Current application of proteomics in biomarker discovery for inflammatory bowel disease

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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 PYY et al. Proteomic applications in IBD

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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.\(^7,8\)

This review examines the current state of biomarkers in IBD, with particular reference to the application of proteomics.

CURRENT BIOMARKERS IN IBD

Biomarkers are measurable substances that can objectively evaluate either physiological processes or therapeutic outcomes\(^9\) and could potentially play a pivotal role in IBD as cheap and non-invasive alternatives to endoscopy.\(^10\) Different biomarkers could be beneficial across all aspects of IBD (illustrated in Figure 1).\(^11\)

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-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...
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

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.
significantly lower concentrations compared to other proteins such as albumin\textsuperscript{[20,26,30]}. 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\textsuperscript{[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\textsuperscript{[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\textsuperscript{[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\textsuperscript{[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\textsuperscript{[35]} identified 16 additional proteins that were expressed differently between active and inactive CD. Kanamura et al\textsuperscript{[32]} 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\textsuperscript{[37]} published a second report which identified a serum protein profile which correlated with infliximab response. Gazouli et al\textsuperscript{[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\textsuperscript{[37]}.

Most recently, Wasinger et al\textsuperscript{[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-
togratin-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

Figure 2 Biomarker “Pipeline” indicating the various stages from biomarker discovery to clinical application[46]. 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.
Table 2  Proteomic studies for discovering diagnostic inflammatory bowel disease biomarkers

| Ref.          | Bio-sample | Sample size          | Proteomic technique          | Results                                                                 |
|--------------|------------|----------------------|------------------------------|-------------------------------------------------------------------------|
| Meuwis et al[27] | Serum      | CD: 30               | SELDI-TOF                    | 4 candidate proteins selected for high diagnostic value; PF4, MRPs, FIBA, Hps 2. PF4 and Hps 2 were also confirmed and correlated using ELISA and immunoblotting |
| Kan姆ura et al[28] | Blood     | CD: 22               | SELDI-TOF                    | Plasma concentrations of HNPl, 2 and 3 were significantly higher in active UC compared to inactive UC, CD and control patients |
| Hatsugai et al[24] | Blood     | CD: 13               | 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 | 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: 15               | Solid-phase extraction MALDI-TOF | 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[25]   | Serum      | UC: 62               | MALDI-TOF                    | 20 protein signals could be used to accurately classify IBD patients |
| Vaiopoulou et al[29] | Serum    | CD: 24 (12 adults, 12 children) | 2-DE                         | Clustered protein was found to be overexpressed in adult CD. Ceruloplasmin and apolipoprotein B-100 was overexpressed in children |
| Han et al[30]     | Intestinal biopsy | CD: 3, UC: 4         | Liquid chromatography quadrupole-TOF | Increased in UC: TTBK2, SYNE2, SUCGL2, POSTN Up-regulated in CD: ANX2, EPX, LAP3, RDX Up-regulated in IBD: S100A8, MPO, DEFA1B Up-regulated in CP (P < 0.05 AND > 2x increase): PRG2, LCP1, PSME1 |
| M’koma et al[31] | Colon      | CD: 27               | Histology directed MALDI-TOF | 5 m/z peaks were identified and cross-validated for the differentiation of UC and CD |
| Seeley et al[32] | Colon      | CD: 26               | 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[33] | Serum     | UC: 27               | MRM                          | SPP24 differentiated IBD patients from healthy controls α-1-microglobulin distinguished patients with UC in remission from healthy controls |
|               |            | CD: 56               |                              |                                                                         |
|               |            | Controls: 14          |                              |                                                                         |
|               |            | RA controls: 12       |                              |                                                                         |

CD: Crohn’s disease; UC: Ulcerative colitis; IBD: Inflammatory bowel disease; MRM: Multiple reaction monitoring; PF4: Platelet factor 4; MRPs: Myeloid related protein 8; FIBA: Fibrinopeptide A; Hps: 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 ≤ 6 cases and controls[23,24,38,56-59]. 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

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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".\(^{20,33,34}\) Consequently, many potential markers are identified in the literature but require further investigation.

The gap between the inherent inaccuracies of the
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 investigations into IBD proteomics should certainly be pursued.

REFERENCES

1 Bernstein CN, Fried M, Krabshuis JH, Cohen H, Eliakim R, Fedail S, Geary R, Goh KL, Hamid S, Khan AG, LeMair AW, Malferrerhein K, 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.20148]

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 Tediosi A, Spiegelhalter DJ, Jass J, Firth J, Dixon M, Leader M, Levison DA, Lindley R, Filipe J, 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, Galandriuk S. The importance of diagnostic accuracy in colonic inflammatory bowel disease. *Am J Gastroenterol* 2000; 95: 3184-3188 [PMID: 11095334 DOI: 10.1016/s0002-9270(00)01992-4]

5 Chen L, Fang B, Giorgianni F, Gingerich 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.20101001]

6 Glen A, Gan CS, Handy FC, Eaton CL, Cross SS, Catto JW, Wright PC, Reham 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, Franzen B, Auer G, Linder S. Cancer proteomics: from identification of novel markers to creation of artificial learning models for tumor classification. *Electrophoresis* 2006; 21: 1210-1217 [PMID: 17058693 DOI: 10.1002/els2.200401216.10.1-AID-ELS1210:3.0.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)00428-8]

9 Jungblut PR, Zimny-Arndt U, Zeindl-Eberhart E, Stulik J, Koupilova K, Pleissner K, Otto A, Müller EC, Sokolowska-Köhler W, Grabher G, Stöfler 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-ELS210:3.0.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.6100107]

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.2005.059476]

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.2010.10.6058]

13 Langhorst J, Elsenbruch S, Koelzer J, Rueffer A, Michelsen 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, Humphrey-Smith I. Progress with gene-product mapping of the Mollicutes: Mycoplasma genitalium. *Electrophoresis* 1995; 16: 1090-1094 [PMID: 7498152 DOI: 10.1002/elps.1100160118]

15 Ferreri AJ, Illerahaus 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+1]

16 Vaipponou LA, Gouzouli 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/s00410-012-2291-4]

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 Bach 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: 23537375 DOI: 10.1155/2013/180605]

20 Rifei 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 Meuvis MA, Fillit M, Geerts P, de Seny L, Lutteri L, Chapelle JP, Bours V, Wenhken J, Belaiche J, Malaisse L, Louis E, Merville MP. Biomarker discovery for inflammatory bowel disease, using proteomic serum profiling. *Biochem Pharmacol* 2007; 73: 1422-1433 [PMID: 17258869 DOI: 10.1016/j.bcp.2006.12.019]

23 Kannutra S, Uto H, Numata M, Hashimoto S, Morichi A, Fujita H, Oketani M, Ido A, Kodama M, Ohi H, Tsushima H. Human neutrophil peptides 1-3 are useful biomarkers in patients with active ulcerative colitis. *Inflamm Bowel Dis* 2009; 15: 909-917 [PMID: 19186280 doi: 10.1002/ijpe.40]
An In Vitro Diagnostic Multivariate Index Assay

2010; Silverberg MS, Lockton S, Stockfisch T, Croner L, 2010; O’Toole D, O’Hare N, Freyne PJ, Weir DG, 2002; Apweiler R, Banks RE, Conaway M, Coon J, 2015; Muller AF, Smith DJ, Newman DJ, Lamb EJ. Renal

28 Tsangaris GT. Serum protein profile of Crohn’s disease treated with Papamichael K, Mantzaris G, Theodoropoulos GE, Anagnostopoulos AK, Gazouli M. Louis E. Proteomics for prediction and characterization of response and chemometric data analysis. Rapid Commun Mass Spectrom 2007; 21: 4142-4148 [PMID: 18029263 DOI: 10.1002/rcm.3323]

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 chromatic data analysis. Rapid Commun Mass Spectrom 2007; 21: 4142-4148 [PMID: 18029263 DOI: 10.1002/rcm.3323]

26 WJGP | www.wjgnet.com

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24 Hatzgiou M, Kurokawa MS, Kouro T, Nagai K, Arito M, Masuko K, Suematsu N, Okamoto K, Ishii F, Kato T. Protein profiles of peripheral blood mononuclear cells are useful for differential diagnosis of ulcerative colitis and Crohn’s disease patients. J Gastroenterol 2010; 45: 488-500 [PMID: 20049485]

23 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 Anal Tech Biomed Life Sci 2009; 877: 3127-3136 [PMID: 19683480 DOI: 10.1016/j.jchromb.2009.08.003]

22 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]

21 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]

20 Zhang Z. An In Vitro Diagnostic Multivariate Index Assay (IVDIMA) for Ovarian Cancer: Harvesting the Power of Multiple Biomarkers. Rev Obstet Gynecol 2012; 5: 35-41 [PMID: 22852125]

19 Rai AJ, Zhang Z, Rosenzeigew J, Shih IE, Pham T, Fung ET, Sokoli L, Chan DW. Proteomic approaches to tumor marker discovery. Arch Pathol Lab Med 2002; 126: 1518-1526 [PMID: 12456215]

18 Ueland F, Desimone C, Seaman L, Ware R, Goodrich S, Podzie-linked I, Smith A, Santoso J, Van Nagel J, Zhang Z. The OVA1 test improves the preoperative assessment of ovarian tumors. Gynecol Oncol 2010; 116: S23

17 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]

16 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]

15 Schreiber S, Hämling J, Zehnter E, Howaldt S, Daer W, Raedler A, Krues W. Renal tubular dysfunction in patients with inflammatory bowel disease treated with mesalazine. Gut 1997; 40: 761-766

14 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]

13 Mahmud N, O’Toole D, O’Hare N, Freyne PJ, Weir DG, Kelleher D. Evaluation of renal function following treatment with 5-aminosalicylate. J Gastroenterol 2001; 36: 191077 DOI: 10.1002/ibd.20854

12 Lee Y, B yards M, Saito SC, Niranjan T, Garcia AM, Gruenewald A, Thomas DB, Shattat IF, Supe K, Wroniecki 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]

11 Makawita S, Diamandis EP. The bottleneck in the cancer biomarker discovery process. J Proteome Res 2010; 9: 6091-6100 [PMID: 21028795 DOI: 10.1021/pr100094k]

10 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/pr060433n]

9 Han NY, Choi W, Park JM, Kim EH, Lee H, Hahn 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]

8 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: 10.1002ibd.21442]

7 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]

6 Meuwis MA, Fillet M, Lutteri L, Marée R, Geurs 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]

5 Gazouli M, Anagnostopoulos AK, Papadopoulou A, Vaiopoulou A, Papamichail K, Mantzaris G, Theodoropoulos GE, Anagnostopoulos AK, 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]

4 Wasinger VC, Yau Y, Duox U, 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.M115.055905]

3 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, Neusuess 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 up to state guidelines. Proteomics Clin Appl 2007; 1: 148-156 [PMID: 17136664 DOI: 10.1002/prca.200600771]
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 Whitehead JP, Lin J, Kennedy J, Hou L, Trute M, Sokol A, Van P, Schoenherr RM, Zhao L, Voytovich UJ, Kelly-Spratt KS, Krasnoseisky A, Gafken PR, Hogan JM, Jones LA, Wang P, Amnon L, Chodosh LA, Nelson PS, Mctintosh 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, Rifi H, Konratovich M, Tezak Z, Mansfield E, Oberg AL, Wright I, Barnes G, Gail M, Mesri M, Kinsinger CR, Rodriguez H, Boja ES. Statistical design for biopspecimen cohort size in proteomics-based biomarker discovery and verification studies. J Proteome Res 2013; 12: 5383-5394 [PMID: 24063748 DOI: 10.1021/pr400132j]

56 Foge F, Jian B, Krieg RC, Wellman A. Protein analysis of mucosal preparations from patients with ulcerative colitis. Mol Med Rep 2008; 1: 51-54 [PMID: 21473977 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/pr100573p]

58 Nanni P, Mezzanotte L, Roda G, Caponi A, Levander F, James P, Roda A. Differential proteomic analysis of HT29 C11.6E and intestinal epithelial cells by LC ESI/QTOF mass spectrometry. J Proteomics 2009; 72: 865-873 [PMID: 19168159 DOI: 10.1016/ j.proteom.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 ML, 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.200652]

61 Araki K, Mikami T, Yoshida T, Kikuchi M, Sato Y, Oh-ishi M, Kodera Y, Maeda T, Okaya I. High expression of HSP74 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, Abbatucci SE, Ackermann BL, Borchers C, Domon B, Deutsch EW, Grant RP, Hoofnagle AN, Kooten MJ, Liebler DC, Liu T, MacLean B, Mani DR, Mansfield F, Neubert H, Paulovich AG, Reiter L, Vitkova O, Whiteley D, Zou J. Characterization of inflammatory bowel disease sera from constipation- and diarrhoea-predominant functional bowel disorders. Eur J Gastroenterol Hepatol 2002; 14: 409-412 [PMID: 11943955 DOI: 10.1002/1477-8787/200204000-00013]

63 Shire 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 2015; 148: 105-109 [PMID: 25957779 DOI: 10.1016/j.cca.2015.09.019]

64 Ruemmele FM, Targan SR, Levy G, Dubinsky M, Braun J, Seidman EG. Diagnostic accuracy of serological assays in pediatric inflammatory bowel disease. Gastroenterology 1995; 118: 822-829 [PMID: 9753483 DOI: 10.1016/S0016-5085(98)70572-5]

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

66 Siipponen T, Björksten CG, Färkäli 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: 20034860 DOI: 10.3109/03005620903483650]

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

68 Vecchi M, Giovannetti P, Bianchi MB, Belluzzi A, Meucci G, Campieri M, de Franchis R. p-ANCA and development of pouchitis disease. Gastroenterology 1994; 107: 886-887 [PMID: 7916418 DOI: 10.1016/0140-6736(94)90219-0]

69 Costa F, Mumolo MG, Cecarelli 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.2004.03406e]
McNicholl AG, Algaba A, López P, López-Palacios N, Calvo M, González-Lama Y, Carneros JA, Velasco M, Matè 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]

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]

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]

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