EVALUATION OF THE IMPACT OF DIFFERENT COMMERCIALLY AVAILABLE DNA EXTRACTION KITS AND LABORATORIES FOR ASSESSING BACTERIAL COMMUNITY STRUCTURE IN Faecal SAMPLES – IMPLICATIONS FOR IBD RESEARCH

PTU-072

Introduction Determining faecal sample bacterial community structure through sequence analysis of DNA has become a very important facet of inflammatory bowel disease (IBD) research. The possible impact of different commercially available DNA extraction kits and the influence of different lab environments on the data generated has however received relatively little attention. The study compared bacterial communities in faecal samples extracted using commercial DNA kits in established gastrointestinal research laboratories.

Methods Faecal samples from 2 healthy volunteers and 2 IBD patients with relapse were investigated. DNA extraction was undertaken using MoBio and Fastprep DNA extraction kits in two established labs. Two protocols were followed for processing samples using the Fastprep kit. Each DNA sample was then split and an aliquot transferred to the other lab. Pyrosequencing PCR of bacterial 16S rRNA genes was performed in both labs on all samples. Quantitative PCR analysis (q-PCR) to validate sequencing data was also performed. Hierarchical clustering was done using the Jaccard and Theta Yue & Clayton similarity coefficients on the pyrosequencing data.

Results DNA extracted using methods FastPrep1, FastPrep2 and the MoBio kit yielded median DNA concentrations of 476 (interquartile range 290–518), 453 (IQR 228–669) and 22 (IQR 9–36) ng/µL respectively. Those obtained with MoBio were significantly lower than FastPrep (p < 0.0001). Hierarchical clustering of sequence data revealed four clusters, with samples clustering by patient. Within each patient cluster, samples clustered by DNA extraction kit. Linear modelling of the effect of patient and kit on relative abundance of common bacterial classes revealed significant differences between MoBio and Fastprep. Ruminococcaceae and Bacteroidetes were significantly increased in MoBio extracted samples, while Lachnospiraceae and Enterobacteriaceae were significantly reduced (p < 0.05 in each case). Q-PCR revealed good correlation with sequencing data, with R² of 0.94, 0.82, 0.69 and 0.57 for Enterobacteriaceae, Bacteroides, Ruminococcaceae and Lachnospiraceae respectively.

Conclusion This study demonstrates significant differences in DNA yield and bacterial DNA composition seen when comparing DNA extracted from the same faecal sample with different extraction kits. This highlights the importance of ensuring that all samples to be analysed together are prepared with the same DNA extraction method, and the need for caution when comparing studies that have used different methods.

Disclosure of Interest None Declared

REFERENCES
3. PTU-072

DOSE INTENSIFICATION WITH ANTI-TNF AGENTS IN INFLAMMATORY BOWEL DISEASE – A SECONDARY CARE EXPERIENCE

Introduction The efficacy of anti-TNF therapy, Infliximab (IFX) and Adalimumab (ADA) has been established in pivotal trials for induction and maintenance of remission of inflammatory bowel disease (IBD). Despite this a proportion of patients lose response and require dose intensification. The aim of our study was to assess the efficacy and durability of dose intensification with IFX/ADA.

Methods A retrospective review of patients with IBD receiving IFX/ADA infusions from October 2011 until April 2012 at our institution was conducted through electronic and case note review, endoscopy, radiology and pathology reports. Patients losing response after 6 months or more of IFX/ADA maintenance therapy and in whom treatment was intensified were included. Dose intensification was defined as an increase in IFX dose per infusion from 5mg to 10mg/kg or increase in frequency of infusions from 8 weeks to 5 – 6 weeks. For ADA this was defined as increasing the dose from 40 mg to 80mg or increasing the frequency from biweekly to weekly.

A positive response was defined by clinician assessment after 3 infusions. Non-response or loss of response was defined as persistent disease related symptoms requiring steroid therapy, hospitalisation, surgery or discontinuation of IFX/ADA.

Results Of 208 patients receiving anti TNF therapy (IFX = 157, ADA = 51), 15 patients (12 female) received dose intensification (IFX = 12, ADA = 3). 6 had Ulcerative Colitis (UC) (4 pan-colonic, 2 left sided) and 9 had Crohn’s Disease (CD) with non stucturing, non penetrating disease (3), active stucturing (3), penetrating (1) and per-anal (2) disease respectively. Disease location was ileal (1), colonic (2) and ileo-colonic in 6. The median age was 40 years (range 18–72 yrs).

Response to intensification after approximately median duration of follow up of 12 months was noted in 8/9 CD and 2/6 UC patients; 9 patients (60%) remained on dose intensification and 5 lost response (33%). In 1 patient treatment was discontinued in the third trimester of pregnancy. Two patients (UC) reverted to their previous dose, 2 non-responders underwent surgery and 1 received Methotrexate. 2 patients are being evaluated for dose intensification. CRP was elevated in 5 patients prior to intensification. Endoscopic assessment of disease was performed in 4 patients with UC showing active colitis in 3. Four patients underwent enterography showing active disease in three.

Conclusion A significant proportion of patients with CD respond to dose intensification but thorough disease assessment does not always appear to precede such critical decision. Anti-TNF trough and antibody levels, an astute assessment for active disease and search for alternative mechanistic explanations for symptoms are imperative prior to embarking on expensive therapy with its inherent risks.

Disclosure of Interest None Declared

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