ADVERSE EVENTS ASSOCIATED WITH MANAGEMENT OF INFLAMMATORY BOWEL DISEASES: INFECTIONS AND SIDE EFFECTS

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1. ABBREVIATION

ApoA1: apolipoprotein A1
BMD: bone mineral density
BMI: body mass index
cAMP: Cyclic adenosine monophosphate
CD: Crohn’s disease
CD40: cluster of differentiation
CDAI: Crohn’s Disease Activity Index
CDP: Clostridium difficile positivity
CMV: cytomegalo virus
CRP: C-reactive protein
CyA: cyclosporine A
DHEA: dehydroepiandrosterone
DXA: Dual-energy X-ray absorptiometry
EIM: extraintestinal manifestations
ELISA: enzyme-linked immunosorbent assay
ESR: erythrocyte sedimentation rate
FC: faecal calprotectin
HDL: high-density lipoprotein
IBD: inflammatory bowel diseases
IBS: irritable bowel syndrome
LDL: low-density lipoprotein
LFA: lateral flow assay
MMP-9: matrix metalloproteinase-9
pMayo score: partial Mayo Score
PTH: parathyroid hormone
TNF-α: tumor necrosis factor
TSH: thyroid stimulating hormone
UC: ulcerative colitis
VZV: varicella zoster virus
2. LIST OF FULL PAPERS RELATED TO THE SUBJECT OF THE THESIS


3. LIST OF FULL PAPERS NOT RELATED TO THE SUBJECT OF THE THESIS


trends in hospitalizations during anti-TNF therapy are associated with time to anti-TNF therapy: Results from two referral centres. Dig Liver Dis. 2014. pii: S1590-8658(14)00595-7.

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4. INTRODUCTION AND AIMS

Inflammatory bowel diseases (IBD: Crohn’s disease – CD – and ulcerative colitis – UC) are chronic, multifactorial conditions characterised by immune-mediated inflammation of the gastrointestinal tract with highest incidence of CD in North America, whereas top incidence of UC is in Europe. Etiology of IBD is not exactly understood; however, basically, it develops due to the combination of genetic and environmental risk factors that contributes to an individual’s disease susceptibility. Latella et al. defined the crucial steps in the natural history of IBD as the occurrence of lesions, the manifestation and severity of symptoms, the need for surgery, the development of complications, disability and mortality; moreover, the main outcomes considered include disease activity and relapse, mucosal healing, need for corticosteroid therapy, hospitalizations, complications, surgery, post-operative recurrence, and mortality.

4.1. Keypoints in the management of IBD – infections and side effects

Management of IBD represents a lifelong medical care as the disorder itself is chronic and permanent with periods of relapses and remissions. However, some special situations modify conventional treatment of IBD like adverse events, infections, pregnancy, grown problems in childhood, nutritional difficulties, and malignancies.

Studies had reported about altered intestinal microflora in IBD, in this manner consider to pay attention of avoidance of dysbiosis and microorganisms like *C. difficile* and *Candida* species that leads even a serious symptom and inflammation in special circumstances, thus had influence on optimization of therapy (Study I).

Microbes have significant concern to differential diagnosis also, because some of them may provoke gut and extraintestinal symptoms similar to IBD. Skin is the most common affected extraintestinal organ with varying cutaneous symptoms. However, other intestinal disorders like *Blastocystis sp.* infection may associate with EIMs also that make a meaningful difficulty in differential diagnosis of IBD (Study II).

Regards to infections, prevention is significantly important step of patient management including the recognition of risk factors for infection, the use of primary or secondary chemoprophylaxis,
careful monitoring before and during use of immunomodulators and the vaccination and education of the patient. Influenza infection is frequent and may have severe complications, however, it is a preventable modality, and thus vaccination is recommended (Study III).

As it mentioned, immunosuppression can be the consequence of immunomodulatory therapy. Not just a risk of subsequent infection could occur in patients on immunosuppressants, but their use can result other side effects also. Corticosteroids still have an important role in the management of acute episodes of IBD. However, the therapeutic benefits are compromised by an extensive spectrum of adverse events. In case of moderate to severe disease activity use of classical systemic corticosteroids seems to be irreplaceable by new formulas, in this reason worth to study the response to different steroid administration types and their side effect profile. (Study IV)

Unfortunately, one third of UC patients is corticosteroid refractory, thus for them cyclosporine A, biological therapy or proctocolectomy is recommended in severe relapse. Adverse events of cyclosporine A that range from mild to severe, may lead even to discontinuation of the drug (Study V). Beyond treatment of acute relapses, patients should be monitored due to control short- and long-term effects of therapy.

4.2. Aims

1. To prospectively assess the frequency of *C. difficile* among our outpatients with IBD in relapse

2. To retrospectively assess the attributes of patients with a confirmed positive *Blastocystis sp.* infection, primarily to examine the occurrence of clinical gastrointestinal symptoms and skin manifestations.

3. To assess the antibody response to the seasonal influenza vaccine in patients with IBD treated with anti-TNF-α alone or combined with immunosuppressive therapy and to compare them with patients receiving non-immunosuppressive therapy.

4. To compare the efficacy, the frequency of side effects and the changes in bone and lipid metabolism in IBD patients using bolus or conventional tapering of methylprednisolone for 12 weeks.
5. To prospectively assess serum cholesterol, triglyceride and creatinine levels before, during, and after CyA therapy in patients with severe, refractory UC and to examine the correlation between plasma lipoprotein levels and other side effects.

5. PATIENTS AND METHODS

5.1. Description of methods

5.1.1. Enzyme-linked immunosorbent assay

Fecal MMP-9 concentrations were measured using ELISA method (Quantikine MMP9 assay, R&D System, UK) in our clinical study. From the collected sera samples, anti-influenza A virus IgG ELISA (Euroimmun, Germany) containing the following antigens “Texas” (H3N2), “Singapore” (H1N1) and “California” (H1N1) strains of influenza A virus was performed according to the manufacturer’s recommendation.

5.1.2. Lateral flow assay

FC levels were measured by a quantitative lateral flow assay (Quantum Blue, Bühlmann Laboratories, Switzerland).

5.1.3. Microbiological examination of stool samples

A microbiological analysis (involving bacteria, fungi and, in reasonable cases, viruses and parasite examination) for the presence of an enteric pathogen was carried out at the Institute of Clinical Microbiology, Szeged.

5.1.4. Statistical analysis

The collected data were analysed statistically. P<0.05 was considered statistically significant. For the analysis, SPSS15.0 (SPSS Inc, Chicago, IL, USA) was used.
5.1.5. Ethical Considerations

The studies were approved by the Regional and Institutional Human Medical Biological Research Ethics Committee of the University of Szeged.

5.2. Patients and methods of clinical studies

We carried out 5 clinical studies connected to the topic. In the Study I, 90 outpatients with IBD who relapse were enrolled prospectively. Clinical data of patients were assessed, and stool cultures; in addition, blood and stool samples were obtained for the determination of serum biochemical factors, FC and faecal MMP-9. IBD patients in clinical remission were selected as a control group.

In second study data of 80 patients with confirmed positive Blastocystis sp. infections were assessed retrospectively. Gastrointestinal and dermatological symptoms and the results of routine biochemical and haematological blood tests of enrolled patients were collected and analysed.

In Study III, 156 immunocompromised IBD patients were vaccinated. 53 patients (control group) refused vaccination. Split virion vaccine and whole virion vaccine were used. Serum samples were obtained for pre- and postimmunisation antibody titres to influenza vaccine (A/California/7/2009 [H1N1], A/Victoria/361/2011 [H3N2], B/Wisconsin/1/2010–like B/Hubei-Wujiagang/158/2009).

In Study IV, 19 IBD patients received intravenous methylprednisolone of 1 mg/kg for 5 days tapered by 4 mg per week. Patients were prospectively randomized in two groups. In “conventional” group (I) steroids were given daily. In “pulse” group (II) weekly dose of steroids were given on special days of the week. The BMI was measured before and after the corticosteroid therapy. Blood samples were collected to assess glucose level, electrolytes, cholesterol and triglycerides levels, inflammatory parameters, cortisol, osteocalcin and crosslaps values. Total body composition analysis was performed at the beginning and at the end of the steroid therapy.

In the last study, clinical data and serum cholesterol, triglyceride, creatininine levels of 72 patients were analysed and compared to a control group treated with infliximab.
6. RESULTS

7.1. Study I: Relapse of IBD and C. difficile

7.2.1. Infection rates and patient follow-up

Out of the 139 enrolled patients, 76 subjects were diagnosed with UC and 63 with CD. Half of the patients were on immunomodulatory therapy in the relapse group (48.8%) and also in the control group (53.1%). Bacteria or fungi were identified in 51 of 90 faecal samples taken from relapsing patients. On the other hand, 14 patients had positive microbiological stool findings in the control group in remission. 46 participants with positivity of C. difficile A and B Toxins had been isolated among the patients in relapse (51.1%), while CDP was observed in 10 patients (20.4%) in the remission group. Other causes of microbiological positivity in the relapse group were due to the presence of Candida species. Previous antibiotic use was shown to have a connection to CDP (p=0.033), but other assessment factors did not predict the presence of Cl.difficile Toxins.

7.2.3. Fecal calprotectin and matrix-metalloproteinase-9 levels

The mean value of FC and MMP-9 in those in relapse was significantly higher compared to the control group. Interestingly, FC and MMP-9 showed greater values in relapsing patients without CDP vs. relapsing patients with positive microbiological stool examination, but there was no significant difference between a C. difficile positive and negative population.

7.2. Study II: Clinical manifestations of Blastocystis sp infection

7.2.1. Frequency of Blastocystis spp.

The occurrence of Blastocystis sp. infection was 6% in the symptomatic patients who required medical attendance in that period.

7.2.2. Results of stool microbiological examination

Of the faecal specimens, 41.1% contained few Blastocystis sp. cells, 5.5% of specimens contained a moderate amount and 53.4% contained a high number of the parasites. In 18.75% of the cases other microorganisms were also present besides Blastocystis sp.
7.2.3. Blastocystis spp. and skin manifestations

Nine out of 80 people had accompanying skin manifestations. Significant differences were revealed between patients with and without skin manifestations regarding laboratory findings. Prevalence of IBD was 9% among Blastocystis sp. positive patients.

7.3. Study III: Efficacy of influenza vaccination

7.3.1. Patient characteristics

209 IBD patients (127 with CD, 82 with UC) were eligible and enrolled in the study. 156 patients received influenza vaccination, while 53 patients (control group) refused the vaccine – the willing to vaccination was 66.3%. Whole virion vaccine was given to 57; split vaccine was given to 99 patients.

7.3.2. Antibody titers for Influenza A and B subtypes

The postimmunisation antibody titers of Influenza A and B subtypes significantly increased in patients immunized with split virion vaccines compared to control subjects. The antibody titers of Influenza A and B significantly increased after the administration of split vaccine compared to whole virion vaccines. The postimmunisation antibody titers of Influenza B also increased significantly after administrating split vs. whole virion vaccine in patients treated with anti TNF-α.

7.3.3. Effect of vaccination on IBD activity

During the 4 months follow-up period, 1 of the control subjects and 21 of the vaccinated patients (8 CD, 13 UC) developed a flare up with an increased diarrhea or bloody stool.

7.3.4. Side effects and development of influenza-like symptoms

Upper respiratory tract infection like symptoms more frequently occurred within the first week in vaccinated vs. control patients. Local and systemic reactions were more common in patients vaccinated with IDFlu9 split vaccine vs. Fluval AB.
7.4. Study IV: Bolus administration of steroid therapy

7.4.1. Patient characteristics, clinical response

Although the male/female ratio was higher in Group II, baseline clinical characteristics of patients did not differ significantly between the two treatment groups. CDAI and pMayo score showed decreasing pattern in both groups during the steroid therapy.

7.4.1. Changes in adrenal glands hormone secretion, in the lipid and bone metabolism after methylprednisolone therapy

In Group I, BMI increased significantly at the end of the steroid therapy. Total body composition analysis showed significant decrease in bone density in Group I. Considering the laboratory parameters, serum cholesterol level increased significantly in Group I patients after steroid therapy. The decrease in serum cortisol level was more remarkable in Group I vs. Group II after steroid therapy.

7.5. Study V: Side effects of cyclosporine A therapy

Side effects occurred in 52 patients (72.2%) during the therapy (Figure 3.). The most frequent side effects were hypertension (15.23%), tremor (13.8%), hypertrichosis (9.72%), myalgia and muscle cramping (11.1% and 4.16%, respectively) and numbness of legs (5.5%). Nephrotoxicity or hepatotoxicity occurred in 6 patients (8.33%). Increased serum cholesterol and triglyceride levels were detected in 47.2% and 19.4% of the patients, respectively. Major side effects resulting in discontinuation of the cyclosporine therapy occurred in 21 patients. Statistically, serum cholesterol levels increased significantly during the therapy and remained higher for one year after the discontinuation of CyA. In the cyclosporine group, cholesterol levels were considerably higher during therapy than in the control group. Cholesterol levels measured after cyclosporine therapy were found to be significantly higher in patients with an adverse reaction vs. patients without adverse reactions. Elevated total cholesterol levels were noted in 65.4% of the patients with detectable side effects. Serum triglyceride levels were elevated in 19.4% of the patients. No difference was found in triglyceride levels compared to the control group.
7. DISCUSSION

Our data showed 40.3% of occurrences of CDP in IBD outpatients, and 51.1% of the patients in the relapse cohort were *C. difficile*-positive. We revealed a significant difference regarding CDP between IBD patients in relapse and those in remission (where \( p<0.001 \)). Our results confirmed an association between previous antibiotic use and *Clostridium difficile* positivity. The occurrences of *C. difficile* and *Candida* positivity were excessively high in patients in an acute relapse, which suggests the importance of intestinal microbiota in IBD and an important role in the relapse, therefore stool analysis is recommended in flare-ups. FC and MMP-9 has no diagnostic value to differentiate between infection-induced and natural relapse.

We discovered that 11.25% of our enrolled patients exhibited skin manifestations associated to *Blastocystis* sp., mainly on the females. The occurrence of *Blastocystis* sp. was 6% in symptomatic patients who required medical attendance in the time period between 2005 and 2013. We did not find significant difference in eosinophilia between patients with vs. without skin manifestations. 73.75% of the patients indicated that they had gastrointestinal symptoms: 40 patients complained of abdominal pain, 17 with blood in their stool, while other symptoms, such as meteorism (15 subjects), weigh loss (8 subjects), perianal pain or itching (6 subjects), passing stool with mucus (5 subjects), vomiting (2 subjects) and fever (2 subjects) were less frequent.

The third study revealed that two thirds of the patients agreed to influenza vaccination. Split virion vaccines proved to be more effective in the vaccination procedure: post-immunisation titres of both subtypes increased significantly after the administration of split virion vaccines compared with the controls and with those patients vaccinated with whole virion vaccines. The antibody titres of Influenza B also increased significantly in patients immunised with split virion vaccine and treated with anti TNF-\( \alpha \) therapy. The high number of cases with pre-existing antibody levels can be explained with previous vaccinations and prior influenza infections, although cross-protection against influenza virus strains could also be present. Although no serious side effects were developed after influenza vaccination, influenza-like symptoms in the first weeks after immunisation occurred more frequently in patients receiving split virion vaccine. Influenza-like symptoms did not differ significantly between vaccinated vs. control patients (8.3% vs. 7.5%). A mild relapse of the disease was observed in only 10% of the patients.
and was more common in vaccinated than in control subjects. In conclusion, our results suggest that IBD patients on immunosuppressive therapy are recommended to be immunised with split virion vaccines and that measuring the antibody responses is worthwhile in patients treated with immunosuppressants to determine the efficacy of influenza vaccination. Larger and more detailed studies are needed to compare the efficacy of these vaccinations and to examine the antibody response in immunocompromised patients.

Our results suggest that bolus tapering of equivalent doses of methylprednisolone administered in conventional daily doses has equivalent clinical efficacy, but more favourable side effect profile. As no significant difference was detected between the two administration types on the clinical and laboratory parameters of disease activity, it appears that bolus administration of corticosteroids can safely and effectively replace the conventional use of methylprednisolone for active IBD. Of course, further controlled, randomized trials are needed to confirm these results that may revolutionize steroid therapy in IBD. It should be noted, significant changes may develop in bone and lipid metabolism during even a short-term steroid therapy.

Serum cholesterol levels showed significant increase during cyclosporine therapy compared to the time before use of the drug and to the control cohort of patients being treated with infliximab and corticosteroids. This elevation remained significant for a year. Serum cholesterol levels for the UC group with adverse events were significantly higher compared to patients who did not develop any side effects. Creatinine levels did not change significantly during cyclosporine therapy. In the control group, cholesterol, triglyceride and creatinine levels did not change significantly during therapy or after its discontinuation. In conclusion, we found increased serum cholesterol levels in severe, steroid-refractory UC patients treated with cyclosporine not only during the therapy, but also after its discontinuation, suggesting that cyclosporine has a long-term effect on serum lipid metabolism. Furthermore, in the presence of other adverse events, cholesterol levels were significantly higher, suggesting that drug-related impairment of cholesterol biosynthesis and other side effects are rather common, therefore practically speaking, monitoring cholesterol levels during CyA therapy is recommended. Considering the high rates of hypercholesterolaemia as a side effect of cyclosporine therapy, this topic is worth to be studied further.
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