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REVIEW ARTICLE

Phenotyping of subjects for large scale studies on patients with IBS

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Key Points

- Irritable bowel syndrome (IBS) is a complex condition with multiple factors contributing to its etiology and pathophysiology, resulting to date in no specific reliable biomarker being identified.
- Large multi-center pan European/World studies carefully phenotyping and characterizing patients may help identify subpopulations with accuracy and consistency, aiding future research and treatment.
- This position paper highlights the necessary requirements to standardize the process of selecting and phenotyping IBS patients and how to organize the collection and storage of patient information/samples in such studies.

Abstract

Background *Irritable bowel syndrome (IBS) is a complex condition with multiple factors contributing to its aetiology and pathophysiology. Aetiologically these include genetics, life-time events and environment, and physiologically, changes in motility, central processing, visceral sensitivity, immunity, epithelial permeability and gastrointestinal microflora. Such complexity means there is currently no specific reliable biomarker for IBS, and thus IBS continues to be diagnosed and classified according to symptom based criteria, the Rome Criteria. Carefully phenotyping and characterisation of a 'large' pool of IBS patients across Europe and even the world however, might help identify sub-populations with accuracy and consistency. This will not only aid future research but improve tailoring of treatment and health care of IBS patients.* ***Purpose*** *The aim of this position paper is to discuss the requirements necessary to standardize the process of selecting and phenotyping IBS patients and how to organise the collection and storage of patient information/samples in such a large multi-centre pan European/global study. We include information on general demographics, gastrointestinal symptom assessment, psychological factors, quality of life, physiological evaluation, genetic/epigenetic and microbiota analysis, biopsy/blood sampling, together with discussion on the organisational, ethical and language issues associated with implementing such a study. The proposed approach and documents selected to be used in such a study was the result of a thoughtful and thorough four-year dialogue amongst experts associated with the European COST action BM1106 GENIEUR (www.GENIEUR.eu).*

Keywords *functional gastrointestinal disorders, irritable bowel syndrome, phenotyping.*

INTRODUCTION

Irritable bowel syndrome (IBS) is a common chronic condition with substantial economic and social implications. It accounts for a considerable demand on health care resources worldwide.[1] A recent meta-analysis reported a pooled IBS prevalence of 11.2%, albeit with large differences between individual studies with figures ranging from 1.1% to 45.0%.[2] This may represent true differences related to demographic factors, but comparison is difficult due to the application of different diagnostic criteria and differences in patient selection. Moreover, differences in access to health care and cultural factors, such as help seeking behaviour,[3] may contribute.

Irritable bowel syndrome is characterized by abdominal pain or discomfort associated with changes in bowel habits, often accompanied by distension and/or bloating.[4] The pathophysiological mechanisms are not fully understood, but a range of different predisposing, precipitating or perpetuating factors may contribute through both central and peripheral mechanisms.[1] Psychological comorbidity,[5,6] differences in central processing, genetic factors, visceral hypersensitivity, abnormal gastrointestinal (GI) motility and secretion,[7] low-grade inflammation and alterations in gut microbiota have all been proposed to underlie the diverse spectrum of symptoms reported by IBS patients.[8,9] Furthermore, dietary factors and food intake, especially the impact of fermentable oligo-, di-, and polysaccharides and polyols (FODMAP) and gluten has gained much attention lately.[10,11] Taken together, IBS is a heterogeneous condition with a variety of different pathophysiological mechanisms, obviously requiring a tailored and pathophysiology-based approach to clinical management. However, although the latter is a very appealing concept, it requires the identification of biomarkers, a challenge that so far has not been solved.

To date, mainly due to a lack of objective diagnostic measures, the diagnosis of IBS is still based on symptoms, as defined currently by the Rome III criteria[4] and forthcoming Rome IV criteria. Although this represents an important tool to standardize IBS diagnosis and decreases the heterogeneity of patient populations

within clinical trials or translational research, the lack of biomarkers makes it difficult to uniformly define patients. Hence, there is a pressing need to establish a set of tools that can be applied to a large set of patients to phenotype and characterize different subpopulations as accurately and consistently as possible. These tools should be widely applicable and feasible in different settings, allowing valid comparisons of findings from different centers. Ideally, the collected data should be combined in large databases. This is essential to garner insight into genetics, epigenetics, microbiota and other potential disease modifiers, which in turn will aid further research and improve tailoring of treatment and health care for patients.

The aim of this paper was to discuss the requirements to standardize the process of selecting and phenotyping IBS patients and how to organize the collection and storage of patient information/samples. The proposed approach and documents selected is the result of a thoughtful and thorough discussion among experts as part of the European COST Action BM1106 GENIEUR (www.GENIEUR.eu).

THE NEED FOR FURTHER PHENOTYPING IBS PATIENTS

As mentioned earlier, the underlying pathophysiology and the clinical presentation of IBS are extremely diverse. Yet, hitherto the classification of IBS patients is limited to differences in defecation pattern yielding four different subgroups or phenotypes, i.e., IBS with constipation, IBS with diarrhea, mixed IBS and unsubtyped IBS.[4] Clearly, this approach has contributed to improved and differential clinical management, yet the heterogeneity within these subpopulations remains tremendous. Especially for epi-/genetic, microbiota and pathophysiological studies, patients should be better stratified and factors influencing the phenotype, such as diet, psychological comorbidity and many others should be inventoried in more detail to achieve a homogeneous population. Biological tests or microscopic examination may deconstruct IBS into individual physiological or mechanistic components reducing complexity and increasing the chance to identify genes or biomarkers crucial to biological processes underlying the pathophysiology of the disease under study. Identification of these so-called intermediate phenotypes (quantitative traits) thus may represent another important approach to improve the homogeneity of IBS subpopulations. Of note, the process of identifying IBS phenotypes or intermediate traits should be based on relevant information from particularly large cohorts, allowing statistically solid and reliable data analysis. Ultimately, the final data collection should allow the assessment of possible links between symptoms, life style, epi-/genetic abnormalities, dysbiosis, and physiological alterations.

STANDARDIZATION OF DATA AND SAMPLE COLLECTION

Standardization of data and sample collection holds many challenges, mostly due to differences in the tools used to collect information or the standard procedures used to collect samples or perform/interpret physiological tests. For instance, the manner in which blood, tissue and stool is sampled for epi-/genetics and microbiota analysis, their short and long-term storage, shipping conditions has clearly to be defined to preserve the material, prevent degradation, and bacterial growth which may impair molecular analysis and bias the study outcome. Furthermore, it is of utmost importance that the information to be collected from a large population is well defined and agreed upon prior to the implementation of a study protocol, not only to standardize the process of data collection but also to prevent collection of unnecessary information. Moreover, data should be collected and registered in a standardized case report form, constructed in such a way that information can be entered easily and stored in a uniform format in a database. The latter requires the use of checkboxes and pick lists rather than free text. As not all centers will have the financial or logistic means to perform all tests or collect the entire data set, a minimal set of information and samples that must be collected in all patients needs to be determined. This information should consist of demographics, general clinical information, ethnicity, diet, standardized questionnaires related to functional GI disorders (FGIDs) and IBS, psychological comorbidity, blood samples, and fecal samples (see below). Relevant information that cannot be collected at all sites should be streamlined in different 'modules' that are performed or collected in a restricted number of centers with the respective expertise. Detailed assessment of dietary intake, collection of biopsies for assessment of permeability, immunohistochemistry, molecular biological testing, measurement of visceral sensitivity (barostat), GI transit, or even functional brain imaging are not established in every center, time consuming and expensive and thus will be restricted to centers of expertise. Nevertheless, standardization with pooling of the acquired information at the different centers will

contribute to more in depth phenotyping in a subpopulation.

GENERAL INFORMATION: DEMOGRAPHICS, ETHNICITY, DIET, AND OTHERS

Inevitably, general information such as demographics, including date of birth, gender, ethnical background, BMI, education, and profession needs to be collected from all subjects as these factors are known to influence the occurrence of symptoms or their reporting.[2] Special attention should be paid to ethnicity as this represents a major determinant in genetic studies.[12] Similarly, family aggregation should be recorded, as the incidence of IBS in siblings and twins is increased,[13,14] providing valuable information to identify new genetic factors in a family/twin study design. Given the potential role of immune activation in IBS[8] the presence of inflammatory bowel diseases (IBD) in family members needs to be checked as well. Similarly, adding celiac disease in the interrogation of family history can be of interest depending on the research questions one wants to tackle.

Known risk factors for the development of IBS should be carefully inventoried and checked for such as adverse early life events, abuse, stress, and onset after a GI infection are critical. Comorbid associations should be investigated and recorded, in particular atopic conditions such as asthma, eczema, and hay fever. These disorders may be more frequent in IBS, suggesting some role for an atopic background to be involved in the pathophysiology of IBS, at least in a subpopulation.[15] A prior history of abdominal surgery, as a trigger of visceral sensitization, and other comorbid conditions suspected to be linked to IBS, i.e., chronic fatigue syndrome, urogynecological symptoms, fibromyalgia, other FGIDs, and psychiatric disorders.[6] As the role of diet is increasingly acknowledged in IBS, food is an important trigger of symptoms,[16] and largely influences the composition of the microbiome, dietary information is becoming more relevant. Some subjects follow dietary restrictions that can be useful or not, others try to modulate symptoms by changing food intake.[17] Therefore, questions regarding gluten or lactose avoidance or on the use of specific diets (vegetarian, raw, vegan, low carb-high fat, high fiber, low FODMAP) should be included in the case record form. It should be emphasized though that a detailed dietary inventory requires a trained dietician, which is time consuming and expensive, and therefore usually restricted to dedicated or specialized centers. To circumvent this, a minimal set of required dietary information should be identified that can be collected in all centers. Special attention should also be given to the medications taken by the patients. One should record antibiotics, antidepressants, and also pro-/pre-/symbiotics, frequently used as self-medication. Recording the latter, as well as recent use of antibiotics, is particularly relevant for studies on the microbiome. Of note, as colonization of the gut starts at birth, the type of delivery, i.e., vaginal *vs* cesarean section, could be of interest to include as well.

Another important set of data concerns the clinical presentation. The type and severity of symptoms are crucial for phenotyping patients[18] and obviously should be always recorded as detailed as possible using standardized questionnaires (see below). As IBS patients from primary care may have different phenotypes compared to patients from tertiary centers, it is recommended to record if patients are recruited from primary, secondary, or tertiary care or from defined groups (i.e., employees of a company). To date, phenotyping of IBS patients is largely based on stool pattern. One of the best tools to determine this variable is the Bristol Stool Form Scale (BSFS) (see below).[19] Indeed, stool form correlates better with whole-gut and colonic transit than defecation frequency.[20] The BSFS is recommended by the Rome committees and also validated in several European languages.[21,22] Finally, although IBS is a symptom-based diagnosis, a minimal set of diagnostic tests should be included to exclude confounding organic conditions. A blood test excluding anemia and inflammation (C-reactive protein) is therefore mandatory. Moreover, conditions mimicking IBS (especially IBS with diarrhea), like lactose malabsorption and celiac disease should ideally be ruled out. Many gastroenterologists perform a lower digestive endoscopy to exclude organic disease, but in the absence of alarm signs, the decision to perform colonoscopy with biopsies remains at the discretion of the individual practitioner.[23] Functional tests (see below) are optional according to the availability in different centers.

Last but not least, standardized criteria have to be defined to select controls. Healthy controls should be subjects with no GI symptoms, as assessed by the questionnaires used (see below), and no chronic disorders that may affect research outcomes.

All data recommend to be collected for IBS phenotyping can be retrieved from the following website: www.GENIEUR.eu.

GI SYMPTOM ASSESSMENT

Assessment of the GI symptom severity and pattern is central in phenotyping IBS patients, especially since

this is a heterogeneous patient group. The key is of course to carefully characterize the IBS symptoms, but due to the frequent overlap with other FGIDs, thorough assessment of overlapping FGIDs should also be included. The gold standard to obtain a careful clinical phenotyping based on the symptom profile is to use validated questionnaires, preferably using combinations of questionnaires to characterize the IBS symptom profile as well as co-existing overlapping FGIDs. It should be emphasized though that there may be some language issues with symptoms, in particular with regard to bloating and distension in English *vs* Spanish. To solve this issue, investigators should consult at a national level with experts and patient groups with regards to the appropriate use of terminology in the questionnaires where such terminology may have potential to be misunderstood due to language issues. Any changes made to the questionnaires clearly need appropriate validation.

Diagnosing FGIDs: Rome III diagnostic questionnaire for adult FGIDs

Functional GI disorders are defined by diagnostic criteria, together with normal findings on a limited number of routine investigations and tests.[24] In parallel with the development of the most recent diagnostic criteria for FGIDs, the Rome III criteria, a thorough process to develop a diagnostic questionnaire for FGIDs was undertaken. This process resulted in the Rome III diagnostic questionnaire for adult FGIDs, designed to make (provisional) diagnoses of all FGIDs.[25] The self-administered questionnaire consists of 93 items about the presence and frequency (but not severity) of all symptoms included in the diagnostic criteria for FGIDs, and it takes about 15–20 min to complete. Different response scales are used; yes/no; 5-, or 7-point ordinal response scales for conditional questions (never or rarely to every day, or always); other response scales for specific questions; and there is a scoring algorithm that identifies provisional (or possible) FGID diagnoses. The questionnaire also contains 15 'red flag' or alarm symptom questions that are not part of the diagnostic algorithms, but may be helpful in determining if further diagnostic studies are needed to exclude other diseases. The questionnaire may also be subdivided into modules for focusing on a specific (group of) FGID(s) (e.g., functional bowel disorders) rather than all FGIDs, depending on the research question. This questionnaire is also valid for use in control subjects participating in research studies to exclude FGIDs, which is important as GI symptoms are very common in the community.[26] A similar questionnaire will be available for the Rome IV criteria when these are published.

Assessment of IBS symptom pattern & severity

Besides confirming the diagnosis of IBS and other FGIDs, it is also of importance to assess the overall severity of IBS, and the severity and pattern of different IBS symptoms. Among several others, the two most widely used questionnaires are the IBS severity scoring system (IBS-SSS),[27] and the Gastrointestinal Symptom Rating Scale (GSRS),[28] which has also been developed into an IBS-specific version (GSRS-IBS). Moreover, as retrospective assessment of bowel habit and IBS subtyping, which is based on stool consistency, seems to be unreliable,[29] a prospective bowel habit diary using the validated BSFS is often advocated.[30,31]

IBS severity scoring system The IBS-SSS was developed as a simple, easy to use scoring system for IBS to reliably capture effects of treatments and or other interventions.[27] It has undergone sufficient validation, and has also been used extensively as an outcome measure in trials assessing different treatment options for IBS.[17,32,33] The questionnaire includes five items; abdominal pain intensity, abdominal pain frequency, abdominal distension, dissatisfaction with bowel habits, influence of IBS on life in general ('life interference'); each scored 0–100. All of the questions use visual analog scales (0–100), except for the abdominal pain frequency question, which collects the number of pain days during the previous 10 days with the response multiplied by 10 to obtain a score between 0 and 100. The total IBS-SSS score thus has a range from 0 to 500, with higher scores indicating more severe symptoms. Frequently accepted cut-off levels are used to divide patients into severity groups: <175, mild IBS; 175–300, moderate IBS; >300, severe IBS. In treatment trials a reduction in IBS-SSS total score of 50 has been found to reliably reflect a clinically meaningful improvement.[27]

Gastrointestinal Symptom Rating Scale The GSRS was originally developed as an interview-based rating scale to evaluate common symptoms in patients with IBS and peptic ulcer disease,[28] but was later modified to become a self-administered questionnaire.[34] The GSRS has 15 items, each scored using a 7-point Likert scale (1–7), combined into five domains (reflux, indigestion, diarrhea, constipation and abdominal pain) identified through factor analysis.[35] The higher the scores the more severe are the symptoms. A more recent development is the GSRS-IBS, the IBS-specific version of the

GSRS.[36] This 13-item questionnaire determines the pattern and severity of IBS-related symptoms during the past week using a similar 7-point Likert scale with descriptive anchors as the original GSRS (ranging from 'no discomfort' to 'very severe discomfort'). The items are divided into five domains: pain, bloating, constipation, diarrhea, and satiety. One advantage with GSRS and GSRS-IBS relative to IBS-SSS for use in large-scale IBS studies is that they also include questions about upper GI symptoms, which is relevant for careful phenotyping. Moreover, with the GSRS and GSRS-IBS it is possible to separately determine the perceived severity of diarrhea and constipation, which is not possible with IBS-SSS, as this questionnaire only asks for dissatisfaction with bowel habits in general.

Bristol Stool Form Scale Irritable bowel syndrome subgrouping is based on stool consistency defined by the widely used BSFS.[4] This is a 7-point scale to describe the stool consistency; 1, separate hard lumps like nuts; 2, sausage shaped but lumpy; 3, like a sausage or snake but with cracks on its surface; 4, like a sausage or snake, smooth and soft; 5, soft blobs with clear cut edges; 6, fluffy pieces with ragged edges, a mushy stool; 7, watery, no solid pieces. The use of the BSFS in bowel habit diaries has been found to be a useful guide to assess intestinal transit time.[30,31] In IBS trials, a 1- or 2-week bowel habit diary is a useful way to objectively define bowel habit (stool frequency and consistency) and to determine the IBS subgroup.[29] Moreover, it serves as a complement to assessment of the perceived severity of the abnormal bowel habit, as measured with IBS-SSS and/or GSRS-IBS.

Assessment of functional dyspepsia The rationale for including a psychometric instrument to measure dyspepsia symptom severity in an IBS cohort is the frequent comorbidity of functional dyspepsia (FD) in IBS. This is not only the case in healthcare seeking patients,[37] but also in the general population.[38] Several validated instruments exist to assess dyspeptic symptoms, such as the Glasgow Dyspesia Severity Score,[39] the Leeds Dyspepsia Questionnaire,[40] and the Canadian Dyspepsia score.[41] One of the most widely used dyspepsia instruments, the Nepean Dyspepsia Index (NDI),[42] seems to be particularly useful for reasons mentioned below.

Short form-Nepean dyspepsia index The short form NDI (SF-NDI), developed and validated by Talley *et al*, quantitates both symptom severity and disease-specific quality of life in patients with (functional) dyspepsia.[43] The first part consists of a symptom checklist using Likert scales to quantitate the frequency [0 (not at all) to 4 (daily)], intensity [0 (not at all) to 5 (very severe)], and bothersomeness [0 (not at all) to 4 (very bothersome)] of 15 upper GI symptoms over the prior 2 weeks. The item scores are added up, yielding a single total symptom severity score.[42] The second part consists of a 10-item disease-specific quality of life measure (which may hence not apply to IBS patients without dyspeptic symptoms), which in turn can be subdivided into five 2-item subscales: tension, interference with daily activities, eating/drinking, knowledge/control, and work/study. Each item is scored on a 5-point Likert scale ranging from 0 (not at all) to 4 (extremely), subscales are calculated as an unweighted sum of the 2 underlying items, and a total score can also be calculated by adding up the subscale scores.[43] Both parts of the SF-NDI have good internal consistency, convergent and discriminant validity, and are responsive to change.[42,43] More recently, minimum clinically important differences have been established.[44] The SF-NDI has been extensively used in FD research and has been translated and validated in many languages.

Assessment of psychological distress and somatic symptom severity ('somatization') The rationale for including instruments to measure presence and severity of depressive and anxiety disorders, as well as (extra-intestinal) somatic symptom severity ('somatization') lies in the observation that IBS patients have elevated levels of anxiety and depression symptoms, as well as of extra-GI bodily symptoms including comorbidity with anxiety, depressive[45] and somatoform/somatic symptom disorders.[6,46,47] Furthermore, higher levels of anxiety, depressive, and extra-intestinal somatic symptoms have been shown to be associated with higher levels of impairment in IBS and treatment responses are associated with improvements in anxiety, depression, and somatization.[48,49]

Patient Health Questionnaire The Patient Health Questionnaire (PHQ) modules on depression, anxiety disorders, and somatic symptom severity ('somatization') have excellent psychometric properties, including criterion validity (sensitivity & specificity based on cut-offs, see below), internal consistency, test–retest reliability, and sensitivity to change. They have been translated to and validated in many languages, and have been validated in a wide variety of populations and medical settings, including many patient groups with somatic symptoms.[50]

Other commonly used measures include the Symptom Checklist-90 (SCL-90[51]; a 90-item questionnaire

including, among others, subscales for the severity of depression, somatization, and different types of anxiety symptoms) as well as the Hospital Anxiety and Depression Scale (HADS)[52] a 14-item instrument which includes subscales for anxiety and depression severity (seven items each) and has validated cut-points for possible and probable diagnosis of anxiety or depressive disorders. The PHQ modules are preferred over the HADS because of recent concerns on the factor structure of the latter questionnaire and more specifically on its inability to differentiate between anxiety and depression thereby rendering it more useful as a general measure of psychological distress.[53]

Depression Module (PHQ-9) The PHQ-9 consists of nine depressive symptom items based on the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) criteria for major depressive episode[54,55]; the frequency of each is rated over the prior 2 weeks on a Likert scale ranging from 0 (not at all) to 3 (almost every day). The total score can be used as a continuous measure of depressive symptom severity ranging from 0 to 27 or, alternatively, cut-off points of 5, 10, 15, and 20 can be used representing mild, moderate, moderately severe, and severe levels of depressive symptoms. The presence of depressive disorder can be determined using a DSM-IV based diagnostic algorithm, or an optimal cut-off point ≥10 (with the latter performing better in terms of sensitivity).[50,56,57]

Anxiety module (GAD-7) The GAD-7 consists of seven anxiety symptoms based on DSM-IV criteria for generalized anxiety disorder,[55,58] which are scored as in the PHQ-9, and can be summed up to generate a continuous anxiety severity score ranging from 0 to 21, with 5, 10, and 15 representing cut-off points for mild, moderate, and severe levels of anxiety symptoms, respectively. In a manner similar to the PHQ-9, ≥10 represents the optimal cut-off point for 'caseness', in this case for generalized anxiety disorder. However, although it was originally developed as an instrument to detect generalized anxiety disorder, the GAD-7 was also shown to have good sensitivity and specificity (at the same cut-off point of ≥10) as a screening tool for panic, social anxiety, and post-traumatic stress disorder,[50] which are also frequently comorbid with IBS.

Somatic symptom severity ('somatization') module (PHQ-15) The PHQ-15 consists of 15 somatic symptom items that account for more than 90% of symptoms seen in primary care, and which also constitute the diagnostic criteria for the now abandoned DSM-IV category of somatization disorder.[55,59–61] Subjects rate how much they have been bothered by each symptom during the past month on a Likert scale ranging from 0 ('not at all') to 2 ('bothered a lot'). The total (sum) score thus ranges from 0 to 30, with cut-off points of 5, 10, and 15 representing thresholds for mild, moderate, and severe somatic symptom severity, respectively. As the PHQ-15 included 3 GI symptom items ('stomach pain', 'constipation, loose bowels, or diarrhea', and 'nausea, gas, or indigestion'), one of which constitutes a core IBS symptom, it is recommended to omit these three items when calculating the total score if the aim is to distinguish GI symptom severity from extra-intestinal symptom severity, or test relationships between both. This reduced version (PHQ-12) has been used and validated in IBS patients by Spiller *et al.*[62]

It should be noted that the PHQ-15 does not provide information on the medically unexplained nature of the somatic symptoms included, nor any data regarding any putative underlying psychological mechanism driving (or resulting from) symptom reporting, two aspects that are central to some, primarily older, conceptualizations of 'somatization'.[50,61,63] However, the first limitation can be overcome by quantitating psychological distress (anxiety, depression) using the two other PHQ modules discussed above.

Visceral Sensitivity Index Contrary to what may be suggested by the name, this instrument measures gastrointestinal symptom-specific anxiety (GSA), which can be defined as 'anxiety related to GI sensations, symptoms or the contexts in which these may occur'.[64] It covers five dimensions of GI-related cognitions and behaviours; worry, fear, vigilance, sensitivity, and avoidance. The rationale to include this instrument in addition to an instrument measuring anxiety in general, lies in the findings that GSA has been shown to be more strongly associated with IBS symptom severity.[64]

The Visceral Sensitivity Index is the only validated instrument to measure GSA, with good psychometric properties consisting of 15 statements, each of which is scored on a 6-point Likert scale ranging from 1 (strongly agree) to 6 (strongly disagree). The items are summed up and the sum is subtracted from 90 to yield a single total score ranging from 0 to 75, with higher scores representing higher levels of GSA.

Assessment of disease-specific quality of Life

Irritable bowel syndrome has a profound impact on quality of life,[65] and therefore validated disease-specific quality of life questionnaires are often included in

large-scale IBS studies. Some of the most widely used instruments are the IBS-QoL,[66] the functional digestive disorders quality of life,[67] and the irritable bowel syndrome quality of life questionnaire (IBSQOL).[68] All of these have been validated and have been used in clinical trials yielding data on responsiveness to change.[67,69,70]

In depth functional/physiological phenotyping

To establish intermediate phenotypes it is important to correlate symptoms and psychological profiling with physiological characteristics, gut microbiota analysis, and studies of gut tissue for immune and permeability assessment. Homogenous subgroups identified through this approach are more likely to yield meaningful results from genetic and epigenetic studies. The methods for performing such assessments have to be evidence based so that they can then be adopted across multiple collaborating centers to develop large data sets. Herein, we discuss the methods for physiological, gut microbiota, blood, and tissue sampling studies.

Physiological testing

The primary aim of testing physiological function within IBS was to differentiate normal from abnormal and to correlate pathophysiology with clinical symptomatology. However, given the current challenges inherent in the pathophysiologic and symptomatic heterogeneity of patients with IBS, current techniques do not have the prerequisite receiver operator characteristics for routine clinical practice. Arguably, this is a consequence of the current manner in which these techniques are evaluated such that they are tested on a relatively small number of IBS patients and are not standardized across different centers. Thus, a standardized approach is required which will not only lead to the generation of large sample sizes of IBS patients, which have hitherto been impossible, but also facilitate the examination of differences in such testing between the different IBS subtypes.

Following detailed literature review and consensus, protocols have been developed (see www.GENIEUR.eu), to standardize research activities on IBS physiological testing (visceral sensitivity and permeability studies), taking into account regulations and ethical requirements, both locally and nationally. In addition, the protocols have been designed such that non-specialist, i.e., non-tertiary centers, may also readily contribute to the cohort.

Visceral sensitivity testing – rectal barostat Mechanical distension of the distal colon can be undertaken to evaluate visceral perception and sensitivity in IBS with previous reports suggesting that up to 60% of patients have heightened sensitivity to distension compared with healthy controls.[71,72] Moreover, visceral hypersensitivity has been proposed as a biomarker in IBS, although it lacks sufficient discriminate sensitivity for routine clinical practice. Although studies have used rectal, sigmoid, and colonic distension, rectal distension has become the 'site of choice' as it is more accessible and thus technically more straightforward and only small perceptive differences have been shown between centers.[73]

Rectal sensitivity can be evaluated by utilizing a distensible polyethylene bag placed in the rectum in conjunction with a barostat, a device that maintains a constant pressure within the aforementioned bag. The barostat can delineate changes in the tone of the rectal wall by measuring alterations in volume and pressure within the bag. During rectal distension, verbal rating scales are used to measure the quality and intensity of pain perception during rising intra-rectal pressure and volume. Rectal distension performed according to specific protocols, during which different pressures are applied to the rectal wall. Although several distension protocols have been used, rapid rectal distension using the ascending method of limits (AML) and random phasic distension (RPD) protocols, are considered to be the most reproducible within individuals and across study centers.[74] However, AML and RPD protocols take up to 60 min to perform. This approach is therefore not widely practical within routine clinical practice and frequently is not undertaken outside the research environment. Sauter *et al.* have recently proposed and validated a Rapid Barostat Bag (RBB) technique, which is as a viable alternative to formal barostat testing in centers where this is not practical.[75] Briefly, for RBB a barostat bag is inflated inside the rectum manually via a dual-channel barostat catheter using a syringe with intra-bag filling pressure monitored and recorded by a handheld pressure gauge. After a 'conditioning' inflation, second 'index' inflation is performed. Threshold volumes for rectal filling sensations are recorded during the second, index distension using a validated visual analog scale (i.e., first perception, urgency, discomfort/pain) also used during conventional barostat studies. Alternatively, a shortened distension protocol can be used, in which only three or four distension steps above minimal distension pressure are applied either in an increasing[76,77] or random fashion.s[74,78] Categorizing patients as hypersensitive, normosensitive, or indeed hyposensitive somewhat

depends on the distension protocol and normal reference range used, but comparable pain thresholds for hypersensitivity have been described using both the shortened distension protocol (≤21 mmHg) and AML (<24 mmHg).[79] The latter study, categorized hyposensitivity when pain thresholds exceeded 38 mmHg.[79]

Colonic transit study Colonic motility is a critical process underlying the major functions of the large bowel. Disorders of colonic motility typically present with constipation or diarrhea. Measurement of colonic transit time is useful in evaluating colonic motility, and allows both the severity of the problem and the response to therapy to be assessed.

Different methods exist to investigate colon transit time. The standard measurement of colonic transit time has been performed with radio-opaque markers or colonic scintigraphy. The traditional approach is to assess the progression time of radio-opaque markers along the large bowel. Colonic scintigraphy can evaluate whole-gut transit.[80] Recently, wireless motility capsules have also been validated as a technique in measuring colon transit time.[81]

For practical reasons, assessment of colon transit is best based on retention of radio-opaque markers seen on abdominal X-ray following their ingestion 3–7 days earlier. These methods have been widely adopted since Hinton *et al.* first described this technique in 1969.[82] They distinguish constipation subgroups such as normal or slow transit constipation, and assess segmental transit times in patients with delayed total colon transit. Retention of ≥5 markers 5 days after ingestion of 24 markers is considered abnormal,[83] and normal values for other variants of colonic transit time measurements also exists.[84] These tests are simple and inexpensive as well as reliable and reproducible. However it requires good compliance of the patient, exposures patients to radiation, and does not measure the transit of a physiological meal.

GENETIC/EPIGENETIC ANALYSIS

Irritable bowel syndrome frequently clusters within families, thus suggesting a degree of heritability.[85–87] Furthermore, twin studies have demonstrated that the genetic heritability is in the order of 22–57% and the reported concordance rates for IBS differ between monozygotic and dizygotic twins, with 33% concordance in the former and 13% in the latter.[88] Hence the collection of data from such 'IBS families' and twins may provide invaluable genetic insights into the pathophysiology of IBS. Although several candidate genes have been investigated in IBS[89] the major weakness of such an approach in IBS studies has been the paucity of replication of findings in independent cohorts and the relatively small sample sizes which in turn results in limited statistical power to detect, what is almost certainly, a small effect. Therefore, there is a large unmet need for international initiatives collecting information and samples in a standardized manner.

Genome-wide association studies (GWAS) and next generation sequencing represent potentially useful techniques for systematically evaluating genetic factors within IBS, but also may provide novel insights into the pathophysiology of the disorder. Many common diseases represent complex disorders of multifactorial origin and have recently been successfully been dissected on genome level (http://www.genome.gov/GWAStudies/). Within other areas of gastroenterology, such as IBD, the GWAS approach has been successfully utilized to identify susceptibility genes with *The International Inflammatory Bowel Disease Genetics Consortium* identifying 163 novel susceptibility loci for IBD.[90] Compared to these GWAS studies on more than 75 000 cases and controls, the number of available IBS patients remains disappointingly small owing to the paucity of international collaborative multi-center efforts in the past.[90]

To date, the largest population based IBS GWAS study has examined more than 500 patients with an IBS-like phenotype, in comparison to 5000 matched controls from a twin registry in the discovery sample and replicated these findings in a further cohort of approximately 3500 IBS patients and controls.[91] Furthermore, as outlined in detail recently, some of the major flaws in IBS genetics research are attributed to the limited phenotype information. Consequently, more detailed phenotyping of larger case–control cohorts is mandatory before meaningful conclusions can be drawn. Similarly, epigenetic changes as a consequence of environmental stresses/nutrition resulting in DNA methylation and/or differential miRNA profiles may also provide important insights into the pathophysiology and stress related exacerbation of symptoms seen in IBS. A recent, albeit small, study has provided preliminary evidence for differential methylation positions using genome wide technology[92] and few miRNA studies generated additional evidence as recently summarized in a recent review[89]).

Therefore, to address these methodological deficiencies, an international consortium needs to be established to collect blood samples suitable for genetic and epigenetic analysis, patient information and functional data from large numbers of patients (see www.GENIEUR.eu). Such an approach will aid in redressing many of the issues regarding sample size that have limited the interpretation of previous studies.

Microbiota analysis

As a consequence of advances in high throughput DNA sequencing over the recent past, quantitation of the human microbiota has become feasible.[93] Given the marked interaction between the gut microbiota and the structure and function of the GI tract, it is not surprising that the microbiota has been the subject of intense research interest within IBS. A number of research groups have used culture-independent techniques to examine the role of the microbiota in different IBS subtypes.[94] Hitherto, the sample sizes of the studied patient cohorts have been relatively small, in addition to a lack of uniformity regarding sampling methods and the collection of phenotypical data between studies, thereby rendering direct comparison a challenge. By standardizing the fecal sample collection (see www.GENIEUR.eu) and the information relevant for microbiota analysis (diet, antibiotic use, psychological trait, and state, etc.), more robust data will be obtained and the interaction with genetic factors of the host can be studied in great detail.

Colonic biopsy sampling

Colonic biopsies can be used to monitor inflammatory events in the intestine as well as changes in neuronal plasticity, neurotransmitter alterations, and intestinal permeability. In addition, differential gene expression (mRNAs, ncRNAs, miRNAs), epigenetic modification of DNA which may impact expression by switching genes on or off in a long-term manner is of utmost interest to gain more insight in the mechanisms underlying IBS. Only by studying biopsies from large cohorts of well phenotyped and characterized IBS patients, new biomarkers and improved insights into the pathophysiology of IBS can be made. Storage of samples in a tissue bank however requires dedicated personnel and logistics, limiting this approach to specialized centers. Again, only if samples are collected using the same standardized operation procedures (see www.GENIEUR.eu), data from different centers can combined to yield large numbers leading to more robust inferences.

IMPLEMENTATION OF LARGE DATABASES: CHANCES AND CHALLENGES

Organizational issues

The development and implementation of a pan-European IBS sample collection and database poses considerable challenges to all partners involved. Numerous obstacles, such a local ethical regulations, validation and translation of questionnaires, harmonization, standardization, best practices, standard operation procedures, data protection, and intellectual property rights all have to be taken into account. Professional electronic custom made databases are costly, not only when design and development is concerned, but also when considering ongoing maintenance. An identical argument holds true for the storage of collected samples. The costs to purchase a professional system easily rises up to €50 000. Clearly, funding should be obtained to finance such tools as not all partners may have the required resources. Of note, funding agencies are currently not enthusiastic to finance such types of enterprises, seriously hampering initiatives aiming to create a multicenter international tissue bank. Nevertheless, ideally, all information on collected tissue, patient information, location of storage, test results, etc. should be hosted on a central server equipped with an appropriate firewall fulfilling security standards. Data and material use at particular sites will be regulated by material transfer agreements between partners and access to data will be given based on 'need to know' basis for the particular project.

Local ethics & legal implications

A substantial hurdle in international sample collection and database implementation are the variable standards at national/local medical ethical committees (METCs). Even within countries, METC standards may vary. Often independent local METCs exist making sample and data collection in different centers even within one country necessary to seek individual approval at sites involved. Of note, this may involve additional costs. At the European level, currently no legislation for pan-European biobanking exists. The current EU framework on data protection and medical confidentiality is based on directive 2004/23/EC, which has important implications for human tissues and cells and human application as well as transplants. Beyond the scope of this directive is human blood (DNA/RNA; CAVE: DNA depending on country treated as personal data). Material transfer agreements and informed consent have therefore to be adapted individually (imports, exports into third countries have to comply with local requirements of the directive, traceability, privacy, security, data safety issues, location of storage, accessibility, longevity, usability for other purposed, and ownership of data. Electronic documentation is needed while data availability and data protection have to be well balanced. Possible disputes (who has access, where, and how is the dispute settled, how to protect the data, what happens

if the database is terminated, who is responsible in case of database damage) have to be identified and agreed upon, according to local law and ethical considerations.

Translation into different languages

When setting up a large pan-European or international sample collection and database, especially related to questionnaires dealing with patient symptomatology and psychological aspects, language considerations are centrally important. Although papers published in peer-reviewed journals usually report data from questionnaires that are available and mostly validated in English, these questionnaires are usually not translated in other languages in a validated manner. Translation is a process that has to incorporate local culture, religion, language-specifics and interpretation.[95] The Rome Foundation for instance employs their own specialist dealing with this issue, the minimal form of translation being back and forth-translation. Locally validated datasets, such as questionnaires, remain the golden standard, yet for countries with smaller population size validated translations often do not exist. In addition, to compound matters further there are also considerations that need to be undertaken concerning copyright issues of translating the respective questionnaires.

CONCLUSION

Despite the challenges discussed above, implementation of a large European or international biobank and database offers valuable opportunities and is fundamental to address many of the knowledge gaps that exist within the field. The necessity of unifying and harmonizing approaches across Europe, and ideally the world, allows greater data compatibility, and larger databases across countries and will improves the quality of multi-centers trials and ultimately patient outcomes.

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