World Journal of Gastrointestinal Pathophysiology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v7.i1.27

World J Gastrointest Pathophysiol 2016 February 15; 7(1): 27-37
ISSN 2150-5330 (online)

Current application of proteomics in biomarker discovery for inflammatory bowel disease

Patrick PY Chan, Valerie C Wasinger, Rupert W Leong

Patrick PY Chan, Valerie C Wasinger, Bioanalytical Mass Spectrometry Facility, Mark Wainwright Analytical Centre, the University of NSW Australia, Kensington, NSW 2052, Australia

Rupert W Leong, Gastroenterology and Liver Services, Concord Repatriation General Hospital, Concord, NSW 2137, Australia

Author contributions: Chan PPY wrote the paper; Wasinger VC and Leong RW reviewed and edited the draft for submission.

Conflict-of-interest statement: No potential conflicts of interest. No financial support.

Correspondence to: Rupert W Leong, MD, MBBS, Gastroenterology and Liver Services, Concord Repatriation General Hospital, Hospital Road, Concord, NSW 2137, Australia. rupert.leong@sswahs.nsw.gov.au
Telephone: +61-2-97676111
Fax: +61-2-97676767

Received: August 31, 2015
Peer-review started: September 2, 2015
First decision: September 29, 2015
Revised: November 13, 2015
Accepted: December 29, 2015
Article in press: January 4, 2016
Published online: February 15, 2016

Abstract

Recently, the field of proteomics has rapidly expanded in its application towards clinical research with objectives ranging from elucidating disease pathogenesis to discovering clinical biomarkers. As proteins govern and/or reflect underlying cellular processes, the study of proteomics provides an attractive avenue for research as it allows for the rapid identification of protein profiles in a biological sample. Inflammatory bowel disease (IBD) encompasses several heterogeneous and chronic conditions of the gastrointestinal tract. Proteomic technology provides a powerful means of addressing major challenges in IBD today, especially for identifying biomarkers to improve its diagnosis and management. This review will examine the current state of IBD proteomics research and its use in biomarker research. Furthermore, we also discuss the challenges of translating proteomic research into clinically relevant tools. The potential application of this growing field is enormous and is likely to provide significant insights towards improving our future understanding and management of IBD.

Key words: Proteomics; Inflammatory bowel disease; Biomarkers; Molecular diagnostic techniques; Mass spectrometry

Core tip: Proteomic methods provide a powerful tool that can be applied to the discovery of disease markers, allowing for rapid identification and quantification of proteins. Inflammatory bowel disease (IBD) currently faces many challenges, ranging from the elucidation of its pathophysiology to the accurate diagnosis in patients. Proteomics has been widely employed in many disease in the search of biomarkers, particularly cancer proteins. It has great potential to improve both our understanding and clinical management of IBD. Our review summarises the current application of proteomics to IBD and discusses challenges relating to translation into clinical practice.

Chan PPY, Wasinger VC, Leong RW. Current application of proteomics in biomarker discovery for inflammatory bowel disease. *World J Gastrointest Pathophysiol* 2016; 7(1): 27-37 Available from: URL: http://www.wjgnet.com/2150-5330/full/v7/i1/27.htm DOI: http://dx.doi.org/10.4291/wjgp.v7.i1.27

INTRODUCTION

Inflammatory bowel disease (IBD) encompasses a group of conditions characterised by chronic gastrointestinal inflammation, with the two major subtypes being Crohn's disease (CD) and ulcerative colitis (UC). Differentiating between subtypes of IBD sometimes has a degree of uncertainty due to overlapping clinical and pathological features[1]. Despite clinical evaluation, radiological, endoscopic and histopathological testing by expert physicians, up to 20% of IBD cases are classified as "indeterminate colitis" or "IBD undifferentiated"[2,3]. However, accurate classification of IBD is essential as response to medication, surgical indications and prognosis can vary between UC and CD[4]. The field of proteomics is a rapidly expanding area of research that has been employed in many diseases such as cancer[5,6], exploring everything from understanding disease pathways to discovering diagnostic markers[7-9]. This review examines the current state of biomarkers in IBD, with particular reference to the application of proteomics.

CURRENT BIOMARKERS IN IBD

Biomarkers are measureable substances that can objectively evaluate either physiological processes or therapeutic outcomes[10] and could potentially play a pivotal role in IBD as cheap and non-invasive alternatives to endoscopy[11]. Different biomarkers could be beneficial across all aspects of IBD (illustrated in Figure 1)[12]. The major commercially available biomarkers are summarised below based on their application in Table 1. Whilst some of these biomarkers demonstrate high diagnostic accuracy, they are currently unable to replace endoscopy entirely and limited only to being adjuncts[11,13]. Therefore, there is a prevailing need for the development of additional non-invasive biomarkers that are sufficiently sensitive and specific in the diagnosis and prognosis of IBD.

PROTEOMICS

The term "proteome" was initially defined as the total protein complement encoded by a given genome[14] but now also encompasses any isoforms, post-translational modifications, interactions and effectively anything "post-genomic"[15]. The study of proteomics involves large scale detection, identification and characterisation of proteins, making it highly promising for biomarker discovery across many diseases[16]. The most common method applied is a combination of two-dimensional electrophoresis (2-DE) and mass-spectrometry. 2-DE provides a powerful tool isolating proteins that differ in abundance between cases and controls[17]. Mass spectrometry can then identify proteins utilising techniques such as "surface enhanced laser desorption/ionisation time-of-flight" (SELDI-TOF) or "matrix-assisted laser desorption/ionisation time-time-of-flight" (MALDI-TOF). Both these technique involve fragmentation of proteins into peptides, determining their mass-to-charge ratio based on their "time-of-flight" within an electric field and comparing their peptide mass signatures to a database of known proteins to identify the original protein.

Although mass spectrometry is not inherently quantitative, many methods have been developed to achieve accurate quantitative data[17,18]. The crux of selecting candidate biomarkers in proteomic studies rely detecting differences in abundances between cases and controls; therefore quantitative proteomics is an essential aspect. Multiple reaction monitoring (MRM) is a quantitative technique that achieves absolute quantitation and has a relatively high sensitivity when detecting peptides in low abundance, suiting it towards application in proteomic biomarker studies[19].

APPLYING PROTEOMICS TO IBD

The process leading up to clinical implementation of a novel proteomic biomarker can be divided into three major stages of a pipeline: Discovery, verification and validation, which all vary in both aim and study design (Figure 2)[20]. At present, the application of proteomics in IBD (and many other diseases) remains largely in its infancy in the initial discovery phase. This stage involves the rapid analysis of entire protein profiles within a target sample (*e.g.,* plasma from an IBD patient), to screen for proteins that have relative differences in abundance compared to control samples[21]. The main disadvantage however, is that these discovery experiments do not provide absolute quantification and are labour intensive (and therefore typically have small sample sizes). The "verification" and "validation" stages addresses these issues by confirming the presence of and quantifying candidate markers in larger populations to assess their value in clinical usage.

Biomarker discovery studies

Proteomic studies involving IBD biomarkers have been divided into those relating to diagnosis and those pertaining to disease characteristics.

The most common approach towards biomarker discovery in proteomics involves assessing relative differences in proteins between cases and controls, for example, identifying which protein is differentially expressed between IBD patients and healthy controls. Furthermore, with the common objective of developing a clinically relevant assay, many groups have analysed

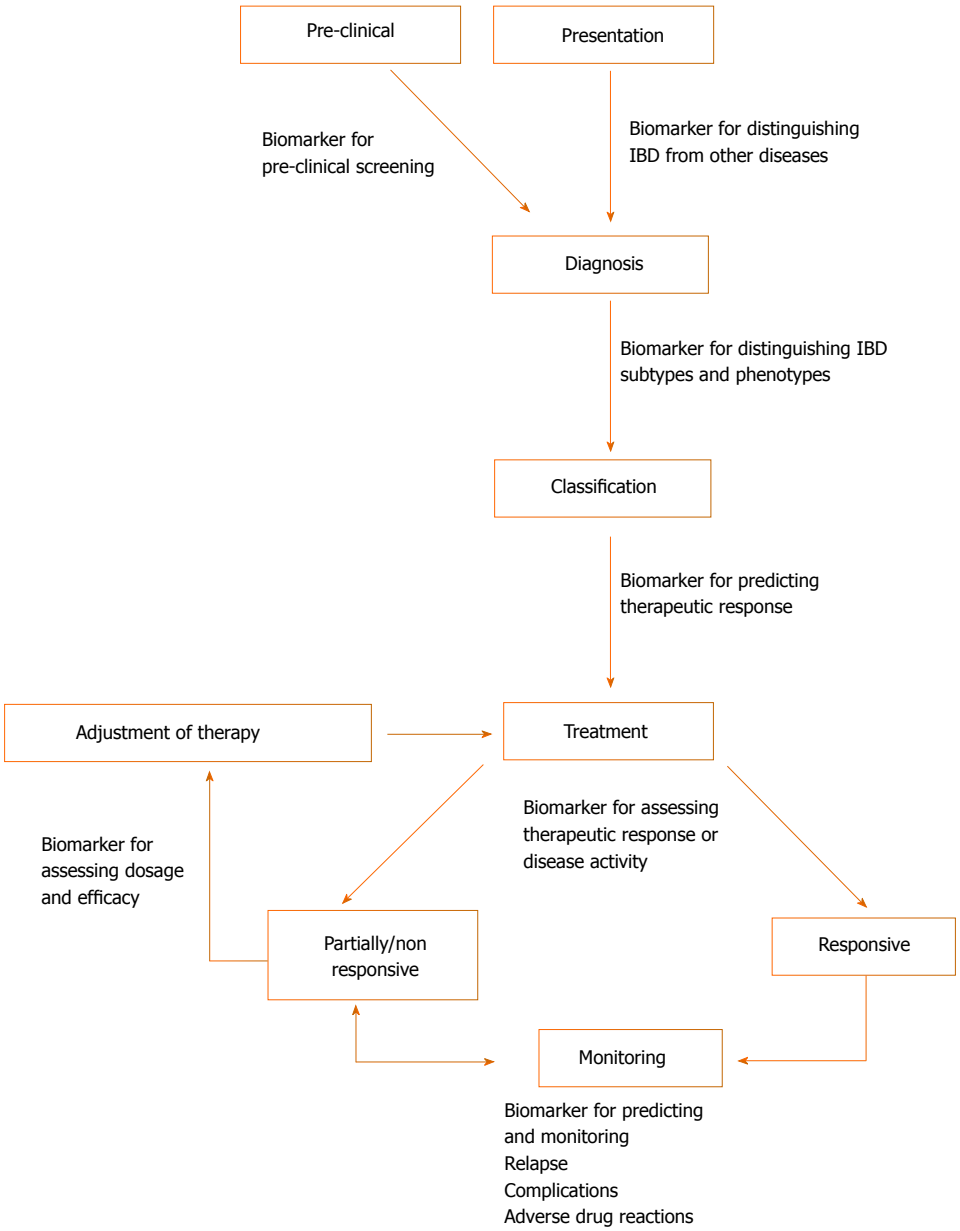

Figure 1 Potential application of biomarkers in inflammatory bowel disease in different stages of clinical management. When presenting clinically, one important use of biomarkers could be in the diagnosis of IBD, as well as differentiating subtypes (*e.g.*, UC *vs* CD) and phenotypes (*e.g.*, fistulising). Whilst not currently part of management, preclinical screening for IBD may be a possibility. Biomarkers can also be used to predict response to therapy and objectively measure therapeutic response and disease severity. Due to the relapsing and remitting course of IBD, monitoring is necessary for assessing relapse, adverse outcomes and complications (*e.g.*, strictures, fistulas and colorectal cancer). Most of these aspects necessitate endoscopic procedures and would benefit from biomarker substitutes. CD: Crohn's disease; UC: Ulcerative colitis; IBD: Inflammatory bowel disease.

plasma/serum for candidate markers (summarised in Table 2).

In 2007, Meuwis *et al*[22] reported a proteomic profile detected with SELDI-TOF MS that could discriminate active UC and CD with a high sensitivity and specificity, performing similarly or better than current ANCA and ASCA serology. From the protein spectra detected, platelet factor 4, myeloid related protein 8, fibrinopeptide A and haptoglobin α2 were considered diagnostically important. Kanmura *et al*[23] examined UC serum samples using SELDI-TOF MS and identified that human neutrophil peptide (HNP) 1-3 was differentially expressed. HNP 1-3 was confirmed by ELISA to differentiate active UC from inactive UC, all CD cases and controls, but not colorectal cancer. Similar studies using variants of mass spectrometry have yielded similar results where protein profiles could accurately distinguish between selected UC and CD cases[24-27]. A recent study by Vaiopoulou *et al*[28] sought to investigate pediatric biomarkers for CD by comparing the proteomic profile between adult and pediatric CD patients. 3 proteins (ceruloplasmin, clusterin and apolipoprotein B-100) were shown to be significantly different between the two cohorts. Whilst the plasma proteome is the most comprehensive collection of proteins, potential biomarkers are more difficult to detect as they exist in

Table 1 Current biomarkers and their utility in inflammatory bowel disease management[12]

Application	Biomarker	Utility
Diagnosis of IBD	Fecal calprotectin[69]	Sensitivity: 89%-98%, specificity: 81%-91%
	Fecal lactoferrin[70]	Sensitivity: 80%, specificity: 82%
	Fecal 100A12[71] (differentiating from IBS)	Sensitivity: 86%, specificity: 96%
	CRP[72-74]	Sensitivity: Approximately equal 100% in CD, approximately equal 50% in UC poor specificity
Distinguishing UC and CD	ASCA[75]	Sensitivity: 40%-50%, specificity: > 90% in CD
	pANCA[75]	Sensitivity: 57%, specificity: 92%
	Escherichia coli antibodies (Anti-OmpC, Anti-I2, Anti-CBir1)[76]	Sensitivity: 18%-55%, specificity: 76%-93%[76]
Marker of disease activity	Fecal lactoferrin[77,78]	Sensitivity: 66%-80% Specificity: 60%-100%
	Fecal calprotectin[77,78]	Sensitivity: 70%-100% Specificity: 44%-100%
	CRP[78]	Sensitivity: 48% Specificity: 91%
Assessing mucosal healing	Fecal calprotectin	Several studies demonstrate significant reduction in biomarker in the presence of mucosal healing with treatment
Predicting disease course	Fecal lactoferrin[77]	May be associated with complications including; structuring or fistulising disease, and small bowel disease
	ASCA pANCA Anti-I2, Anti-OmpC[12]	pANCA may predict aggressive UC and pouchitis following surgery[79]
Predicting Relapse within 12 mo	Fecal calprotectin[80,81]	Sensitivity: 69%-90% Specificity: 69%-82% Positive predictive value: 81%/87% (UC/CD) Negative predictive value: 90%/43% (UC/CD)
	Fecal lactoferrin[81]	Sensitivity: 62% Specificity: 65%
Predicting therapeutic response	pANCA[82]	Conflicting reports, possible lower response rate to infliximab in patients with a positive serology
	Anti-I2[83]	94% responded to fecal diversion

CD: Crohn's disease; UC: Ulcerative colitis; IBD: Inflammatory bowel disease; ASCA: Anti-Saccharomyces cerevisiae; pANCA: Perinuclear antineutrophil cytoplasmic antibodies; CRP: C-Reactive protein; PF4: Platelet factor 4.

significantly lower concentrations compared to other proteins such as albumin[20,29,30]. The alternative approach that has also become popular involves sampling "proximal fluid", as any biological material directly sampled from the site of disease is likely to contain greater concentrations of potential biomarkers relative to plasma[20,30-32]. Employing a similar rationale, direct sampling of diseased tissue in IBD (a far simpler task compared to other diseases due to routine endoscopic biopsies) has been utilised for proteomic experiments (Table 2). Shkoda *et al*[33] reported the first proteomic study of intestinal tissue, identifying nine statistically significant proteins delineating inflamed IBD tissue from non-inflamed controls. Furthermore, 40 proteins were further detected between inflamed and non-inflamed UC tissue, although only two pairs of patient samples were analysed. Similarly, Han *et al*[34] identified a large number of differentially expressed proteins (37 relevant for CD, 27 for UC and 11 associated with general IBD) that were seen as candidate biomarkers. M'koma and colleagues conducted two studies that identified spectral peaks representing unknown protein profiles and reported being able to accurately distinguish between the UC and CD using an algorithm[35,36]. These tissue findings however are likely to require validation in plasma samples as the aim involves develop a clinical assay such as a blood test.

A similar demand for objective biomarkers exists across all aspects of IBD patient management, as such these markers have been investigated in a number of studies (summarised in Table 3). Han *et al*[34] identified 16 additional proteins that were expressed differently between active and inactive CD. Kanmura *et al*[23] associated a higher level of HNP 1-3 with a positive response following induction of corticosteroid therapy, whilst non-responders had lower HNP 1-3 levels. Meuwis *et al*[37] published a second report which identified a serum protein profile which correlated with infliximab response. Gazouli *et al*[38] performed a similar study using MALDI-TOF MS, identifying 15 proteins that were differentially expressed amongst patients that responded differently to infliximab. They were however, unable to confirm the findings by Meuwis *et al*[37].

Most recently, Wasinger *et al*[39] reported a panel of protein markers that were progressed into the "validation" stage using MRM. Two proteins [phosphoprotein 24 (SPP24) and α-1 microglobulin], were reported to be able to differentiate IBD patients and health controls whilst guanylin and secretogranin-1 differentiated UC and CD. Furthermore, three of these proteins (secre-

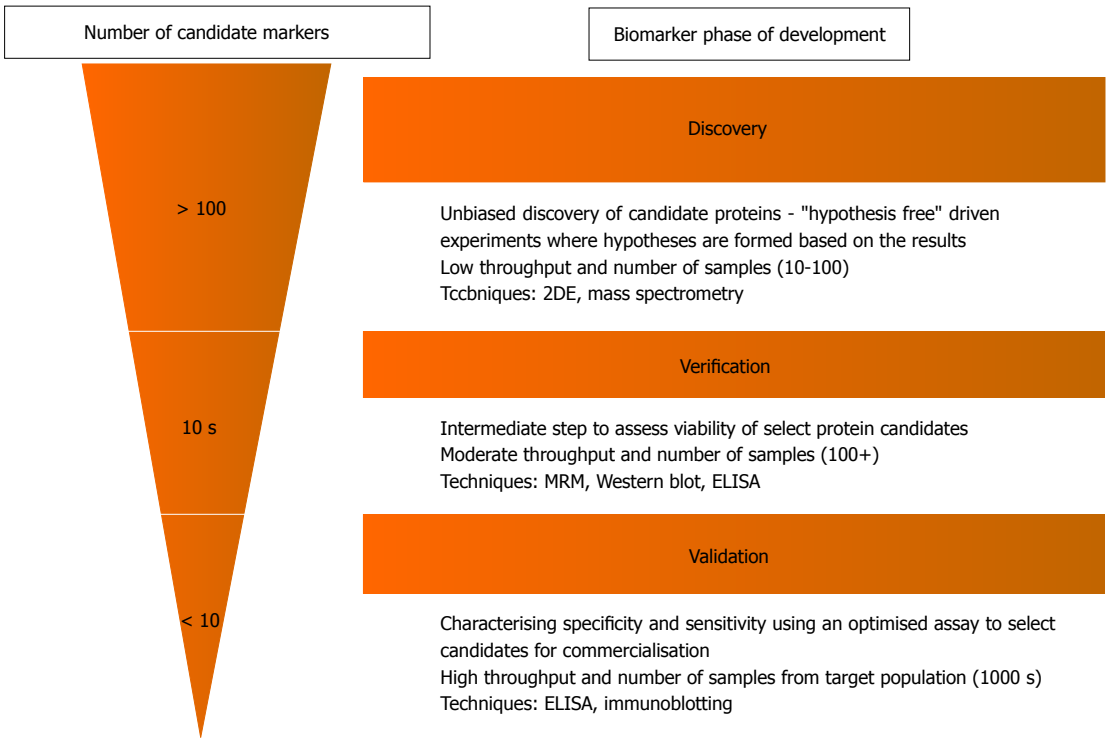

Figure 2 Biomarker "Pipeline" indicating the various stages from biomarker discovery to clinical application[20]. The number of candidate proteins (rough estimate of numbers indicated in figure) is narrowed down significantly in each step, selecting only the best candidates for further assessment and characterisation in a larger sample. The methodology also varies between the different phases. The early discovery phase uses low throughput methods such as 2-DE and mass spectrometry to screen large numbers of proteins in a low number of samples. Verification and validation require much more accurate quantitative methods as candidate proteins are narrowed down from the discovery phase and are assessed for their clinical utility in a large target population. This requires higher throughput methods such as MRM and immunoassays such as ELISA. CD: Crohn's disease; UC: Ulcerative colitis; IBD: Inflammatory bowel disease; MRM: Multiple reaction monitoring.

togranin-1, SPP24 and α-1 microglobulin), were able to distinguish between active and quiescent disease in UC and CD.

An important consideration when investigating IBD biomarkers is that a single protein may not provide the clinical utility desired, but rather a panel of markers governed by a scoring index or algorithm[40]. An existing example is the Brignola score which predicts relapse risk in asymptomatic Crohn's patients by measures erythrocyte sedimentation rate, white blood cell count, hemoglobin, albumin, alpha 2-globulin, serum iron, C-reactive protein, alpha 1-glycoprotein, and alpha 2-antitrypsin[41]. This has been hinted at in several IBD proteomic studies which differentiated UC and CD using protein profiles rather than discrete markers[22,24]. The role of multiple biomarkers is highlighted by OVA1, the first Food and Drug Administration approved proteomic panel of biomarkers, consisting of 5 markers as a multivariate index assay. This assay combines multiple variables in an algorithm that produces a single diagnostic result[42]. These markers were identified using SELDI[43] and predicts the probability of a malignancy in a woman undergoing surgery for an adnexal mass[44]. Similarly, Plevy *et al*[45] used a combined panel of 8 serological markers, 4 genetic markers and 5 inflammatory aimed at discriminating CD from UC. The utility of this test however still requires validation in a prospective cohort. Furthermore, as it was a North American cross-sectional study, this warrants additional investigation into its validity when considering factors such as stability of markers over time[45] and ethnical variations[46].

An area that has yet to be addressed relates to the influence of IBD medications on protein abundance levels. Schreiber *et al*[47] reported the possible link between high dose 5-aminosalicylic acid (5-ASA) and modulated urinary protein concentrations. However, other groups have suggested that these urinary proteins reflect renal extra-intestinal manifestations rather than 5-ASA toxicity[48,49]. Derici *et al*[50] identified an association between similar urinary proteins and disease activity in UC, however none of these have been conclusive. Similarly, Mishima *et al*[51] detected elevated plasma levels of osteopontin in IBD patients, whilst Lorenzen *et al*[52] suggested a possible association between increased urinary osteopontin expression and steroid induced nephrotic syndrome. Whilst the relation between medications and their effect on protein expression is currently unclear, there are a number of implications in the context of biomarker discovery. Depending on the clinical question, the influence of medications would require strict experimental design and patient selection to avoid confounders. Additionally, biomarkers predicting or identifying adverse drug

Table 2 Proteomic studies for discovering diagnostic inflammatory bowel disease biomarkers

Ref.	Bio-sample	Sample size	Proteomic technique	Results
Meuwis *et al*[22]	Serum	CD: 30 UC: 30 Inflammatory control: 30 Healthy controls: 30	SELDI-TOF	4 candidate proteins selected for high diagnostic value; PF4, MRP8, FIBA, Hpα 2. PF4 and Hpα 2 were also confirmed and correlated using ELISA and immunoblotting
Kanmura *et al*[23]	Blood	CD: 22 UC: 48 Colorectal Cancer: 5 Infectious colitis: 6 Healthy controls: 13	SELDI-TOF	Plasma concentrations of HNP1, 2 and 3 were significantly higher in active UC compared to inactive UC, CD and control patients
Hatsugai *et al*[24]	Blood	CD: 13 UC: 17 Healthy controls: 17	2-DE MALDI-TOF	Multivariate analysis of peripheral blood mononuclear cells protein profile 58 protein) allowed for accurate discrimination between UC and CD
Nanni *et al*[25]	Blood	Healthy controls: 48 CD: 15	Liquid chromatography quadrupole-TOF SELDI-TOF	Exopeptidase activity may distinguish CD from UC. Label free method developed could accurately distinguish synthetic spiked samples of serum
Sumramanian *et al*[26]	Serum	CD: 48 UC: 62		Protein signature of 12 mass: Charge peaks could classify CD with approximately equal 95% sensitivity/specificity 4 proteins identified as clinically useful
Nanni *et al*[27]	Serum	Healthy controls: 48 CD: 15 UC: 26	Solid-phase extraction MALDI-TOF	20 protein signals could be used to accurately classify IBD patients
Vaiopoulou *et al*[28]	Serum	CD: 24 (12 adults, 12 children)	2-DE MALDI-TOF	Clusterin was found to be overexpressed in adult CD. Ceruloplasmin and apolipoprotein B-100 was over-expressed in children
Han *et al*[34]	Intestinal biopsy	CD: 3 UC: 4 Inflammatory polyps: 2 Normal colon: 3	Liquid chromatography quadrupole-TOF	Increased in UC: TTBK2, SYNE2, SUCLG2, POSTN Up-regulated in CD: ANXA2, EPX, LAP3, RDX Up-regulated in IBD: S100A8, MPO, DEFA1B Up-regulated in CD ($P < 0.05$ AND > 2x increase): PRG2, LCP1, PSME1
M'koma *et al*[35]	Colon samples	CD: 27 UC: 24	Histology directed MALDI-TOF	5 m:z peaks were identified and cross-validated for the differentiation of UC and CD
Seeley *et al*[36]	Colon samples	CD: 26 UC: 36	Histology directed MALDI-TOF	Using a support vector machine and 25 m:z peaks, UC and CD cases were predicted in 93.3% and 60.4% respectively. A lower spectral accuracy cut-off increased sensitivity
Wasinger *et al*[39]	Serum	UC: 27 CD: 56 Controls: 14 RA controls: 12	MRM	SPP24 differentiated IBD patients from healthy controls α-1-microglobulin distinguished patients with UC in remission from healthy controls

CD: Crohn's disease; UC: Ulcerative colitis; IBD: Inflammatory bowel disease; MRM: Multiple reaction monitoring; PF4: Platelet factor 4; MRP8: Myeloid related protein 8; FIBA: Fibrinopeptide A; Hpα2: Haptoglobin α2.

reactions introduces an additional area of research as IBD often requires lifelong medical therapy.

It is clear that proteomics could play a potentially significant role towards improving the clinical management of IBD. Despite this, the value of these studies and their findings remain unknown and require validation in future studies.

FUTURE CONSIDERATIONS FOR IBD PROTEOMICS

Current limitations

Despite significant advancements in discovery-phase technologies and protocols, the rate at which new diagnostic protein assays are being introduced remains static, averaging 1.5/year[29]. The stagnation occurs at the verification stage, effectively obstructing any progression towards the development of a clinical assay[53,54]. This is clearly evident by the inundation of IBD discovery-phase experiments published in the recent decade with little to no candidate proteins undergoing validation.

One common criticism of many proteomic studies is the lack of strict experimental design, resulting in questionable results that cannot be reproduced; in particular, a small sample size and insufficient statistical power biomarker discovery[40,55]. This issue holds true across the aforementioned studies in IBD as out of 19 bio-sample based discovery experiments, 8 studies used $\leqslant$ 6 cases and controls[33,34,38,56-59]. In an effort to address this issue, Skates *et al*[55] designed a statistical model that estimates the statistical power of discovery and verification studies in tissue and plasma. Statistical power is estimated using 5 parameters: Biospecimen used (serum/tissue/proximal fluid), number of candidate proteins selected during discovery, number of cases/controls, percentage of cases where the biomarker is expressed and the difference in standard deviation between the biomarker signal in cases compared to controls. In addition, biomarkers typically occur in low abundance and may randomly exceed machine

Table 3 Proteomic studies for discovering inflammatory bowel disease management biomarkers

Ref.	Bio-sample	Sample size	Proteomic technique	Results
Disease activity biomarkers				
Han *et al*[34]	Intestinal tissue	CD: 3 UC: 4 Inflammatory Polyps: 2 Normal colon: 3	LC-QTOF	16 proteins distinguishing active/inactive CD 4 proteins distinguishing active/inactive UC
Wasinger *et al*[39]	Serum	UC: 27 CD: 56 Controls: 14 RA controls: 12	MRM	SPP24 was able to differentiate active and quiescent disease in both UC and CD
Prognostic biomarkers				
May *et al*[57]	Intestinal epithelial cells	Non-dysplastic tissue from non-progressors: 5 Non-dysplastic tissue from progressors: 5 Highly dysplastic tissue from UC progressors: 5	High-performance liquid chromatography quadrupole -TOF	155 candidate proteins were expressed differentially by > 2x between dysplastic/cancerous and non-dysplastic UC tissue. They were identified as mitochondrial, cytoskeletal, apoptotic and RAS superfamily proteins
Response to therapy biomarkers				
Meuwis *et al*[37]	Serum	Infliximab responders: 40 Infliximab non-responders: 40	SELDI-TOF	3 proteins (PF4, sCD40L and IL-6) were identified infliximab non-responders, although PF4 and sCD40L could not be confirmed or correlated with ELISA
Kanmura *et al*[23]	Blood samples	CD: 22 UC: 48 Colorectal cancer: 5 Infectious colitis: 6 Healthy controls: 13	SELDI-TOF	Plasma concentration of HNP1, 2 and 3 decreased following successful corticosteroid therapy compared to non-responders
Gazouli *et al*[38]	Serum	Infliximab responders: 6 Infliximab non-responders: 6 Infliximab partial responders: 6	2-DE, MALDI-TOF	7 proteins were increased in CD patients who did not achieve remission on infliximab. 4 were increased in patients who achieved remission. 3 proteins were lower in remission patients

CD: Crohn's disease; UC: Ulcerative colitis; IBD: Inflammatory bowel disease; MRM: Multiple reaction monitoring.

sensitivity limits, resulting in artificial differences between samples. This combined with inherent biological variations between patient samples further emphasises the importance of achieving sufficient statistical power[60,61]. It has already been noted that the concentration of candidate IBD biomarkers may be more concentrated in the intestinal tissue compared to serum, potentially reducing the chance of false discoveries. This highlights one advantage of analysing tissue samples over serum, although it is unknown which would yield better results[20]. Significant efforts have been made to address such limitations including: Recent requirements on reporting, inclusion of standards, and superior methods. These all aim to improve accuracy and reliability and will all contribute to translatable proteomic markers for disease[62,63].

Hanash *et al*[30] identified a number of confounding factors that could contribute to variations and false discoveries when identifying potential biomarkers. Patient factors include genetic variations, metabolic state, acute phase reactants and non-specific changes such as cell death. The use of model systems such as cell cultures and animal models, provides an alternative approach that could control for confounding environmental and genetic factors[20,30]. At least 66 different animal models of IBD exist, however these may not accurately reflect the true pathophysiology of IBD. Differences in methodology that could produce artificial differences include: Sample collection and preparation, improper characterisation and randomisation, and sample/statistical analysis. Zhang *et al*[64] hypothesize that many are likely site specific, suggesting that "multisite sampling" may suffice in the absence of careful prospective sample collection and randomization. This would theoretically reduce the impact of these factors and improve the likelihood of clinically useful candidate biomarkers being detected[64].

The issues highlighted above demonstrate the requirement for standardisation of protocols in large-scale proteomics experiments or at least stringent experimental design to increase the chances of discovering valid biomarkers.

Towards verification and validation

The process of validation differs significantly from the initial discovery stage as candidate proteins are tested in thousands of samples. This phase uses reliable high throughput methods (*e.g.*, immunoassays) in order to evaluate the biomarker's utility in the target population. Unfortunately this phase requires significant financial investment and produces a major barrier to validating the numerous proteins identified as "candidate biomarkers"[20,53,54]. Consequently, many potential markers are identified in the literature but require further investigation.

The gap between the inherent inaccuracies of the

discovery phase and the prohibitive cost of validation gave rise to the notion of an intermediate "verification" stage, aimed towards bridging this gap. This is achieved by quantification of selected candidate biomarkers in a larger sample that better represents the target population[55]. Ideally this is performed using reliable and established immunoassays, however, commercial antibodies are unavailable for the majority of protein targets, especially novel candidate markers. Assays must then be developed specifically for testing of the biomarker, an extremely costly endeavour when considering the large numbers of biomarkers[54]. Mass spectrometry can be further utilised here through quantitative techniques. Methods such as MRM have emerged as a viable alternatives towards cost-effectively triaging proteins of interest for further validation[20,53,54,65] and has been published for a number of biomarkers in other diseases[66-68].

CONCLUSION

The field and application of proteomics has expanded greatly in recent years and could have profound implications on the clinical diagnosis and management of IBD through the discovery of novel biomarkers. Many groups have already begun the "discovery" process and have identified many potential candidates. Although the transition into clinical validation is challenging, the tremendous potential of proteomics has garnered great interest and success in other diseases and further investigation into IBD proteomics should certainly be pursued.

REFERENCES

1 **Bernstein CN**, Fried M, Krabshuis JH, Cohen H, Eliakim R, Fedail S, Gearry R, Goh KL, Hamid S, Khan AG, LeMair AW, Malfertheiner Q, Rey JF, Sood A, Steinwurz F, Thomsen OO, Thomson A, Watermeyer G. World Gastroenterology Organization Practice Guidelines for the diagnosis and management of IBD in 2010. *Inflamm Bowel Dis* 2010; **16**: 112-124 [PMID: 19653289 DOI: 10.1002/ibd.21048]

2 **Price AB**. Overlap in the spectrum of non-specific inflammatory bowel disease--'colitis indeterminate'. *J Clin Pathol* 1978; **31**: 567-577 [PMID: 670413 DOI: 10.1136/jcp.31.6.567]

3 **Theodossi A**, Spiegelhalter DJ, Jass J, Firth J, Dixon M, Leader M, Levison DA, Lindley R, Filipe I, Price A. Observer variation and discriminatory value of biopsy features in inflammatory bowel disease. *Gut* 1994; **35**: 961-968 [PMID: 8063225 DOI: 10.1136/gut.35.7.961]

4 **Farmer M**, Petras RE, Hunt LE, Janosky JE, Galandiuk S. The importance of diagnostic accuracy in colonic inflammatory bowel disease. *Am J Gastroenterol* 2000; **95**: 3184-3188 [PMID: 11095339 DOI: 10.1016/s0002-9270(00)01992-4]

5 **Chen L**, Fang B, Giorgianni F, Gingrich JR, Beranova-Giorgianni S. Investigation of phosphoprotein signatures of archived prostate cancer tissue specimens via proteomic analysis. *Electrophoresis* 2011; **32**: 1984-1991 [PMID: 21739434 DOI: 10.1002/elps.201100101]

6 **Glen A**, Gan CS, Hamdy FC, Eaton CL, Cross SS, Catto JW, Wright PC, Rehman I. iTRAQ-facilitated proteomic analysis of human prostate cancer cells identifies proteins associated with progression. *J Proteome Res* 2008; **7**: 897-907 [PMID: 18232632 DOI: 10.1021/pr070378x]

7 **Alaiya AA**, Franzén B, Auer G, Linder S. Cancer proteomics: from identification of novel markers to creation of artifical learning models for tumor classification. *Electrophoresis* 2000; **21**: 1210-1217 [PMID: 10786893 DOI: 10.1002/(SICI)1522-2683(20000401)21:6<1210::AID-ELPS1210>3.0.CO;2-S]

8 **Celis JE**, Gromov P. Proteomics in translational cancer research: toward an integrated approach. *Cancer Cell* 2003; **3**: 9-15 [PMID: 12559171 DOI: 10.1016/S1535-6108(02)00242-8]

9 **Jungblut PR**, Zimny-Arndt U, Zeindl-Eberhart E, Stulik J, Koupilova K, Pleissner KP, Otto A, Müller EC, Sokolowska-Köhler W, Grabher G, Stöffler G. Proteomics in human disease: cancer, heart and infectious diseases. *Electrophoresis* 1999; **20**: 2100-2110 [PMID: 10451122 DOI: 10.1002/(sici)1522-2683(19990701)20:10<2100::aid-elps2100>3.0.co;2-d]

10 **Wagner JA**, Williams SA, Webster CJ. Biomarkers and surrogate end points for fit-for-purpose development and regulatory evaluation of new drugs. *Clin Pharmacol Ther* 2007; **81**: 104-107 [PMID: 17186007 DOI: 10.1038/sj.clpt.6100017]

11 **Vermeire S**, Van Assche G, Rutgeerts P. Laboratory markers in IBD: useful, magic, or unnecessary toys? *Gut* 2006; **55**: 426-431 [PMID: 16474109 DOI: 10.1136/gut.2005.069476]

12 **Lewis JD**. The utility of biomarkers in the diagnosis and therapy of inflammatory bowel disease. *Gastroenterology* 2011; **140**: 1817-1826.e2 [PMID: 21530748 DOI: 10.1053/j.gastro.2010.11.058]

13 **Langhorst J**, Elsenbruch S, Koelzer J, Rueffer A, Michalsen A, Dobos GJ. Noninvasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases: performance of fecal lactoferrin, calprotectin, and PMN-elastase, CRP, and clinical indices. *Am J Gastroenterol* 2008; **103**: 162-169 [PMID: 17916108 DOI: 10.1111/j.1572-0241.2007.01556.x]

14 **Wasinger VC**, Cordwell SJ, Cerpa-Poljak A, Yan JX, Gooley AA, Wilkins MR, Duncan MW, Harris R, Williams KL, Humphery-Smith I. Progress with gene-product mapping of the Mollicutes: Mycoplasma genitalium. *Electrophoresis* 1995; **16**: 1090-1094 [PMID: 7498152 DOI: 10.1002/elps.11501601185]

15 **Ferreri AJ**, Illerhaus G, Zucca E, Cavalli F. Flows and flaws in primary central nervous system lymphoma. *Nat Rev Clin Oncol* 2010; **7**: 193-197 [PMID: 20700952 DOI: 10.1038/nrclinonc.2010.9-c1]

16 **Vaiopoulou A**, Gazouli M, Theodoropoulos G, Zografos G. Current advantages in the application of proteomics in inflammatory bowel disease. *Dig Dis Sci* 2012; **57**: 2755-2764 [PMID: 22740064 DOI: 10.1007/s10620-012-2291-4]

17 **Ong SE**, Mann M. Mass spectrometry-based proteomics turns quantitative. *Nat Chem Biol* 2005; **1**: 252-262 [PMID: 16408053 DOI: 10.1038/nchembio736]

18 **Bachi A**, Bonaldi T. Quantitative proteomics as a new piece of the systems biology puzzle. *J Proteomics* 2008; **71**: 357-367 [PMID: 18640294 DOI: 10.1016/j.jprot.2008.07.001]

19 **Wasinger VC**, Zeng M, Yau Y. Current status and advances in quantitative proteomic mass spectrometry. *Int J Proteomics* 2013; **2013**: 180605 [PMID: 23533757 DOI: 10.1155/2013/180605]

20 **Rifai N**, Gillette MA, Carr SA. Protein biomarker discovery and validation: the long and uncertain path to clinical utility. *Nat Biotechnol* 2006; **24**: 971-983 [PMID: 16900146 DOI: 10.1038/nbt1235]

21 **Srinivas PR**, Verma M, Zhao Y, Srivastava S. Proteomics for cancer biomarker discovery. *Clin Chem* 2002; **48**: 1160-1169 [PMID: 12142368]

22 **Meuwis MA**, Fillet M, Geurts P, de Seny D, Lutteri L, Chapelle JP, Bours V, Wehenkel L, Belaiche J, Malaise M, Louis E, Merville MP. Biomarker discovery for inflammatory bowel disease, using proteomic serum profiling. *Biochem Pharmacol* 2007; **73**: 1422-1433 [PMID: 17258689 DOI: 10.1016/j.bcp.2006.12.019]

23 **Kanmura S**, Uto H, Numata M, Hashimoto S, Moriuchi A, Fujita H, Oketani M, Ido A, Kodama M, Ohi H, Tsubouchi H. Human neutrophil peptides 1-3 are useful biomarkers in patients with active ulcerative colitis. *Inflamm Bowel Dis* 2009; **15**: 909-917 [PMID:

19107772 DOI: 10.1002/ibd.20854]
24 **Hatsugai M**, Kurokawa MS, Kouro T, Nagai K, Arito M, Masuko K, Suematsu N, Okamoto K, Itoh F, Kato T. Protein profiles of peripheral blood mononuclear cells are useful for differential diagnosis of ulcerative colitis and Crohn's disease. *J Gastroenterol* 2010; **45**: 488-500 [PMID: 20049485]
25 **Nanni P**, Levander F, Roda G, Caponi A, James P, Roda A. A label-free nano-liquid chromatography-mass spectrometry approach for quantitative serum peptidomics in Crohn's disease patients. *J Chromatogr B Analyt Technol Biomed Life Sci* 2009; **877**: 3127-3136 [PMID: 19683480 DOI: 10.1016/j.jchromb.2009.08.003]
26 **Subramanian V**, Subramanian D, Pollok RC. S1182 Serum Protein Signatures Determined By Mass Spectrometry (SELDI-ToF) Accurately Distinguishes Crohn's Disease (CD) from Ulcerative Colitis (UC). *Gastroenterology* 2008; **134**: A-196 [DOI: 10.1016/S0016-5085(08)60904-X]
27 **Nanni P**, Parisi D, Roda G, Casale M, Belluzzi A, Roda E, Mayer L, Roda A. Serum protein profiling in patients with inflammatory bowel diseases using selective solid-phase bulk extraction, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and chemometric data analysis. *Rapid Commun Mass Spectrom* 2007; **21**: 4142-4148 [PMID: 18022963 DOI: 10.1002/rcm.3323]
28 **Vaiopoulou A**, Gazouli M, Papadopoulou A, Anagnostopoulos AK, Karamanolis G, Theodoropoulos GE, M'Koma A, Tsangaris GT. Serum protein profiling of adults and children with Crohn disease. *J Pediatr Gastroenterol Nutr* 2015; **60**: 42-47 [PMID: 25250685 DOI: 10.1097/MPG.0000000000000579]
29 **Anderson NL**. The clinical plasma proteome: a survey of clinical assays for proteins in plasma and serum. *Clin Chem* 2010; **56**: 177-185 [PMID: 19884488 DOI: 10.1373/clinchem.2009.126706]
30 **Hanash SM**, Pitteri SJ, Faca VM. Mining the plasma proteome for cancer biomarkers. *Nature* 2008; **452**: 571-579 [PMID: 18385731 DOI: 10.1038/nature06916]
31 **Kennedy S**. Proteomic profiling from human samples: the body fluid alternative. *Toxicol Lett* 2001; **120**: 379-384 [PMID: 11323197 DOI: 10.1016/S0378-4274(01)00269-7]
32 **Teng PN**, Bateman NW, Hood BL, Conrads TP. Advances in proximal fluid proteomics for disease biomarker discovery. *J Proteome Res* 2010; **9**: 6091-6100 [PMID: 21028795 DOI: 10.1021/pr100904q]
33 **Shkoda A**, Werner T, Daniel H, Gunckel M, Rogler G, Haller D. Differential protein expression profile in the intestinal epithelium from patients with inflammatory bowel disease. *J Proteome Res* 2007; **6**: 1114-1125 [PMID: 17330946 DOI: 10.1021/pr060433m]
34 **Han NY**, Choi W, Park JM, Kim EH, Lee H, Hahm KB. Label-free quantification for discovering novel biomarkers in the diagnosis and assessment of disease activity in inflammatory bowel disease. *J Dig Dis* 2013; **14**: 166-174 [PMID: 23320753 DOI: 10.1111/1751-2980.12035]
35 **M'Koma AE**, Seeley EH, Washington MK, Schwartz DA, Muldoon RL, Herline AJ, Wise PE, Caprioli RM. Proteomic profiling of mucosal and submucosal colonic tissues yields protein signatures that differentiate the inflammatory colitides. *Inflamm Bowel Dis* 2011; **17**: 875-883 [PMID: 20806340 DOI: 10.1002/ibd.21442]
36 **Seeley EH**, Washington MK, Caprioli RM, M'Koma AE. Proteomic patterns of colonic mucosal tissues delineate Crohn's colitis and ulcerative colitis. *Proteomics Clin Appl* 2013; **7**: 541-549 [PMID: 23382084 DOI: 10.1002/prca.201200107]
37 **Meuwis MA**, Fillet M, Lutteri L, Marée R, Geurts P, de Seny D, Malaise M, Chapelle JP, Wehenkel L, Belaiche J, Merville MP, Louis E. Proteomics for prediction and characterization of response to infliximab in Crohn's disease: a pilot study. *Clin Biochem* 2008; **41**: 960-967 [PMID: 18489908 DOI: 10.1016/j.clinbiochem.2008.04.021]
38 **Gazouli M**, Anagnostopoulos AK, Papadopoulou A, Vaiopoulou A, Papamichael K, Mantzaris G, Theodoropoulos GE, Anagnou NP, Tsangaris GT. Serum protein profile of Crohn's disease treated with infliximab. *J Crohns Colitis* 2013; **7**: e461-e470 [PMID: 23562004 DOI: 10.1016/j.crohns.2013.02.021]
39 **Wasinger VC**, Yau Y, Duo X, Zeng M, Campbell B, Shin S, Luber R, Redmond D, Leong RW. Low mass blood peptides discriminative of inflammatory bowel disease (IBD) severity: A quantitative proteomic perspective. *Mol Cell Proteomics* 2016; **15**: 256-265 [PMID: 26530476 DOI: 10.1074/mcp.M115.055095]
40 **Mischak H**, Apweiler R, Banks RE, Conaway M, Coon J, Dominiczak A, Ehrich JH, Fliser D, Girolami M, Hermjakob H, Hochstrasser D, Jankowski J, Julian BA, Kolch W, Massy ZA, Neususess C, Novak J, Peter K, Rossing K, Schanstra J, Semmes OJ, Theodorescu D, Thongboonkerd V, Weissinger EM, Van Eyk JE, Yamamoto T. Clinical proteomics: A need to define the field and to begin to set adequate standards. *Proteomics Clin Appl* 2007; **1**: 148-156 [PMID: 21136664 DOI: 10.1002/prca.200600771]
41 **Brignola C**, Campieri M, Bazzocchi G, Farruggia P, Tragnone A, Lanfranchi GA. A laboratory index for predicting relapse in asymptomatic patients with Crohn's disease. *Gastroenterology* 1986; **91**: 1490-1494 [PMID: 3770373]
42 **Zhang Z**. An In Vitro Diagnostic Multivariate Index Assay (IVDMIA) for Ovarian Cancer: Harvesting the Power of Multiple Biomarkers. *Rev Obstet Gynecol* 2012; **5**: 35-41 [PMID: 22582125]
43 **Rai AJ**, Zhang Z, Rosenzweig J, Shih IeM, Pham T, Fung ET, Sokoll LJ, Chan DW. Proteomic approaches to tumor marker discovery. *Arch Pathol Lab Med* 2002; **126**: 1518-1526 [PMID: 12456215]
44 **Ueland F**, Desimone C, Seamon L, Ware R, Goodrich S, Podzielinski I, Smith A, Santoso J, Van Nagell J, Zhang Z. The OVA1 test improves the preoperative assessment of ovarian tumors. *Gynecol Oncol* 2010; **116**: S23
45 **Plevy S**, Silverberg MS, Lockton S, Stockfisch T, Croner L, Stachelski J, Brown M, Triggs C, Chuang E, Princen F, Singh S. Combined serological, genetic, and inflammatory markers differentiate non-IBD, Crohn's disease, and ulcerative colitis patients. *Inflamm Bowel Dis* 2013; **19**: 1139-1148 [PMID: 23518807 DOI: 10.1097/MIB.0b013e318280b19e]
46 **Prideaux L**, De Cruz P, Ng SC, Kamm MA. Serological antibodies in inflammatory bowel disease: a systematic review. *Inflamm Bowel Dis* 2012; **18**: 1340-1355 [PMID: 22069240 DOI: 10.1002/ibd.21903]
47 **Schreiber S**, Hämling J, Zehnter E, Howaldt S, Daerr W, Raedler A, Kruis W. Renal tubular dysfunction in patients with inflammatory bowel disease treated with aminosalicylate. *Gut* 1997; **40**: 761-766 [PMID: 9245930]
48 **Fraser JS**, Muller AF, Smith DJ, Newman DJ, Lamb EJ. Renal tubular injury is present in acute inflammatory bowel disease prior to the introduction of drug therapy. *Aliment Pharmacol Ther* 2001; **15**: 1131-1137 [PMID: 11472315 DOI: 10.1046/j.1365-2036.2001.01041.x]
49 **Mahmud N**, O'Toole D, O'Hare N, Freyne PJ, Weir DG, Kelleher D. Evaluation of renal function following treatment with 5-aminosalicylic acid derivatives in patients with ulcerative colitis. *Aliment Pharmacol Ther* 2002; **16**: 207-215 [PMID: 11860403 DOI: 10.1046/j.1365-2036.2002.01155.x]
50 **Derici U**, Tuncer C, Ebinç FA, Mutluay R, Yakaryilmaz F, Kulaksizoglu S, Soylemezoglu O, Sindel S. Does the urinary excretion of alpha1-microglobulin and albumin predict clinical disease activity in ulcerative colitis? *Adv Ther* 2008; **25**: 1342-1352 [PMID: 19002407 DOI: 10.1007/s12325-008-0109-8]
51 **Mishima R**, Takeshima F, Sawai T, Ohba K, Ohnita K, Isomoto H, Omagari K, Mizuta Y, Ozono Y, Kohno S. High plasma osteopontin levels in patients with inflammatory bowel disease. *J Clin Gastroenterol* 2007; **41**: 167-172 [PMID: 17245215 DOI: 10.1097/MCG.0b013e31802d6268]
52 **Lorenzen J**, Shah R, Biser A, Staicu SA, Niranjan T, Garcia AM, Gruenwald A, Thomas DB, Shatat IF, Supe K, Woroniecki RP, Susztak K. The role of osteopontin in the development of albuminuria. *J Am Soc Nephrol* 2008; **19**: 884-890 [PMID: 18443355 DOI: 10.1681/ASN.2007040486]
53 **Makawita S**, Diamandis EP. The bottleneck in the cancer biomarker

pipeline and protein quantification through mass spectrometry-based approaches: current strategies for candidate verification. *Clin Chem* 2010; **56**: 212-222 [PMID: 20007861 DOI: 10.1373/clinchem.2009.127019]

54 **Whiteaker JR**, Lin C, Kennedy J, Hou L, Trute M, Sokal I, Yan P, Schoenherr RM, Zhao L, Voytovich UJ, Kelly-Spratt KS, Krasnoselsky A, Gafken PR, Hogan JM, Jones LA, Wang P, Amon L, Chodosh LA, Nelson PS, McIntosh MW, Kemp CJ, Paulovich AG. A targeted proteomics-based pipeline for verification of biomarkers in plasma. *Nat Biotechnol* 2011; **29**: 625-634 [PMID: 21685906 DOI: 10.1038/nbt.1900]

55 **Skates SJ**, Gillette MA, LaBaer J, Carr SA, Anderson L, Liebler DC, Ransohoff D, Rifai N, Kondratovich M, Težak Ž, Mansfield E, Oberg AL, Wright I, Barnes G, Gail M, Mesri M, Kinsinger CR, Rodriguez H, Boja ES. Statistical design for biospecimen cohort size in proteomics-based biomarker discovery and verification studies. *J Proteome Res* 2013; **12**: 5383-5394 [PMID: 24063748 DOI: 10.1021/pr400132j]

56 **Fogt F**, Jian B, Krieg RC, Wellmann A. Proteomic analysis of mucosal preparations from patients with ulcerative colitis. *Mol Med Rep* 2008; **1**: 51-54 [PMID: 21479377 DOI: 10.3892/mmr.1.1.51]

57 **May D**, Pan S, Crispin DA, Lai K, Bronner MP, Hogan J, Hockenbery DM, McIntosh M, Brentnall TA, Chen R. Investigating neoplastic progression of ulcerative colitis with label-free comparative proteomics. *J Proteome Res* 2011; **10**: 200-209 [PMID: 20828217 DOI: 10.1021/pr100574p]

58 **Nanni P**, Mezzanotte L, Roda G, Caponi A, Levander F, James P, Roda A. Differential proteomic analysis of HT29 Cl.16E and intestinal epithelial cells by LC ESI/QTOF mass spectrometry. *J Proteomics* 2009; **72**: 865-873 [PMID: 19168159 DOI: 10.1016/j.jprot.2008.12.010]

59 **Shkoda A**, Ruiz PA, Daniel H, Kim SC, Rogler G, Sartor RB, Haller D. Interleukin-10 blocked endoplasmic reticulum stress in intestinal epithelial cells: impact on chronic inflammation. *Gastroenterology* 2007; **132**: 190-207 [PMID: 17241871 DOI: 10.1053/j.gastro.2006.10.030]

60 **Alex P**, Gucek M, Li X. Applications of proteomics in the study of inflammatory bowel diseases: Current status and future directions with available technologies. *Inflamm Bowel Dis* 2009; **15**: 616-629 [PMID: 18844215 DOI: 10.1002/ibd.20652]

61 **Araki K**, Mikami T, Yoshida T, Kikuchi M, Sato Y, Oh-ishi M, Kodera Y, Maeda T, Okayasu I. High expression of HSP47 in ulcerative colitis-associated carcinomas: proteomic approach. *Br J Cancer* 2009; **101**: 492-497 [PMID: 19603022 DOI: 10.1038/sj.bjc.6605163]

62 **Carr SA**, Abbatiello SE, Ackermann BL, Borchers C, Domon B, Deutsch EW, Grant RP, Hoofnagle AN, Hüttenhain R, Koomen JM, Liebler DC, Liu T, MacLean B, Mani DR, Mansfield E, Neubert H, Paulovich AG, Reiter L, Vitek O, Aebersold R, Anderson L, Bethem R, Blonder J, Boja E, Botelho J, Boyne M, Bradshaw RA, Burlingame AL, Chan D, Keshishian H, Kuhn E, Kinsinger C, Lee JS, Lee SW, Moritz R, Oses-Prieto J, Rifai N, Ritchie J, Rodriguez H, Srinivas PR, Townsend RR, Van Eyk J, Whiteley G, Wiita A, Weintraub S. Targeted peptide measurements in biology and medicine: best practices for mass spectrometry-based assay development using a fit-for-purpose approach. *Mol Cell Proteomics* 2014; **13**: 907-917 [PMID: 24443746 DOI: 10.1074/mcp.M113.036095]

63 **Percy AJ**, Chambers AG, Smith DS, Borchers CH. Standardized protocols for quality control of MRM-based plasma proteomic workflows. *J Proteome Res* 2013; **12**: 222-233 [PMID: 23245390 DOI: 10.1021/pr300893w]

64 **Zhang Z**, Chan DW. The road from discovery to clinical diagnostics: lessons learned from the first FDA-cleared in vitro diagnostic multivariate index assay of proteomic biomarkers. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 2995-2999 [PMID: 20962299 DOI: 10.1158/1055-9965]

65 **Meng Z**, Veenstra TD. Targeted mass spectrometry approaches for protein biomarker verification. *J Proteomics* 2011; **74**: 2650-2659 [PMID: 21540133 DOI: 10.1016/j.jprot.2011.04.011]

66 **Cho CK**, Drabovich AP, Batruch I, Diamandis EP. Verification of a biomarker discovery approach for detection of Down syndrome in amniotic fluid via multiplex selected reaction monitoring (SRM) assay. *J Proteomics* 2011; **74**: 2052-2059 [PMID: 21624510 DOI: 10.1016/j.jprot.2011.05.025]

67 **Drabovich AP**, Jarvi K, Diamandis EP. Verification of male infertility biomarkers in seminal plasma by multiplex selected reaction monitoring assay. *Mol Cell Proteomics* 2011; **10**: M110.004127 [PMID: 21933954 DOI: 10.1074/mcp.M110.004127]

68 **Kim K**, Kim SJ, Han D, Jin J, Yu J, Park KS, Yu HG, Kim Y. Verification of multimarkers for detection of early stage diabetic retinopathy using multiple reaction monitoring. *J Proteome Res* 2013; **12**: 1078-1089 [PMID: 23368427 DOI: 10.1021/pr3012073]

69 **von Roon AC**, Karamountzos L, Purkayastha S, Reese GE, Darzi AW, Teare JP, Paraskeva P, Tekkis PP. Diagnostic precision of fecal calprotectin for inflammatory bowel disease and colorectal malignancy. *Am J Gastroenterol* 2007; **102**: 803-813 [PMID: 17324124 DOI: 10.1111/j.1572-0241.2007.01126.x]

70 **Gisbert JP**, McNicholl AG, Gomollon F. Questions and answers on the role of fecal lactoferrin as a biological marker in inflammatory bowel disease. *Inflamm Bowel Dis* 2009; **15**: 1746-1754 [PMID: 19363798 DOI: 10.1002/ibd.20920]

71 **Kaiser T**, Langhorst J, Wittkowski H, Becker K, Friedrich AW, Rueffer A, Dobos GJ, Roth J, Foell D. Faecal S100A12 as a non-invasive marker distinguishing inflammatory bowel disease from irritable bowel syndrome. *Gut* 2007; **56**: 1706-1713 [PMID: 17675327 DOI: 10.1136/gut.2006.113431]

72 **Beattie RM**, Walker-Smith JA, Murch SH. Indications for investigation of chronic gastrointestinal symptoms. *Arch Dis Child* 1995; **73**: 354-355 [PMID: 7492204 DOI: 10.1136/adc.73.4.354]

73 **Poullis AP**, Zar S, Sundaram KK, Moodie SJ, Risley P, Theodossi A, Mendall MA. A new, highly sensitive assay for C-reactive protein can aid the differentiation of inflammatory bowel disorders from constipation- and diarrhoea-predominant functional bowel disorders. *Eur J Gastroenterol Hepatol* 2002; **14**: 409-412 [PMID: 11943955 DOI: 10.1097/00042737-200204000-00013]

74 **Shine B**, Berghouse L, Jones JE, Landon J. C-reactive protein as an aid in the differentiation of functional and inflammatory bowel disorders. *Clin Chim Acta* 1985; **148**: 105-109 [PMID: 3995779 DOI: 10.1016/0009-8981(85)90219-0]

75 **Ruemmele FM**, Targan SR, Levy G, Dubinsky M, Braun J, Seidman EG. Diagnostic accuracy of serological assays in pediatric inflammatory bowel disease. *Gastroenterology* 1998; **115**: 822-829 [PMID: 9753483 DOI: 10.1016/S0016-5085(98)70252-5]

76 **Peyrin-Biroulet L**, Standaert-Vitse A, Branche J, Chamaillard M. IBD serological panels: facts and perspectives. *Inflamm Bowel Dis* 2007; **13**: 1561-1566 [PMID: 17636565 DOI: 10.1002/ibd.20226]

77 **Sipponen T**, Björkesten CG, Färkkilä M, Nuutinen H, Savilahti E, Kolho KL. Faecal calprotectin and lactoferrin are reliable surrogate markers of endoscopic response during Crohn's disease treatment. *Scand J Gastroenterol* 2010; **45**: 325-331 [PMID: 20034360 DOI: 10.3109/00365520903483650]

78 **Sipponen T**, Savilahti E, Kolho KL, Nuutinen H, Turunen U, Färkkilä M. Crohn's disease activity assessed by fecal calprotectin and lactoferrin: correlation with Crohn's disease activity index and endoscopic findings. *Inflamm Bowel Dis* 2008; **14**: 40-46 [PMID: 18022866 DOI: 10.1002/ibd.20312]

79 **Vecchi M**, Gionchetti P, Bianchi MB, Belluzzi A, Meucci G, Campieri M, de Franchis R. p-ANCA and development of pouchitis in ulcerative colitis patients after proctocolectomy and ileoanal pouch anastomosis. *Lancet* 1994; **344**: 886-887 [PMID: 7916418 DOI: 10.1016/S0140-6736(94)92859-2]

80 **Costa F**, Mumolo MG, Ceccarelli L, Bellini M, Romano MR, Sterpi C, Ricchiuti A, Marchi S, Bottai M. Calprotectin is a stronger predictive marker of relapse in ulcerative colitis than in Crohn's disease. *Gut* 2005; **54**: 364-368 [PMID: 15710984 DOI: 10.1136/gut.2004.043406]

81 **Gisbert JP**, Bermejo F, Pérez-Calle JL, Taxonera C, Vera I,

McNicholl AG, Algaba A, López P, López-Palacios N, Calvo M, González-Lama Y, Carneros JA, Velasco M, Maté J. Fecal calprotectin and lactoferrin for the prediction of inflammatory bowel disease relapse. *Inflamm Bowel Dis* 2009; **15**: 1190-1198 [PMID: 19291780 DOI: 10.1002/ibd.20933]

82 **Dubinsky MC**, Mei L, Friedman M, Dhere T, Haritunians T, Hakonarson H, Kim C, Glessner J, Targan SR, McGovern DP, Taylor KD, Rotter JI. Genome wide association (GWA) predictors of anti-TNFalpha therapeutic responsiveness in pediatric inflammatory bowel disease. *Inflamm Bowel Dis* 2010; **16**: 1357-1366 [PMID: 20014019 DOI: 10.1002/ibd.21174]

83 **Spivak J**, Landers CJ, Vasiliauskas EA, Abreu MT, Dubinsky MC, Papadakis KA, Ippoliti A, Targan SR, Fleshner PR. Antibodies to I2 predict clinical response to fecal diversion in Crohn's disease. *Inflamm Bowel Dis* 2006; **12**: 1122-1130 [PMID: 17119386 DOI: 10.1097/01.mib.0000235833.47423.d7]

P- Reviewer: Bonaz BL, Ciccone MM, Gazouli M, Maric I, Naito Y, Sorrentino D, Tandon R, Vecchi M
S- Editor: Qiu S **L- Editor**: A **E- Editor**: Jiao XK

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com