Chapter 3
Irritable Bowel Syndrome: Diagnosis

Marcin Włodarczyk and Aleksandra Sobolewska-Włodarczyk

Abstract Irritable bowel syndrome (IBS) is a chronic gastrointestinal disorder diagnosed on symptom-based criteria without inclusion of any objective parameter measurable by known diagnostic methods. Heterogeneity of patient's symptoms and overlapping with more serious organic diseases increase uncertainty for the physician's work and enhance the cost of confirming the diagnosis. In 2016 Rome IV criteria of functional disorders were published. These criteria are the basis to make a diagnosis in the daily work of medical doctors, especially general practitioners and gastroenterologists. Recent studies showed that in the future a combination of several new biomarkers could improve the diagnostic process of IBS. Among the studied biomarkers, most evidence is provided for fecal calprotectin. However, cut-off values for fecal calprotectin still have to be investigated prior to inclusion in the IBS diagnostic algorithm. In this chapter diagnosis criteria of IBS will be discussed.

According to the National Collaborating Centre for Nursing and Supportive Care (NICE) guidelines, prevalence of IBS is between 10 and 20 % worldwide, with women to men ratio 2:1 [1]. The need for a reliable and standard method to properly discriminate functional gastrointestinal disorders (FGIDs) has led to the development of symptom-based criteria by the Rome Foundation. Accordingly, diagnosis

M. Włodarczyk (✉) · A. Sobolewska-Włodarczyk
Department of Biochemistry, Faculty of Medicine, Medical University of Lodz,
Mazowiecka 6/8, 92-215, Lodz, Poland
e-mail: dr.mwlodarczyk@gmail.com

A. Sobolewska-Włodarczyk
e-mail: olasobolewska1@poczta.onet.pl

M. Włodarczyk
Department of General and Colorectal Surgery, Faculty of Military Medicine,
Medical University of Lodz, Hallera Sq 1, 90-647, Lodz, Poland

A. Sobolewska-Włodarczyk
Department of Gastroenterology, Faculty of Military Medicine,
Medical University of Lodz, Hallera Sq 1, 90-647, Lodz, Poland

J. Fichna (ed.), *Introduction to gastrointestinal diseases Vol. 1*,
DOI 10.1007/978-3-319-49016-8_3

of IBS is established on clinical background with exclusion of "red flag" symptoms (age >50, rectal bleeding, anemia, short-term symptoms, and weight loss) [1]. Because there are usually no physical signs to definitively diagnose IBS, diagnosis is often a process of ruling out other conditions. To make the right identification, general practitioner or gastroenterologist needs to following diagnostic steps.

3.1 Step I

Healthcare professionals should consider assessment for IBS if the person reports having any of the following symptoms for at least 6 months:

- Abdominal pain
- Bloating
- Change in bowel habit.

Bloating means fullness or swelling in the abdomen that often occurs after meals.

During the medical interview, the occurrence of diarrhea, constipation or both problems should be considered. It is very important to ask the patient how many times per day he or she has bowel movements related with visit in the bathroom and about their stool consistency. Sometimes doctors can use the Bristol Scale of stool [2] (Fig. 3.1).

3.2 Step II

All the patients presenting with possible IBS symptoms should be asked if they have any of the following "red flag" indicators and should be referred to secondary care for further investigation if any are present.

- Unintentional and unexplained weight loss
- Rectal bleeding
- A family history of bowel or ovarian cancer
- A change in bowel habit to looser and/or more frequent stools persisting for more than 6 weeks in a person aged over 60 years.

'Red flag' symptoms can be connected to cancer and they are considered as alarming signs. Each time the patient reports even only one of the above symptoms, a specialist's diagnosis is necessary. Special attention must be applied to patients with a positive family history of cancer.

Bristol Stool Chart

	Type 1	Separate hard lumps, like nuts (hard to pass)	Very constipated
	Type 2	Lumpy and sausage like	Slightly constipated
	Type 3	A sasuage shape with cracks in the surface	Normal
	Type 4	Like a sausage or snake, smooth and soft	Normal
	Type 5	Soft blobs with clear-cut edges	Lacking fibre
	Type 6	Fluffy pieces with ragged edges, a mushy stool	Inflammation
	Type 7	Liquid consistency with no solid pieces	Inflammation

Fig. 3.1 Bristol stool chart

3.3 Step III

All the patients presenting with possible IBS symptoms should be assessed and clinically examined for the following "red flag" indicators and should be referred to secondary care for further investigation if any are present.

- Anaemia
- Abdominal masses
- Rectal masses
- Inflammatory markers for inflammatory bowel disease.

In any of these cases, physical examination and imaging studies such as ultrasound should be performed.

If there is a significant concern that symptoms may suggest ovarian cancer, gynecologist's consultation and pelvic examination should also be considered.

3.4 Step IV

The final diagnosis of IBS should be considered only if the person has abdominal pain that is either relieved by defecation or associated with altered bowel frequency or stool form. This should be accompanied by at least two of the following four symptoms:

- Altered stool passage (straining, urgency, incomplete evacuation)
- Abdominal bloating (more common in women than men), distension, tension or hardness
- Symptoms made worse by eating
- Passage of mucus.

Other features such as lethargy, nausea, backache and bladder symptoms are common in people with IBS and may be used to support the diagnosis.

3.5 Step V

3.5.1 Basic Diagnostic Tests

In people who meet the IBS diagnostic criteria, the following fundamental test should be undertaken to exclude other diagnoses and diagnose the IBS:

- Full blood count (FBC)
- Erythrocyte sedimentation rate (ESR) or plasma viscosity

- C-reactive protein (CRP)
- Antibody testing for coeliac disease (endomysial antibodies [EMA] or tissue transglutaminase [TTG]).

To perform above test the venous blood from the peripheral vessels should be drawn.

3.6 Additional Testing

Additional diagnostic testing in patients who meet the IBS diagnostic criteria the tests for celiac disease may be warranted in patients presenting with diarrhea as their predominant symptom. However, the extensive diagnostic testing is unnecessary for patients without alarm symptoms. In some cases addressing disease-related concerns, discussing reasonable treatment goals and expectations, educating and empowering patients, and addressing somatization issues with patients may provide greater benefit than extensive testing. However, even if the above mentioned diagnostic scheme is performed, many of IBS patients need additional clinical tests to exclude the other infections, inflammatory or neoplastic diseases. Additional tests include:

- Abdominal ultrasound
- Rigid/flexible sigmoidoscopy
- Colonoscopy; barium enema
- Thyroid function test
- Faecal ova and parasite test
- Faecal occult blood
- Hydrogen breath test (for lactose intolerance and bacterial overgrowth).

After all of the above steps are completed, IBS may be recognized. However, to systematize the diagnosis of IBS, the Rome III Diagnostic Criteria were introduced in 2006 and Rome IV criteria in 2016 [3].

There were systematic approaches that attempted to classify the then hazy area of FGIDs as early as 1962, when Chaudhary and Truelove published a retrospective review of IBS patients at Oxford, England. Later on, the “Manning Criteria” for IBS were derived from a paper published in 1978 by Manning and colleagues. This seminal classification started a new era and from then on, scientific work on functional gastrointestinal disorders proceeded with increased enthusiasm.

The “Rome process” is an international effort to create scientific data to help in the diagnosis and treatment of functional gastrointestinal disorders (FGIDs), such as IBS, functional dyspepsia and rumination syndrome. The Rome Diagnostic Criteria are set forth by the Rome Foundation, a non- profit organization, under the professional management of Hilliard Associates based in Raleigh, North Carolina.

The Rome criteria have been evolving from the first set, issued in 1989 through the Rome Classification System for FGIDs (1990), the Rome I Criteria for IBS (1992) and the FGIDs (1994), the Rome II Criteria for IBS (1999) and the FGIDs (1999), the Rome III Criteria (2006) and to the most recent Rome IV criteria (2016). Currently the Rome III Diagnostic Criteria for FGIDs is still the 'Gold Standard' for the diagnosis of IBS [4].

3.7 According to Rome III Criteria for IBS Is as Follows

Recurrent abdominal pain at least 3 days/month* in the last 3 months associated with two or more of the following:

- Improvement with defecation
- Onset associated with a change in frequency of stool
- Onset associated with a change in form (appearance) of stool.

Diagnostic Criterion fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis.

* Criterion fulfilled for the last months with symptom onset at least months prior to diagnosis.

In pathophysiology research and clinical trials, a pain/discomfort frequency of at least 2 days a week during screening evaluation is recommended for subject eligibility [4].

Patients with IBS are divided into subgroups based on their predominant symptoms:

(a) diarrhea predominant (IBS-D),
(b) constipation predominant (IBS-C),
(c) mixed type with diarrhea and constipation (IBS-M),
(d) undetermined IBS (IBS-U).

Around 75 % of patients are alternators, which illustrates the instability of symptoms over time in the same patient [5].

The Rome III diagnostic questionnaire for IBS contains 10 items and answers to questions are on an ordinal scale with individual frequency thresholds for each question. The qualification of patient to an appropriate subgroup is performed on the answers to questions regarding bowel movements habits, frequency and consistency of stools (Table 3.1).

Table 3.1 ROME III criteria—Questionnaire (Rome Foundation)

1. In the last 3 months, how often did you have discomfort or pain anywhere in your abdomen?	0. Never → 1. Less than one day a month 2. One day a month 3. Two to three days a month 4. One day a week 5. More than one day a week 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 1. Yes 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?	0. No 1. Yes	
4. How often did this discomfort or pain get better or stop after you had a bowel movement?	0. Never or rarely 1. Sometimes 2. Often 3. Most of the time 4. Always	
5. When this discomfort or pain started, did you have more frequent bowel movements?	0. Never or rarely 1. Sometimes 2. Often 3. Most of the time 4. Always	
6. When this discomfort or pain started, did you have less frequent bowel movements?	0. Never or rarely 1. Sometimes 2. Often 3. Most of the time 4. Always	
7. When this discomfort or pain started, were your stools (bowel movements) looser?	0. Never or rarely 1. Sometimes 2. Often 3. Most of the time 4. Always	
8. When this discomfort or pain started, how often did you have harder stools?	0. Never or rarely 1. Sometimes 2. Often 3. Most of the time 4. Always	
9. In the last 3 months, how often did you have hard or lumpy stools?	0. Never or rarely 1. Sometimes 2. Often 3. Most of the time 4. Always	Alternative scale: 0. Never or rarely 1. About 25 % of the time 2. About 50 % of the time 3. About 75 % of the time 4. Always, 100 % time

(continued)

Table 3.1 (continued)

10. In the last 3 months, how often did you have loose, mushy or watery stools?	0. Never or rarely 1. Sometimes 2. Often 3. Most of the time 4. Always	Alternative scale: 0. Never or rarely 1. About 25 % of the time 2. About 50 % of the time 3. About 75 % of the time 4. Always, 100 % time

Criteria for IBS-C (question 9 > 0) and (question 10 = 0)
Criteria for IBS-D (question 9 = 0) and (question 10 > 0)
Criteria for IBS-M (question 9 > 0) and (question 10 > 0)
Criteria for IBS-U (question 9 = 0) and (question 10 = 0)

3.8 Biomarkers for IBS

In 2001, Biomarkers Definitions Working Group defined the term "biomarker" as "a characteristic that is measured and evaluated as an indicator of normal biological processes, pathogenetic processes or pharmacologic responses to a therapeutic agent" [6, 7]. Noninvasive biomarkers are particularly desired, as their application would reduce costs and minimize unnecessary diagnostic tests.

There are several obstacles in the search for relevant biological biomarkers in IBS. They include:

- eterogeneity of symptoms between patients and temporal instability of the symptoms in the same patient
- overlapping of IBS symptoms with other functional gastrointestinal disorders (FGIDs) and more serious organic diseases
- unclear understanding of the pathophysiology of IBS and other disorders [8].

Nevertheless, several new markers in IBS have already been proposed.

1. C-reactive protein (CRP)

C-reactive protein (CRP), a member of pentraxin family is an annular (ring-shaped), pentameric protein found in blood plasma, whose levels rise in response to inflammation. It is an acute-phase protein of hepatic origin that increases following interleukin-6 secretion by macrophages and T cells. Its physiological role is to bind to lysophosphatidylcholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via the C1Q complex [9].

CRP is synthesized by the liver in response to factors released by macrophages and fat cells (adipocytes) [10–12]. Importantly, it is not related to C-peptide (insulin) or protein C (blood coagulation).

CRP is usually used to assess the degree of inflammation and therapeutic success in diseases such as Crohn's disease and ulcerative colitis. In the study conducted by Hod et al. researchers tried to confirm the hypothesis of elevated high sensitivity CRP (hs-CRP) as a marker of microinflammation in IBS [13]. Hs-CRP levels were higher in IBS patients than HC, but still in the normal laboratory range. This may reflect the low-grade gut inflammation believed to occur in IBS and support its existence. In the study, hs-CRP levels were highest in patients with diarrhea-predominant IBS and in patients with greater disease severity. A cut-off value of 1.08 mg L(-1) demonstrated a sensitivity of 60.2 % and a specificity of 68 % for differentiating IBS from HC. The clinical relevance of CRP values in assessing IBS disease severity or therapy follow-up has not yet been proven.

2. Erythrocyte sedimentation rate (ESR)

The erythrocyte sedimentation rate (ESR), also called a sedimentation rate or Westergren ESR, is the rate at which red blood cells sediment in a period of one hour. It is a common hematology test, and is a non-specific measure of inflammation. The ESR is governed by the balance between pro-sedimentation factors, mainly fibrinogen, and those factors resisting sedimentation, namely the negative charge of the erythrocytes. When an inflammatory process is present, the high proportion of fibrinogen in the blood causes red blood cells to stick to each other [14].

ESR, like CRP, is also hypothesized to be a nonspecific marker for microinflammation [15]. Hauser et al. hypothesized that mild inflammation in IBS patients could be detected by ESR, which could be a sensitive, yet cheap and ubiquitous test [15]. Furthermore, Hauser et al. assumed that ESR would be related with the disease severity index and decreased general and disease-specific health-related quality of life (HRQoL). The preliminary results of a pilot study showed that IBS patients with higher ESR expressed lower disease-specific HRQoL (e.g. they expressed more bowel symptoms, social and emotional disturbances related to disease). No significant correlations were found between ESR and the disease severity as well as general HRQoL.

3. Cortisol

Cortisol belongs to the glucocorticoid class of steroid hormones and is produced in humans by the zona fasciculata of the adrenal cortex within the adrenal gland [16]. It is released in response to stress and low blood-glucose concentration.

Cortisol functions to increase blood sugar through gluconeogenesis, to suppress the immune system, decreases bone formation, and to aid in the metabolism of fat, protein, and carbohydrates [17, 18].

Cortisol is known as the "stress hormone", involved in the body response to stress. The level of cortisol in the blood depends on hypothalamic-pituitary-adrenal (HPA) axis activity. One of the theories suggested that disturbances in HPA axis underlie the development of IBS. Recent studies illustrated that risk factors such as early life trauma and chronic stress increased susceptibility to IBS, with symptoms

manifesting after exposure to triggers like changes in enteric flora composition, infection and dietary factors. Therefore, the idea of measuring cortisol levels in these patients and searching for disturbances in the HPA axis seems a logical way of proceeding with research into the origin of disorders [19, 20]. In the study conducted by Kennedy et al., salivary cortisol levels were measured in response to the Trier Social Stress Test (TSST). The authors found greater total cortisol output in response to acute stress in IBS patients compared with healthy subjects [21]. Patients with IBS exhibit sustained HPA axis activity, and often developed different gastrointestinal symptoms in response to acute experimental psychosocial stress.

In 2009, a similar study was performed by FitzGerald et al. The authors measured cortisol levels in women with IBS-D after lumbar puncture as representative of a physical stressor. Results of this study showed an attenuated response of the HPA axis in patients with IBS compared with healthy controls. The impaired tone of the HPA axis was attributed to adaptive changes in brain response to chronic stress to which IBS patients are considered to be more often exposed in comparison with healthy individuals [22]. Women with IBS display blunted adrenocorticotropic hormone and cortisol responses to the lumbar puncture along with a profile of affective responsiveness suggestive of chronic psychosocial stress, although no CRF(CSF) differences between groups were observed.

4. Chromogranin A (CgA)

Chromogranin A (CgA) is a precursor to several functional peptides which negatively modulate the neuroendocrine function of the releasing cell (autocrine signaling) or nearby cells (paracrine signaling). CgA induces and promotes generation of secretory granules including those containing insulin in pancreatic islet beta cells [23]. It is used as an indicator for pancreas and prostate cancer and in carcinoid syndrome [24, 25]. It may also play a role in early neoplastic progression. CgA is cleaved by an endogenous prohormone convertase to produce several peptide fragments.

The chromogranin family recently have been highlighted in the search for the ideal biomarker for IBS. Popularity of this proteins increased since it was discovered that chromogranin family can modulate intestinal inflammation and present active communication between the neuroendocrine and immune system [26]. In the study conducted by Sidhu et al. an elevated CgA serum level in a subset of IBS-D patients was found [27]. The results confirmed the hypothesis about enterochromaffin cell hyperplasia in post-infectious (PI)-IBS patients [28, 29].

The role of chromogranin as an inflammation marker has yet to be proven. In contrast, El-Salhy et al. found no increase in CgA blood level compared with healthy controls and considered that changes in CgA levels in blood are clinically insignificant. Instead, they found reduced density of CgA-containing cells in the duodenum and colon of both IBS-D and IBS-C patients [30]. Because of this finding altered density of intestinal CgA cells as a potential histopathological marker for IBS was proposed [30].

To conclude, a recent study performed by Öhman et al. showed elevated levels of CgA and secretogranins II and III in patients with IBS-D and IBS alternators (IBS-A) [31]. One of the most important observations in this study was that there is a strong negative correlation between the colonic transit time and fecal levels of mentioned granins. This discovery opens the door to new questions and hypotheses regarding the role of fecal granins in IBS.

5. Fecal calprotectin (FC)

Fecal calprotectin (FC) is a biochemical measurement of calprotectin in the stool. Elevated fecal calprotectin indicates the migration of neutrophils to the intestinal mucosa, which occurs during intestinal inflammation, including inflammation caused by inflammatory bowel disease. Under a specific clinical scenario, the test may eliminate the need for invasive colonoscopy or radio-labelled leukocyte scanning [31, 32].

The main diseases that cause an increased excretion of fecal calprotectin are infectious colitis, Crohn's disease, ulcerative colitis, and neoplasms (cancer) [33]. Moreover, the levels of fecal calprotectin seem to be in a normal range in patients with IBS [34].

However, newer studies keep trying to find relevant cutoff FC stool levels that could—with great certainty—distinguish IBS from IBD and reduce unnecessary invasive diagnostic tools. In 2002, Tibble et al. established that the cut-off FC levels of 30 mg/kg combined with Rome I criteria can serve as a clear proof of IBS with no need for further examination [35]. The report published in 2013 showed that FC is confirmed as a highly specific and sensitive biomarker for IBD and the value of 50 mcg/g showed 93 % sensitivity and 94 % specificity in differentiating IBD from IBS [36]. Waugh et al. concluded that FC can be a highly sensitive way of detecting IBD, although there are inevitably trade-offs between sensitivity and specificity, with some false positives (IBS with positive calprotectin) if a low calprotectin cut-off is used. In most cases, a negative calprotectin rules out IBD, thereby sparing most people with IBS from having to have invasive investigations, such as colonoscopy.

In 2014 Chang et al. reported an interesting finding that higher FC levels in IBS patients correlate with disease activity more significantly than serum CRP levels [37]. Findings of elevated FC should be investigated further, because these may increase the sensitivity and specificity of tests performed in the diagnostic algorithm to confirm IBS. Another positive remark on FC is the opinion that FC level correlates with a reduced physical component of health related quality of life (HRQoL) [38]. This means that FC can be used to monitor the response to therapy. Measurement of FC levels should be included in the IBS diagnostic algorithm, regardless of whether it is used to confirm microinflammation and to choose an adequate therapy approach for these patients or to exclude the diagnosis of IBD and minimize unnecessary invasive procedures.

6. Human β-defensin-2 (HBD-2)

Defensins form a family of microbicidal and cytotoxic peptides made by neutrophils. Members of the defensin family are highly similar in protein sequence [39].

HBD-2 is produced by a number of epithelial cells and exhibits potent antimicrobial activity against Gram-negative bacteria and *Candida*, but not Gram-positive *S. aureus*. It has been speculated that HBD-2 may contribute to the infrequency of Gram-negative infections of the skin and lung tissue [40].

In 2009, Langhorst et al. found significantly higher levels of HBD-2 in patients with IBS compared with healthy controls [41]. The results indicate significantly elevated levels of HBD-2 in patients with IBS compared with controls and similar to those with active UC. The results confirm the theory of an activation of the mucosal innate defense system toward a proinflammatory response in IBS patients in the absence of macroscopic signs of inflammation. Langhorst et al. suggested that HBD-2 presents another potential biomarker whose clinical role in IBS has not been adequately investigated so far.

3.9 Conclusion

IBS is still a symptom-based diagnosis disorder that reduces patients' quality of life and which imposes a significant economic burden to the healthcare system. Many healthcare providers view IBS as a static disorder that is hard to define, difficult to diagnose and impossible to treat. These popular views are just several of the most common misconceptions related to the diagnosis and treatment of IBS. The truth, however, is that IBS is a dynamic field characterized by significant changes in diagnostic strategies and therapeutic options over the last decade. The search for a new, cheap and reliable biomarker seems to be the future in diagnosis of IBS.

References

1. National Collaborating Centre for Nursing and Supportive Care (2008) irritable bowel syndrome in adults: diagnosis and management of irritable bowel syndrome in primary care
2. Amarenco G (2014) Bristol Stool Chart: étude prospective et monocentrique de «l'introspection fécale» chez des sujets volontaires [Bristol stool chart: prospective and monocentric study of 'stools introspection' in healthy subjects]. Progrès en Urologie (in French) 24 (11):708–713
3. Rome IV. Functional Gastrointestinal Disorders. Disorders of Gut-Brain Interaction. Douglas A. Drossman, Senior Editor. 2016
4. http://www.romecriteria.org/
5. El-Salhy M (2012) Irritable bowel syndrome: diagnosis and pathogenesis. World J Gastroenterol 18(37):5151–5163

6. Aronson J (2005) Biomarkers and surrogate endpoints. Br J Clin Pharmacol 59(5):491–494. doi:10.1111/j.1365-2125.2005.02435.x.
7. Biomarkers Definitions Working Group (2001) Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 69(3):89–95. doi:10.1067/mcp.2001.113989
8. Clarke G, Quigley EMM, Cryan JF, Dinan TG (2009) Irritable bowel syndrome: towards biomarker identification. Trends Mol Med 15(10):478–489. doi:10.1016/j.molmed.2009.08.001
9. Thompson D, Pepys MB, Wood SP (1999) The physiological structure of human C-reactive protein and its complex with phosphocholine. Structure 7(2):169–177. doi:10.1016/S0969-2126(99)80023-9
10. Pepys MB, Hirschfield GM (2003) C-reactive protein: a critical update. The Journal of Clinical Investigation 111(12):1805–1812. doi:10.1172/JCI18921
11. Lau DC, Dhillon B, Yan H, Szmitko PE, Verma S (2005) Adipokines: molecular links between obesity and atheroslcerosis. Am J Physiol Heart Circ Physiol 288(5):H2031–H2041. doi:10.1152/ajpheart.01058.2004
12. Mantovani A, Garlanda C, Doni A, Bottazzi B (2008) Pentraxins in innate immunity: from C-reactive protein to the long pentraxin PTX3. J Clin Immunol 28(1):1–13. doi:10.1007/s10875-007-9126-7
13. Hod K, Dickman R, Sperber A et al (2011) Assessment of high-sensitivity CRP as a marker of micro-inflammation in irritable bowel syndrome. Neurogastroenterol Motil 23(12):1105–1110. doi:10.1111/j.1365-2982.2011.01788.x
14. ESR (2013) MedlinePlus: U.S. National Library of Medicine and National Institutes of Health. Retrieved 8 July 2013
15. Hauser G, Tkalcic M, Pletikosic S, Grabar N, Stimac D (2012) Erythrocyte sedimentation rate—possible role in determining the existence of the low grade inflammation in Irritable bowel syndrome patients. Med Hypotheses 78(6):818–820. doi:10.1016/j.mehy.2012.03.020
16. Scott E (2011) Cortisol and stress: how to stay healthy. About.com. Retrieved 29 Nov 2011
17. Hoehn K, Marieb EN (2010) Human anatomy and physiology. Benjamin Cummings, San Francisco. ISBN 0-321-60261-7
18. Chyun YS, Kream BE, Raisz LG (1984) Cortisol decreases bone formation by inhibiting periosteal cell proliferation. Endocrinology 114(2):477–480. doi:10.1210/endo-114-2-477
19. Chitkara DK, van Tilburg MAL, Blois-Martin N, Whitehead WE (2008) Early life risk factors that contribute to irritable bowel syndrome in adults: a systematic review. Am J Gastroenterol 103(3):765–774. doi:10.1111/j.1572-0241.2007.01722.x
20. Blanchard EB, Lackner JM, Jaccard J et al (2008) The role of stress in symptom exacerbation among IBS patients. J Psychosom Res 64(2):119–128. doi:10.1016/j.jpsychores.2007.10.010
21. Kennedy PJ, Cryan JF, Quigley EM, Dinan TG, Clarke G (2014) A sustained hypothalamic-pituitary-adrenal axis response to acute psychosocial stress in irritable bowel syndrome. Psychol Med 44(14):3123–3134. doi:10.1017/s003329171400052x
22. FitzGerald LZ, Kehoe P, Sinha K (2009) Hypothalamic-pituitary-adrenal axis dysregulation in women with irritable bowel syndrome in response to acute physical stress. West J Nurs Res 31(7):818–836. doi:10.1177/0193945909339320
23. Helman LJ, Ahn TG, Levine MA, Allison A, Cohen PS, Cooper MJ, Cohn DV, Israel MA (1988) Molecular cloning and primary structure of human chromogranin A (secretory protein I) cDNA. J Biol Chem 263(23):11559–11563
24. Wu JT, Erickson AJ, Tsao KC, Wu TL, Sun CF (2000) Elevated serum chromogranin A is detectable in patients with carcinomas at advanced disease stages. Ann Clin Lab Sci 30(2):175–178
25. Nikou GC, Lygidakis NJ, Toubanakis C, Pavlatos S, Tseleni-Balafouta S, Giannatou E, Mallas E, Safioleas M (2005) Current diagnosis and treatment of gastrointestinal carcinoids in a series of 101 patients: the significance of serum chromogranin-A, somatostatin receptor scintigraphy and somatostatin analogues. Hepatogastroenterology 52(63):731–741

26. Zhang D, Shooshtarizadeh P, Laventie B-J et al (2009) Two chromogranin a-derived peptides induce calcium entry in human neutrophils by calmodulin-regulated calcium independent phospholipase A2. PLoS One 4(2). doi:10.1371/journal.pone.0004501.e4501
27. Sidhu R, McAlindon ME, Leeds JS, Skilling J, Sanders DS (2009) The role of serum chromogranin A in diarrhoea predominant irritable bowel syndrome. J Gastrointest Liver Dis 18(1):23–26
28. Dunlop SP, Jenkins D, Neal KR, Spiller RC (2003) Relative importance of enterochromaffin cell hyperplasia, anxiety, and depression in postinfectious IBS. Gastroenterology 125 (6):1651–1659. doi:10.1053/j.gastro.2003.09.028
29. Sidhu R, Drew K, McAlindon ME, Lobo AJ, Sanders DS (2010) Elevated serum chromogranin A in irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD): a shared model for pathogenesis? Inflamm Bowel Dis 16(3):361. doi:10.1002/ibd.20982
30. El-Salhy M, Lomholt-Beck B, Hausken T (2010) Chromogranin a as a possible tool in the diagnosis of irritable bowel syndrome. Scand J Gastroenterol 45(12):1435–1439. doi:10.3109/00365521.2010.503965
31. Öhman L, Stridsberg M, Isaksson S, Jerlstad P, Simrén M (2012) Altered levels of fecal chromogranins and secretogranins in IBS: relevance for pathophysiology and symptoms? Am J Gastroenterol 107(3):440–447. doi:10.1038/ajg.2011.458
32. Brophy MB, Nolan EM (2015) Manganese and microbial pathogenesis: sequestration by the mammalian immune system and utilization by microorganisms. ACS Chem Biol 10(3):641–651. doi:10.1021/cb500792b
33. Chen CC, Huang JL, Chang CJ, Kong MS (2012) Fecal calprotectin as a correlative marker in clinical severity of infectious diarrhea and usefulness in evaluating bacterial or viral pathogens in children. J Pediatr Gastroenterol Nutr 55(5):541–547
34. Costa F, Mumolo MG, Ceccarelli L, Bellini M, Romano MR, Sterpi C, Ricchiuti A, Marchi S, Bottai M (2005) Calprotectin is a stronger predictive marker of relapse in ulcerative colitis than in Crohn's disease. Gut 54(3):364–368
35. Tibble JA, Sigthorsson G, Foster R, Forgacs I, Bjarnason I (2002) Use of surrogate markers of inflammation and Rome criteria to distinguish organic from nonorganic intestinal disease. Gastroenterology 123(2):450–460. doi:10.1053/gast.2002.34755
36. Waugh N, Cummins E, Royle P et al (2013) Faecal calprotectin testing for differentiating amongst inflammatory and non-inflammatory bowel diseases: systematic review and economic evaluation. Health Technol Assess 17(55). doi:10.3310/hta17550
37. Chang M-H, Chou J-W, Chen S-M et al (2014) Faecal calprotectin as a novel biomarker for differentiating between inflammatory bowel disease and irritable bowel syndrome. Molecular Medicine Reports. 10(1):522–526. doi:10.3892/mmr.2014.2180
38. Tkalcic M, Pletikosic S, Hausr G (2014) Biological and psychological determinants of health related quality of life in irritable bowel syndrome patients. J Psychosom Res 76(6):516. doi:10.1016/j.jpsychores.2014.03.086
39. Sawai MV, Jia HP, Liu L, Aseyev V, Wiencek JM, McCray PB Jr, Ganz T, Kearney WR, Tack BF (2001) The NMR structure of human beta-defensin-2 reveals a novel alpha-helical segment. Biochemistry 40(13):3810–3816
40. Schröder JM, Harder J (1999) Human beta-defensin-2. Int J Biochem Cell Biol 31(6):645–651
41. Langhorst J, Junge A, Rueffer A et al (2009) Elevated human β-defensin-2 levels indicate an activation of the innate immune system in patients with irritable bowel syndrome. Am J Gastroenterol 104(2):404–410