cross (X) anywhere on the line between 0 - 100% in order to indicate as possible the severity of your symptoms***

## PART A: SEVERITY SCORE

FOR OFFICE USE ONLY

1.

*a)* ***Do you currently suffer from abdominal (tummy) pain?***

YES NO

*b)* ***If yes, how severe is your abdominal (tummy) pain?***


*c)* ***Please enter the number of days that you get the pain in every 10 days.***
For example if you enter 4 it means that you get pain 4 out of 10 days.
If you get pain every day enter 10

Number of days with pain ☐ X 10

2.

*a)* ***Do you currently suffer from abdominal distension? (bloating, swollen, tense or tight tummy)***

YES NO

*b)* ***If yes, how severe is your abdominal distension/tightness***


3. ***How satisfied are you with your bowel habit?***


4. ***Please indicate with a cross on the line below how much your Irritable Bowel Syndrome is affecting or interfering with your life in general***

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