Novel immunoassays for detection of faecal calprotectin and neutrophil extracellular traps in the gut

Faecal calprotectin (CP) is a valuable biomarker for inflammatory bowel disease (IBD). Nevertheless, commercial assays do not display comparable clinical sensitivities and specificities. Dr Magne Fagerhol at the Oslo University Hospital, Norway, explored the structure of faecal CP to understand the sources of variation and developed new monoclonal antibody mixtures and immunoassay procedures that improved the sensitivity. Moreover, Fagerhol demonstrated the presence of neutrophil extracellular traps (NETs) released from activated neutrophils in the gut. He proposes three novel immunoassays for improved quantitative analysis of faecal CP and detection of NETs in stools.

Inflammatory bowel disease (IBD) describes the chronic and recurrent inflammatory conditions of the gastrointestinal (GI) tract. Unfortunately, the frequency of IBD is rising globally due to the increased intake of calories from added sugars and saturated fats. In 2030, more than ten million people in the Western world are forecast to be living with IBD. Though the aetiology of the disease is largely unknown, defects in the immune system and abnormally immune activity against commensal microorganisms in the GI tract are believed to play crucial roles in driving the pathogenicity of IBD.

Neutrophils are the most abundant leukocytes and the first cells to reach acute inflammatory sites. They show antimicrobial activity by engulfing the microbes, releasing multiple antimicrobial agents to the site of infection and forming neutrophil extracellular traps (NETs). NETs are web-like structures consisting of DNA-histone complexes and antimicrobial proteins. While NETs function to trap and kill pathogens, excessive NETs are associated with host tissue damage and the progression of gut diseases, including IBD.

NETs contain calprotectin (CP), a major cytosolic protein in neutrophils discovered in 1980 by Dr Magne Fagerhol and colleagues. This finding paved the way for further research on CP and its roles in inflammatory diseases. CP exerts its antimicrobial activity by binding of zinc and other trace metals. Faecal CP is now an established non-invasive biomarker in IBD patients.

Most methods to quantify faecal CP are based on the enzyme-linked immunosorbent assay (ELISA), a powerful method that employs antibodies linked to enzymes for detecting and quantifying a specific protein in a mixture. Sandwich-type ELISA is the most frequently used immunoassay, where at least two antigenic epitopes on the protein are required for binding. The immobilised antibody in the microwell of the ELISA plate and the detection antibody in the solution form a ‘sandwich’ with the protein to be detected. Mostly, the horseradish peroxidase (HRP) enzyme is conjugated to the detection antibody to obtain a detectable signal. A standard curve is generated by plotting the known concentrations of a standard protein against the absorbance values to quantify the target protein.

In recent years, different commercial CP assay kits have been developed. Though used widely, the commercial kits lack standardisation, have low sensitivity and specificity, and produce different results mainly due to differences in antibodies, extraction procedures, and standards used. The results obtained with different kits are not interchangeable, limiting the use of faecal CP in monitoring patients with IBD.

Back in 1992, Fagerhol and colleagues described an ELISA-based method for extraction and quantification of faecal CP and reported elevated levels in the stools of patients with IBD. COMMERCIAL ASSAYS LACK STANDARDISATION

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Together with Dr Jarle Rugtveit, Fagerhol undertook this challenge to develop novel and reliable quantitative methods for the detection of faecal CP and studying NET generation. To do that, they first demonstrated that the structure of faecal CP is different from that in the standard protein. This is a major source of variation in the results obtained with the commercial CP assay kits. Moreover, though CP is a heterodimeric protein complex with S100A8 and S100A9 subunits, faecal CP was shown to lack the subunit S100A8. This means that if the standards included in the commercial CP assay kits contain the subunit S100A8, falsely low concentrations will be estimated. A similar result will be obtained if the antibodies of the commercial kit bind to both subunits.

The researchers selected a mixture of novel monoclonal antibodies targeting the S100A9 subunit only - the first assay standard compatible with faecal CP using chromatographic methods for an objective assessment of faecal CP levels. Another advantage of the offered standard is that it maintains stability even after storage at 5°C for four months. NOVEL ELISA METHODS

CP is a NET-associated protein bound to chromatin DNA filaments. Fagerhol and Rugtveit demonstrated the heterogeneity of faecal CP and emphasised that the use of commercial CP assay kits would only be acceptable when employing a mixture of monoclonal antibodies targeting all the subfractions of the protein as obtained by chromatographic methods. The researchers also underlined the possibility that CP protein structure might be altered,
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My research has always been stimulated by an interest in biology, in particular pathophysiology. Reading Harrison’s Principles of Internal Medicine during medical school (1954–1960), particularly the first 400 pages on the pathogenesis of disease down to the molecular level, ignited my interest in these phenomena. In the 14th edition, some results from my 1970 doctoral thesis (describing the Pi system) are mentioned, namely that some mutants of alpha-1-antitrypsin cause emphysema due to insufficient inhibition of granulocyte elastase. I decided to find a granulocyte protein whose level in plasma might reflect the turnover of such cells. This became calprotectin and subsequently the topic of five doctoral theses’ work in my laboratory.

Behind the Research

Dr Magne K Fagerhol

What inspired you to conduct this research?

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Dr Fagerhol developed new methods for the assessment of faecal calprotectin.

Research Objectives

Dr Fagerhol developed new methods for the assessment of faecal calprotectin.

Detail

Bio

Magne Fagerhol received his MD from Bergen University Hospital in 1960 and a PhD from Oslo University Hospital in 1970. He is presently Professor at the Department of Immunology and Transfusion Medicine, Oslo University Hospital, Ullevål. Dr Fagerhol discovered the Pi system of genetic variants of serum alpha-1-antitrypsin (1965) and calprotectin (1980) and developed the faecal calprotectin assay (1992).

References


Personal Response

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