DOI: 10.15386/cjmed-496

SERUM BIOMARKERS FOR IRRITABLE BOWEL SYNDROME

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Abstract

Background and aim. *Irritable bowel syndrome (IBS) is one of the most frequent and common functional gastrointestinal diseases. For its diagnosis, clinical criteria are still used. Our objective was to asses if there are specific serum biomarkers for the diagnosis of IBS, and as secondary purpose we aimed to analyze the specificity and sensitivity – where determined – for the proposed biomarkers.*

Methods. *We performed a review in order to find potential serum biomarkers useful for the diagnosis of IBS. MEDLINE and Cochrane databases were searched in May 2015. Inclusion criteria were: original studies that assessed serological markers in IBS patients, markers potentially useful for diagnosing IBS or in differentiating subtypes of IBS. Exclusion criteria were biomarkers assessed in IBS patients not for the diagnosis of IBS, but used in order to exclude other conditions or diseases in these patients; or markers that were not addressed to IBS; or papers that assessed only fecal biomarkers, or histological or surrogate - indirect biomarkers.*

Results. *From the 268 papers retrieved by our initial search, using a modified strategy we identified 58 papers. Out of the 58 papers retrieved by the search, six papers were selected and other nine studies were eventually analyzed. Of the results of the computerized search, a number of papers were not included for various reasons: some were not related to the subject (26), others were not appropriate for the subject (19) because they addressed inflammatory bowel disorders, in others fecal markers were the subject of the study, six were reviews, others were impossible to gain access to (1). Twelve out of the 14 studies included are case-control studies, IBS diagnosis being established in all of the selected results based on the Rome criteria. A higher sensitivity of 81% was found using a combination of markers but with lower specificity, while one study that assessed also a combination of markers, found a higher specificity but sensitivity was only 50 %; none reached the characteristics for an ideal biomarker.*

Conclusions. *For the moment, just one serum biomarker with a high specificity and sensitivity useful in the diagnosis of IBS was identified. We consider that in the future a combination of several biomarkers could better identify IBS than a single biomarker. Therefore, clinical criteria are still to be used for the diagnosis of IBS in attendance for newer research or validation of results.*

Keywords*:* biomarkers, irritable bowel syndrome

Introduction

Irritable bowel syndrome (IBS) is one of the most frequent and common functional gastrointestinal diseases [1,2].

IBS is defined by the association of pain or abdominal discomfort with altered bowel transit [3]. IBS is a chronic disease that impairs the quality of life [4].

In the absence of "alarm signs", IBS diagnosis is currently established using symptom-based criteria - Rome III criteria [2].

Current guidelines for diagnosing IBS used in United

Manuscript received: 12.06.2015
Accepted: 30.06.2015
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States and Great Britain use symptom-based diagnostic criteria for the diagnosis of IBS [5,6]. Though the criteria reach 70% sensitivity (Se) and 80% specificity (Sp) they are similar as performance [7,8] to the first criteria used – Manning [9].

Like for other diseases for which a biopsychosocial model was established [10,11], this biopsychosocial model was later described also for IBS [12]. According to this model there are multiple factors -- genetic, demographic and environmental -- which interact with psychosocial factors and might lead to the typical clinical manifestations [12].

Although numerous studies have attempted to elucidate the etiopathogenetic pathways, the physiopathology of IBS is not entirely decrypted [13].

Many hypotheses have been proposed to explain the etiopathogenesis of IBS, some of them confirmed and others completed or rejected.

At present, the etiopathogenesis of IBS is considered to be multifactorial [3,14], including both central and peripheral mechanisms. Some of the mechanisms involved are: altered gastrointestinal motility [15], visceral hypersensitivity [16], altered neuro-endocrine-immune pathways [17].

Intestinal inflammation has been proposed as a potential etiopathogenetic pathway for IBS since 1960 when increased number of mastocytes in the muscular external layer of cecal and terminal colic biopsies was evidenced [18,19]. Low grade inflammation might be evidenced in IBS patients (pts.) [20] including those with postinfectious IBS [21].

Mast cells and their mediators that were found in IBS patients have been shown to act on the enteric nervous system, a mechanism that might contribute to IBS symptoms [22-24].

Besides mastocytes, in the intestinal mucosa of IBS patients also an elevated number of imunocytes: CD4+ and CD8+ lymphocites, T lymphocites might be found, in comparison to controls [25].

Data in literature emphasize the role of inflammation, even subclinical, in the pathogenesis of IBS [26].

Pro and anti-inflammatory cytokines are important modulators of inflammatory responses and might play different roles in intestinal inflammation [27].

Literature provides some data regarding cytokines genes expression and protein secretion determined from mucosa of IBS patients colonic biopsies [28].

There is one meta-analysis which evaluated potential biomarkers for IBS from sera or colonic biopsies also in IBS patients [29]. This meta-analysis showed an imbalance of the two investigated cytokines (proinflammatory TNF-α and anti-inflammatory IL-10) [29].

While the diagnosis of IBS relies on clinical complaints, a quest for serological markers for diagnosis is undertaken.

A biomarker can be determined objectively - by quantification (measured). An ideal biomarker should fulfill following criteria: high Se and Sp, easy use, reproducible, low inter-observer variability, affordable and acceptable for and by the patient [30]. For all these reasons, there is growing interest for these biomarkers.

Many biomarkers have been proposed for the diagnosis and/or evaluation of the therapeutic effects of different pharmacological drug classes used in IBS [31]. We aimed at reviewing serum biomarkers only, suggested for IBS diagnosis, and critically analyzing their diagnostic value, and as secondary purpose we aimed at analyzing the specificity and sensitivity – where determined – for some of the markers proposed as potential markers for IBS.

Materials and methods

We performed a literature search in order to find out the studies dedicated to biomarkers in IBS. Our initial search on MEDLINE and Cochrane databases yielded 268 results using “IBS, biomarkers” strategy (May 2015).

Including criteria were: original studies that assessed serological markers for diagnosing IBS or differentiating subtypes of IBS. Excluding criteria were biomarkers assessed in IBS patients but used in order to exclude other conditions or diseases in these patients, or markers that were not addressed to IBS, or papers that assessed only fecal biomarkers, or histological or surrogate - indirect biomarkers.

In a more detailed search using “irritable bowel syndrome, serum biomarkers” strategy 58 results were retrieved. These were all checked and potentially relevant studies were found. Of the results of the online search, a number of papers were not included for various reasons (see Figure 1): some were not related to the subject (26), others were not appropriate for the subject (19) because were addressed to inflammatory bowel disorders (IBD), in others fecal markers were the subject of the study, six were reviews, for others full text was not accessible (1). We identified six studies that corresponded to our search criteria and purpose. Full text (where applicable) was read and reference lists were checked in order to find other pertinent data. Out of nine papers retrieved by this strategy, eight more papers were eventually included.

Of these studies some addressed serum but also fecal biomarkers, and some included also patients with IBD, but due to the paucity of the results applicable only to serum biomarkers, the ones considered suitable were also included.

There is one study that evaluated biomarkers specific to certain pathways [32].

After reanalyzing our obtained data and after the exclusion of another paper because it was not appropriate to our purpose, we eventually analyzed the 14 studies (see Table I). We further searched only the serum biomarkers investigated in these studies (see Table II).

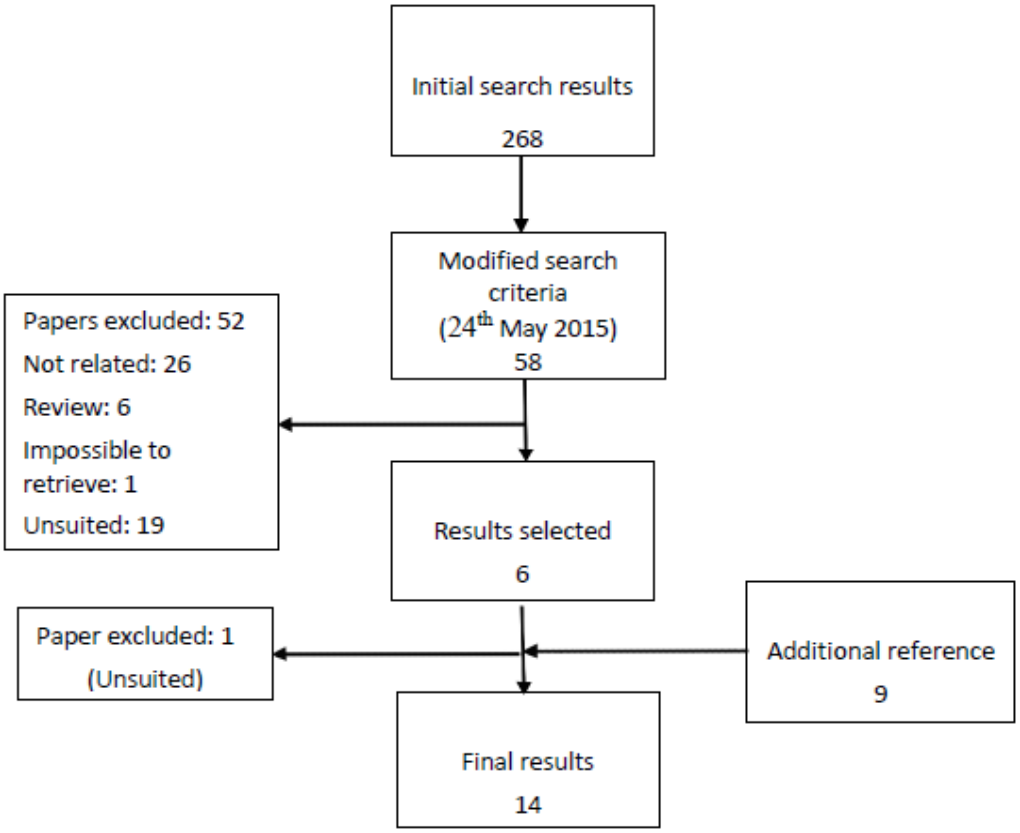

Figure 1. Flow chart of the selection process, results obtained from the literature search for those studies related to our subject.

Search results

The first study that assessed specific biomarkers for IBS [14] targeted multiple pathways, therefore a combination of these markers were proposed.

Out of the 140 biomarkers that were proposed, a combination of 10 biomarkers were found to have a positive predictive value of 81%, 64% negative predictive value and 50% IBS prevalence in the validation cohort [14]. The 10 biomarkers are: interleukin-1ß (Il-1ß), growth-related oncogene-a (GRO-a), brain-derived neurotrophic factor (BDNF), anti-saccharomyces cerevisiae antibody (ASCA IgA), antibody against CBir1 (Anti-CBir1), antihuman tissue transglutaminase (tTG), tumour necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK), antineutrophil cytoplasmic antibody (ANCA), tissue inhibitor of metalloproteinase-1 (TIMP-1), neutrophil gelatinase-associated lipocalin (NGAL) [14].

Another paper published in 2014 studied a set of markers – a combination of markers, serological and gene expression markers [33]. Beside the 10 markers proposed by Lembo et al. other 10 markers (histamine, prostaglandin E2, tryptase, serotonin, P substance, Il-12, Il-6, Il-8, Il-10, TNF-α) and 14 genes were added [33]. Results show that the proposed combination might differentiate IBS patients from healthy subjects with a Se of 83% and a Sp of 86%. By adding to the 34 markers four psychological markers (anxiety, depression, stress and non-gastrointestinal somatic symptoms) the AUC has raised from 0.93 to 0.94 [33].

One study assessed immune (cellular and humoral) responses in functional gastrointestinal diseases (FGID) compared to healthy subjects [34]. In this study a subset of FGID – IBS cases - were included. Il-5, Il-10, Il-13, IFN-γ, TNF-α, Il-10 and Il-12 and these stimulated levels were determined in FGID and in healthy volunteers. Results show that stimulated lymphocyte expression of Il-5 and Il-13 were higher in IBS ($P<0.05$) compared to controls, and stimulated monocytic Il-12 and lymphocytic Il-10 expression were reduced in IBS [34].

A study conducted in Mexico determined a number of cytokines in a group of volunteers subdivided into IBS patients, those that fulfilled Rome II criteria and healthy volunteers [35]. Il-10 levels were found to be significantly lower in IBS patients than controls ($P<0.010$), while TNF-α values were higher ($P=0.010$) [35].

Data from previous studies showed that overall, patients with IBS showed significantly ($P<0.017$) higher

Table I. Studies included, study type, markers investigated, subjects included, results.

First author, year, reference number	Study type	Total no. mk	No. of subjects	Results
1. Lembo et al., 2009, [14]	Prospective, case-control	10	1721 (876 IBS, 398 IBD, 155 FGID, 57 CD, 235 healthy sb.)	Se=50%, Sp=88%, PPV=81%, NPV=64% IBS prevalence 50%
2. Jones et al., 2014, [33]	Prospective, case-control	34	244 (168 IBS, 76 matched controls) all the 34 markers, 25 sb. - 28 markers, 25 sb. - 24 markers	Se=81 %, Sp=64 %
3. Kindt et al., 2009, [34]	Prospective, case-control	7	100 (32 healthy, volunteers, 68 FGID - 30 IBS)	Se, Sp not shown
4. Schmulson et al., 2012, [35]	Prospective, case-control	2	178 volunteers (randomized 62 IBS, 116 controls)	Se, Sp not shown
5. Liebregts et al., 2007, [36]	Prospective, case-control	6	91 (55 IBS, 36 healthy controls)	Se, Sp not shown
6. Buckley et al., 2014, [37]	Prospective, interventional	2	Humans 12 (IBS- 6, healthy volunteers 6) and animals (rats) – 36	Se, Sp not shown Student's t-tests
7. Chang et al., 2012, [38]	Prospective, case-control	24	85 (45 IBS, 41 healthy controls)	Se, Sp not shown
8. Darkoh et al., 2014, [39]	Prospective, case-control	18	100 (60 IBS, 40 healthy volunteers)	Se, Sp not shown
9. Rana et al., 2012, [27]	Prospective, case-control	3	125 (63 IBS-D, 62 healthy sb.) 45 pts were exclude out of the 108 screened	Se, Sp not shown
10. Semnani et al., 2009, [40]	Prospective, case-control	1	160 (80 IBS, 80 healthy sb.)	Se, Sp not shown
11. Mckernan et al., 2011, [41]	Prospective, case-control	15	60 (30 IBS, 30 healthy controls)	Se, Sp not shown Student's t-test (two-tailed)
12. Dinan et al., 2006, [42]	Prospective, case-control	6	151 (76 IBS, 75 controls; of which 49 IBS and 48 controls Cytokine were determined)	Se, Sp not shown Student's t-test (two-tailed)
13. Hauser et al., 2014, [43]	Prospective, pilot	2	86 (IBS)	Se, Sp not shown
14. Pimentel et al., 2015, [44]	Prospective, case-control	2	2681 (2375 IBS-D, 43 healthy sb., 121 CD, 142 IBD)	Se, Sp not shown

baseline values for TNF- α , Il-1β, and Il-6 in comparison with healthy controls [36].

A study published in 2014 evaluated Il-6 in IBS patients, Crohn's disease patients and controls [37]. Levels of Il-6 were similar in the groups analyzed.

Another study published in 2012 evaluated multiple cytokines in IBS patients and controls [38]. The results showed significant differences in the serum levels of cytokines determined (Il-1β, Il-6, Il-8, Il-10, Il-12, and TNF-α) between IBS and controls [38].

A set of serum and fecal biomarkers were determined in another study: MCP-1, MIP-1 β, TNF-α, IFN-γ, Il-1β, Il-10, Il-4, Il-13, and CXCL16 [39]. IFN-γ, Il-1β, and TNF-α were also determined and serum levels were found significantly higher in IBS patients than in healthy volunteers [39]. This is also the first study that determined two chemokines (MCP-1 and MIP) levels in IBS patients and controls. Chemokines regulate the migration and distribution of leukocytes at inflammation sites. The study published in 2014, found significant values of MCP-1 and MIP both in sera and also feces of the IBS patients comparative with the controls [39].

Il-6, TNF- α and Il-10 serum levels were measured in IBS-D patients and compared with healthy volunteers [27]. Il-6 and TNF-α serum values were higher in IBS-D patients, significantly statistic (p values $p<0.001$ and $p<0.05$ respectively) [27].

A study that aimed to evaluate serum levels of leptin and their relation with IBS found that lower levels of leptin in IBS than in controls [40].

A panel of markers (Il-2, Il-4, Il-5, Il-10, Il-12, Il-13, IFN-γ, IL-1β, Il-6, Il-8 and TNF-α) were analyzed in IBS patients and controls [41]. T-test Student (with correction for multiple comparisons) confirmed to be significantly

elevated levels of IL-6 (P=0.008), IL-8 (P=0.028) in IBS patients than in healthy controls [41].

Table II. Serum biomarkers investigated.

Study	Serum biomarkers
1. Lembo et al., 2009, [14]	Il-1β, GRO-a, BDNF, ASCA IgA, Anti-CBir1, tTG, TWEAK, ANCA, TIMP-1, NGAL
2. Jones et al., 2014, [33]	histamine, prostaglandin E2, tryptase, serotonin, P substance, Il-12, Il-6, Il-8, Il-10, TNF-α
3. Kindt et al., 2009, [34]	Il-5, Il-10, Il-13, IFN-γ, TNF-α, Il-10, Il-12
4. Schmulson et al., 2012, [35]	Il-10, TNF-α
5. Liebregts et al., 2007, [36]	TNF-α, Il-1β, Il-6
6. Buckley et al., 2014, [37]	Il-6
7. Chang et al., 2012, [38]	Il-1β, Il-6, Il-8, Il-10, Il-12, TNF-α
8. Darkoh et al., 2014, [39]	MCP-1, MIP-1 β, TNF- α, IFN-γ, Il-1 β, Il-10, Il-4, Il-13, CXCL16
9. Rana et al., 2012, [27]	Il-6, TNF-α, Il-10
10. Semnani et al., 2009, [40]	leptin
11. Mckernan et al., 2011, [41]	Il-2, Il-4, Il-5, Il-10, Il-12, Il-13, IFN-γ, IL-1 β, Il-6, Il-8, TNF-α
12. Dinan et al., 2006, [42]	Il-6, Il-8, Il-10, sIl-6 R, TNF-α
13. Hauser et al., 2014, [43]	ESR
14. Pimentel et al., 2015, [44]	anti-vinculin antibodies, anti-CdtB

Il-6, Il-8, Il-10, sIl-6 R and TNF-α were determined in IBS patients and controls [42]. Increased levels of Il-6 and Il-8 cytokines were found in IBS patients [42].

Erythrocyte sedimentation rate (ESR) was proposed in a study as a potential marker for IBS [43]. Data on 86 patients showed that there was no significant correlation between the ESR and disease activity, nor ESR and disease-specific health-related quality of life evaluated [43].

Latest literature data propose an association of antibodies: anti-vinculin antibodies and antibodies against Cytolethal distending toxin B (anti-CdtB) in differentiating a subset of IBS – diarrhea predominant IBS (IBS-D) [44]. Results show that the two biomarkers had higher values in IBS-D in comparison with controls and IBD patients (Pimentel 2015). These biomarkers might be useful in differentiating IBS-D patients from patients with inflammatory bowel disease [44].

Discussion

The growing interest for biomarkers has led to a great number of research studies in this field, some of them showing promising new insights into the potential future IBS diagnosis or its exclusion diagnosis.

Though there are studies that aimed to determine a specific biomarker for IBS results are do not point to just one biomarker. A more realistic approach seems to be a combination or a "panel" of biomarkers that target multiple pathways.

A secondary purpose was to analyze the Sp and Se – where determined – for some of the markers proposed as potential markers for IBS.

A higher Se of 81% was found using a combination of 34 markers but with lower specificity (64%) found by Jones et al. [34]. By contrast, the highest specificity was found by Lembo et al., but the Se was only 50% [14]. Limitations in our analysis was due to the fact that there were no data regarding Se and Sp in the other papers studied [27,34–44].

Of the 14 papers, 12 were case-control studies. All studies used Rome criteria to diagnose IBS.

Most of the studies here analyzedevaluated not only serum but also colonic biopsies (various analyses) and compared multiple variables, though only serological diagnosis accomplishes the criteria for a marker.

The latest study seems to bring encouraging evidence to support further research regarding serum biomarkers for IBS diagnosis [44], taking into account that recently the serum biomarkers proposed by Pimentel et al. are already available on the market - a new blood test that identifies the presence of the two antibodies (anti-CdtB and anti-vinculin) [45].

Conclusions

Until now there is not an accepted panel of serum biomarkers shown to be accurate for the diagnosis of IBS. Though there are a number of studies that evaluated a number of potential biomarkers, there are limited data to favor of one biomarker or a combination of maximum three serum biomarkers. Most of the studies have several limitations: in size, due to difficulty in obtaining funds or in enrolling patients, but also methodological regarding the reproducibility of some of the proposed biomarkers. For now, in our opinion, symptom-based criteria are still to be used for the diagnosis of IBS, in attendance for new research data.

Aknowledgment

This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/138776.

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