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Inflammatory bowel disease activity in previously hospitalised patients with extended spectrum beta-lactamase producing *Enterobacteriaceae* presence in the gut

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ABBREVIATIONS

BMI – body mass index

CD – Crohn's disease

CDAI – Crohn's disease activity index

CRP – C reactive protein

ECCO – European Crohn's and Colitis Organisation

ESBL – extended spectrum beta-lactmases

ESBL-E – extended spectrum beta-lactmase producing *Enterobacteriaceae*

ESR – erythorcyte sedimentation rate

EUCAST – European Committee on Antimicrobial Susceptibility Testing

HBI – Harvey-Bradshaw index

Hgb – hemoglobin

IBD – inflammatory bowel diseases

ICD-10 – International Classification of Diseases version 10.

MDRM – multidrug resistant microorganisms

MRSA – methicillin-resistant Staphylococcus aureus

PSKUS – Pauls Stradins Clinical University Hospital

RAKUS – Riga East Clinical University Hospital

RSU – Rīga Stradiņš University

UC – ulcerative colitis

USA – the United States of America

VRE – vancomycin-resistant Enterococcus

INTRODUCTION

Inflammatory bowel diseases (IBD), most commonly include ulcerative colitis (UC) and Crohn's disease (CD), and they manifest as chronic inflammation of the gastrointestinal tract with extra-intestinal manifestations (Lichtenstein et al., 2018; Magro et al., 2017).

Due to frequent contact with the medical system, hospitalisation, use of immunomodulatory drugs, antimicrobial therapy and IBD pathogenesis itself, IBD patients are more likely to be colonsised by multidrug resistant microorganisms (MDRM), including extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-E), which are the most common MDRM found in IBD patients (Leung et al., 2012; Vaisman et al., 2013). ESBL-E in IBD patients are found up to ten times more frequently than other common MDRM such as methicillin-resistant Staphylococcus aureus (MRSA) or vancomycin-resistant enterococcus (VRE) (Leung et al., 2012; Vaisman et al., 2013).

Gut colonisation with ESBL-E in IBD patients is associated with ESBL-E spread in the medical system and faster development of ESBL-E determined infections, which is further associated with increased morbidity, prolonged hospitalisation, increased cost, further development of antimicrobial resistance and increased mortality (ECDC, 2014; ECDC, 2015). Therefore, the presence of ESBL-E in IBD patients' gut is important from the public health and infection control, as well as clinical perspective (CDC, 2018; ECDC, 2014; ECDC, 2015; Lynch et al., 2013; Martinez-Martinez et al., 2017).

Previous studies have looked at ESBL-E presence in IBD patients from the epidemiological point of view (Leung et al., 2012; Vaisman et al., 2013; Li et al., 2015). They have analyzed the prevalence and risk factors for gut colonisation with ESBL-E in IBD patients in North American and Asian countries. However, despite the increasing evidence that the presence of

Enterobacteriaceae in the gut microbiota has a potential role in etiology and pathogensis of IBD, as well as gut colonisation with MDRM, including ESBL-E, none have looked at the association between ESBL-E presence in the gut of IBD patients and IBD clinical activity.

This study looks at differences in UC and CD clinical disease activity in previously hospitalised UC and CD patients with and without ESBL-E in their gut, by using UC and CD clinical disease activity scores, according to the European Crohn's and Colitis Organisation (ECCO) guidelines, and determining the presence of ESBL-E in patients' gut, according to the European Committee on Antimicrobial Susceptibility Detection (EUCAST) guidelines. The study shows that higher IBD clinical activity is observed in previously hospitalised UC and CD patients with ESBL-E found in their gut. However, further studies are needed to determine the association between ESBL-E and IBD cinical activity.

1. HYPOTHESIS, AIM AND OBJECTIVES

Hypothesis

Higher inflammatory bowel disease (IBD) clinical activity is observed in previously hospitalised ulcerative colitis (UC) and Crohn's disease (CD) patients if extended spectrum beta-lactamase producing *Enterobacteriaceae* (ESBL-E) are present in their gut.

Aim

The aim of the study was to determine the difference in IBD clinical activity in previosuly hospitalised UC and CD patients with and without ESBL-E presence in their gut.

Objectives

1. To describe the presence of ESBL-E (frequency of presence, bacterial species, bacterial plasmid genes that determine ESBL production) in the gut of previously hospitalised UC and CD patients.
2. To describe the clinical disease activity and extent of IBD in previously hospitalised UC and CD patients.
3. To compare the IBD clinical activity in cases with and without ESBL-E presence in the gut of previously hospitalised UC and CD patients.
4. To provide practical recommendations for further development of research, improvement of public health and clinical practice.

2. LITERATURE REVIEW

2.1. Definition of inflammatory bowel disease

Inflammatory Bowel Diseases (IBD) comprise a number of diseases with chronic gastrointestinal inflammation and extra-intestinal manifestations. This term most commonly refers to two IBD forms – ulcerative colitis (UC) and Crohn's disease (CD). (Crohn's and Colitis foundation, 2014; O'Brian & Downward, 2017)

2.2. Most commonly used clinical disease activity indices, scoring systems and classifications

2.2.1. Ulcerative colitis

The complete Mayo score is the most commonly used scoring system in clinical studies and daily practice for determining the clinical disease activity in UC (MD Calc, Mayo score) (See Appendix 1). The complete Mayo score provides measurement of UC clinical disease activity at a specific time point with a single number (MD Calc, Mayo Score; Sutherland et al., 1987). It ranges from 0 to 12, with higher scores indicating a more active disease. It consists of 4 subcategories. Each subcategory gives points from 0 (normal or inactive disease) to 3 (severe disease), which add up to make the full Mayo score (ECCO, 2016; IG-IBD Scores, Mayo Score; ECCO, Mayo Score).

The partial Mayo score is commonly used in the daily clinical practice when no endoscopic examination (colonoscopy) description is available (See Appendix 1). It consists of the sum of 3 subsections excluding the endoscopic description of the mucous membrane appearance. The partial Mayo score ranges from 0 to 9, with a higher score indicating a more severe disease activity. The

distribution of points in 3 categories is identical to the full Mayo score (Alberta Health Services, Partial Mayo Score 2016).

The adapted Truelove and Witt's index is based on patient's bloody stool count per day, which, in combination with several other objective clinical and laboratory measures, shows the UC clinical disease activity (ECCO, 2016; ECCO, Truelove & Witt's Score) (See Appendix 1).

Montreal classification categorises UC by its macroscopic distribution and clinical disease activity (ECCO, 2016; ECCO, Montreal Classification; Dignass et al., 2012) (See Appendix 1).

2.2.2. Crohn's disease

Different calculators are used to calculate the Crohn's disease activity score (CDAI); therefore, the calculated score may differ significantly depending on the calculator used and its algorithm (See Appendix 2). This study uses the CDAI calculator provided in the ECCO guidelines and resources (ECCO, CDAI). The CDAI calculator consists of 8 criteria; each of them gives a certain number of points and each of them has a specific weighting factor in the calculation of the CDAI. The score of each individual criteria added up makes the sum of the CDAI (ECCO, CDAI; IG-IBD Scores, CDAI).

Harvey-Bradshaw index (HBI) was designed as a simplified version of the CDAI to facilitate systematic collection of clinical data in patients with CD (Harvey & Bradshaw, 1980; ECCO, HBI) (See Appendix 2). HBI takes into account five clinical parameters, each of which gives a specific score. The use of HBI compared to CDAI is simpler and does not require laboratory test results. HBI is based on the clinical data from the previous day (Alberta Health Services, Harvey Bradshaw Index, 2016).

The Montreal classification for CD consists of three sections and includes age, disease localisation, and clinical behavior of the disease (ECCO, Montreal Classification) (see Appendix 2).

2.3. Extended spectrum beta-lactamase producing *Enterobacteriaceae*

Extended spectrum beta-lactamases (ESBLs) is a group of enzymes that inactivate beta-lactam antibiotics by hydrolysing the beta-lactam ring in their structure. In this way ESBLs makes these antibiotics ineffective against the microorganisms that produce the ESBLs and, therefore, determines the resistance of these microorganisms to beta-lactam antibiotics, including extended spectrum (third generation) cephalosporins (El Salabi et al., 2013; Jacoby et al., 2005; Oteo et al., 2010).

Most commonly, ESBLs are produced by *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter* species (El Salabi et al., 2013; Jacoby et al., 2005; Oteo et al., 2010). The most common *Enterobacteriaceae* that produce ESBLs are *E. coli* and *Klebsiella* species (Jacoby et al., 2005; Oteo et al., 2010).

ESBL production is determined by genes. Most often, these genes are located in bacterial plasmids – the mobile genetic elements localised outside the bacterial chromosomal deoxyribonucleic acid (DNA) structure (El Salabi et al., 2013; Jacoby et al., 2005; Lynch et al., 2013).

To date, clinically the most important ESBL groups are CTX-M enzymes, followed by TEM and SHV ESBLs. These enzymes are encoded by the respective palmids genes – *bla*CTX-M, *bla*TEM and *bla*SHV (Martinez-Martinez et al., 2017).

2.4. Extended spectrum beta-lactamase producing *Enterobacteriaceae* detection

In Europe, ESBL-E are diagnosed according to the EUCAST guidelines (Martinez-Martinez et al., 2017). The same principles of ESBL-E detection are used in the USA guidelines defined by the Clinical and Laboratory Sandtandarts Institute (CLSI, 2013; CDC, 2010). The recommended strategy for the ESBL-E detection is based on the initial screening for ESBL-E – resistance detection to indicator antibiotics – oximino-cephalsporins, followed by a phenotypic and in some cases genotypic confirmatory test for ESBL-E (Martinez-Martinez et al., 2017).

2.5. Extended spectrum beta-lactamase producing *Enterobacteriaceae* in inflammatory bowel diseases

Due to the impaired immune system, frequent contact with healthcare facilities and regular use of immunomodulatory drugs and antibiotics, IBD patients are more likely to get colonised by MDRM (Leung et al., 2012). IBD patients are more commonly colonised and experience more frequent infections with MRSA, ESBL-E and VRE, compared to patients without IBD (Vaisman et al., 2013).

ESBL-E are the most common MDRM that colonise the intestinal tract of IBD patients. In IBD patients, ESBL-E are found 4 to 10 times more frequently than other common MDRM, such as MRSA and VRE (Leung et al., 2012; Li et al., 2015; Vaisman et al., 2013). It is known that the prevalence of ESBL-E in the IBD patient population is higher (4.1–19 %, mean 11.1 %) than in patients without IBD (4.1 – 6.6 %, mean 5.5 %) (Karanika et al., 2016; Leung et al., 2012; Li et al., 2015; Vaisman et al., 2013).

It is known that more than 25 % of IBD patients are hospitalised due to infections, including infections caused by MDRM (Vaisman et al., 2013) and these infections are associated with a 4-fold higher mortality than in general patient population (Vaisman et al., 2013). These statistics are becoming increasingly important as the number of hospitalised IBS patients continue to rise (Vaisman et al., 2013). Patients who are colonised with MDRM at admission have a higher risk of developing a MDRM infection (Vaisman et al., 2013).

3. MATERIALS AND METHODS

This cross-sectional study was conducted at two tertiary medical centers in Riga, Latvia between 2015 and 2017 and included the IBD clinical activity data and information about the ESBL-E presence in patients' gut in previously hospitalised UC and CD patients.

3.1. Patient selection

The previously hospitalised IBD patient group was selected deliberately, because previously hospitalised IBD patients are more likely to repeatedly come into contact with the medical system and promote further spread of ESBL-E if found in their gut (ECDC, 2014; ECDC, 2015; Leung et al., 2012; Vaisman et al., 2013). UC and CD patients who were previously hospitalised at least once during a 7-year period (January 1, 2010 to December 31, 2016) at either of the two largest tertiary medical care centers in Latvia – Riga East Clinical University Hospital (RAKUS) and Pauls Stradins Clinical University Hospital (PSKUS) with diagnoses of K50 – Crohn's disease and K51 – ulcerative colitis (803 unique IBD patients identified) were invited to participate in the study and evaluated according to the inclusion and exclusion criteria. 177 patients – 122 UC and 55 CD patients met the inclusion and exclusion criteria and were included in the analysis.

3.2. Inclusion and exclusion criteria

Inclusion criteria: 1) Patient has a confirmed K51 (ulcerative colitis) (clinical, laboratory, endoscopic and histological findings) or K50 (Crohn's disease) (clinical, laboratory, radiological or endoscopic and histologic findings) diagnosis. 2) Patient has been previously hospitalised at least once. 3) Patients of

any gender between the age of 18 and 80. 4) Patient with any IBD clinical activity (clinical remission, mild, moderate and severe). 5) Patient can be contacted and can come to the study visit.

Exclusion criteria: 1) Patient does not have a confirmed K50 (Crohn's disease) or K51 (ulcerative colitis) diagnosis as defined by the inclusion criteria; 2) Patient is hospitalised during the study; 3) Patient has never been hospitalised before; 4) Patient is being treated for ESBL-E infection at the time of the study; 5) Patient cannot be contacted; 6) Patient does not want to participate in the study, is unable to attend or does not attend the study visit.

3.3. Clinical disease activity evaluation in UC and CD

UC and CD patients have been analysed separately because of the different principles in scoring systems used to determine the clinical disease activity in both diseases. According to the guidelines of the European Crohn's and Colitis Organisation (ECCO), clinical disease activity and disease extent of UC was evaluated using the full and the partial Mayo score, adapted Truelove and Witt's index and Montreal classification (see Appendix 1), while clinical disease activity and disease extent of CD was evaluated using Crohn's disease activity index (CDAI), Harvey-Bradshaw index (HBI) and Montreal Classifications (see Appendix 2) (ECCO, 2016).

3.4. Identification of ESBL-E and ESBL bacterial plasmid gene detection

A rectal swab with fecal biomaterial was obtained from the previously hospitalised IBD patients. ESBL-E were isolated and bacterial plasmid genes (*bla*CTX-M, *bla*TEM and *bla*SHV) that determine the ESBL production were

detected according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (Martinez-Martinez et al., 2017).

3.5. Comparable patient groups

Clinical disease activity of previously hospitalised UC and CD patients was compared in cases with and without the presence of ESBL-E in patients' gut.

3.6. Statistical analysis

The required sample size for the study was calculated using OpenEpi (OpenEpi) online software. Statistical analysis of the data was performed using parametric and non-parametric descriptive and analytical statistical methods on *IBM SPSS Statistics 25.0.* Statistical significance was determined at 5 % and a p value of < 0.05 was considered statistically significant.

3.7. Ethical principles

The study was conducted in accordance with the Helsinki Declaration of the World Medical Association (WMA, 2018). Approvals were obtained from the RSU and RAKUS Medical and Biomedical Research Ethics Committees.

4. RESULTS

The presence of ESBL-E in the gut was determined in 177 previously hospitalised IBD patients, including 122 UC patients (69 % of the study patients) and 55 CD patients (31 % of the study patients). The presence of ESBL-E in the gut was found in 19 (11 %) IBD patients. ESBL-E were detected in the gut of 13 (11 %) UC patients and 6 (11 %) CD patients.

4.1. Characterisation of the previously hospitalised IBD patients

Demographics of the UC and CD patients can be found in Table 4.1.

Table 4.1

Demographics of the UC and CD patients

	UC (n = 122)	**CD (n = 55)**
Female, Patient count (%)	58 (48 %)	24 (44 %)
Age, years		
Mean (SD)	44.14 (SD = 15.5)	38.11 (SD = 15.25)
Min	18	19
Max	79	77

4.1. Characterisation of ESBL-E present in the gut of previously hospitalised IBD patients

4.2.1. ESBL-E species

In both UC and CD patients, *E. coli* was the most commonly found ESBL-E in the patients' gut (77 % and 83 %, respectively) (See Figure 4.1).

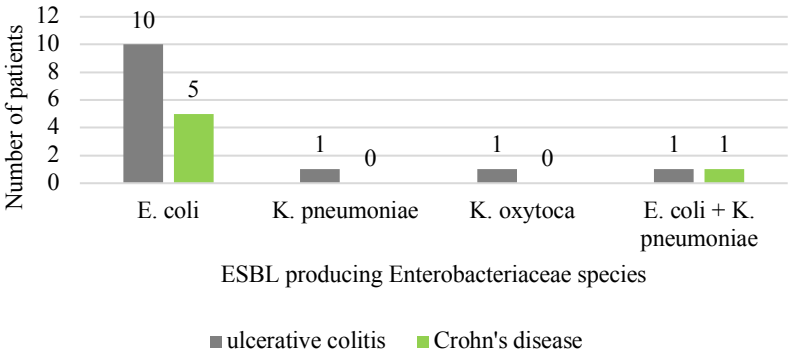

Figure 4.1. **ESBL-E species found in the gut of IBD patients**

4.2.2. Bacterial plasmid genes that determine ESBL production

In both UC and CD patients, CTX-M was the most commonly detected bacterial plasmid gene in the ESBL-E found in the patient's gut (71 % and 56 %, respectively) (See Figure 4.2).

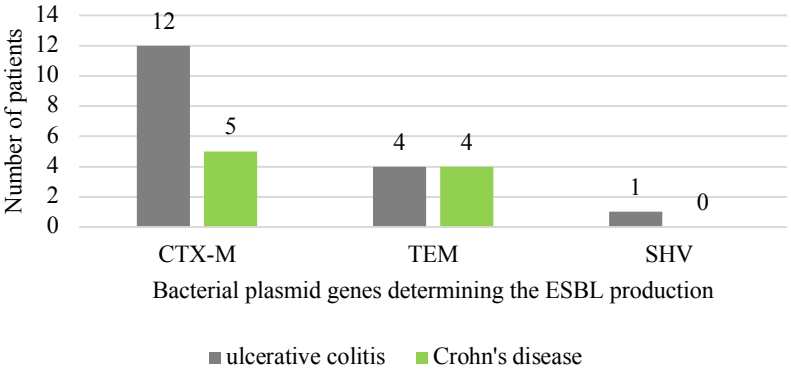

Figure 4.2. **Bacterial plasmid genes that determine ESBL production in IBD patients**

ESBL producing *E.coli* plasmids most commonly contained CTX-M gene that determined ESBL production in these bacteria (See Figure 4.3).

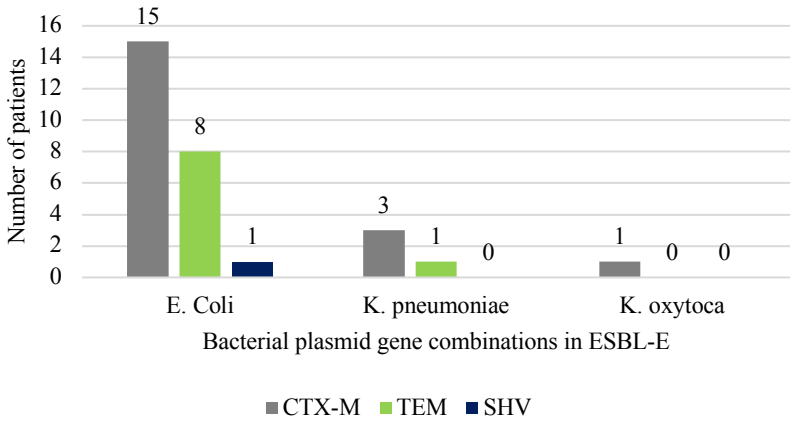

Figure 4.3. **Bacterial plasmid gene combinations in ESBL-E in IBD patients**

4.3. Comparison of IBD clinical activity and disease extent in previously hospitalised IBD patients with and without ESBL-E presence in the gut

4.3.1. Ulcerative colitis

Full Mayo score

Higher clinical disease activity according to the full Mayo score was observed in UC patients with ESBL-E found in their gut – moderate disease activity (Mdn = 5, IQR = 6), whereas mild cinical disease activity (Mdn = 3, IQR = 2) according to the full Mayo score was observed in UC patients without ESBL-E presence in their gut (U = 423, p = 0.016) (See Figure 4.4).

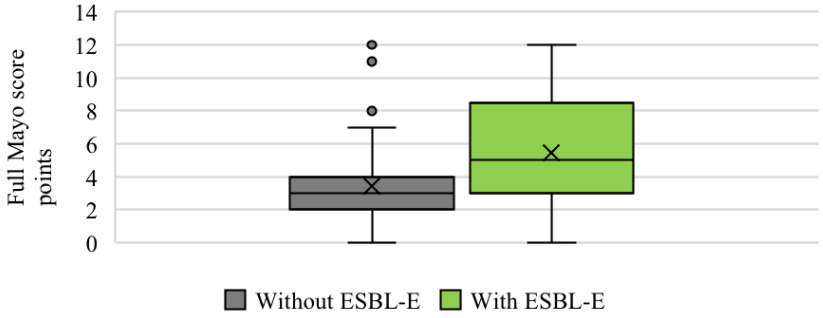

Figure 4.4. **Clinical disease activity points according to the full Mayo score in UC patients with (n = 13) and without (n = 109) ESBL-E presence in the gut (p = 0.016)**

Partial *Mayo score*

When analyzing the differecnes in prevously hospitalised UC patients with and without ESBL-E presence in their gut according to the partial Mayo score, no statistically significant differences were found between patients with (Mdn = 3, IQR = 5) and without (Mdn = 2, IQR = 2) ESBL-E presence in their gut (U = 482, p = 0.052).

Montreal classification

The presence of ESBL-E in the gut had a significant effect on the clinical disease activity of UC patients, as assesed by the Montreal classification X2 (3, n = 122) = 15.54 p = 0.001, $\phi_C = 0.37$ (See Figure 4.5). Moderate (S2) and severe (S3) disease activity was predominantly found in UC patients with ESBL-E in their gut, whereas clinical remission (S0) and mild (S1) clinical disease activity was predominantly found in UC patients without ESBL-E presence in their gut.

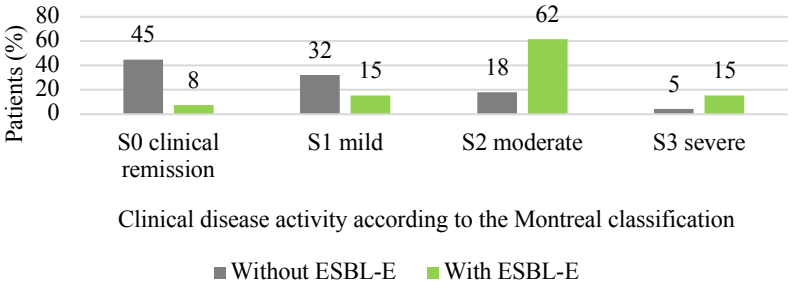

Figure 4.5. **Clinical disease activity according to the Montreal classification in UC patients with (n = 13) and without (n = 109) ESBL-E presence in the gut (p = 0.001)**

The presence of ESBL-E in the gut showed a moderate effect on the UC disease extent in the colon, as assesed by the Montreal classification. X2 (2, n = 122) = 6.35 p = 0.042, ϕ_C = 0.18 (See Figure 4.6). Left-sided (E2) and total or extensive colitis (E3) were more commonly observed in UC patients with ESBL-E found in their gut and, in contrast to the UC patients without ESBL-E in their gut, proctitis (E1) in these patients was not observed at all.

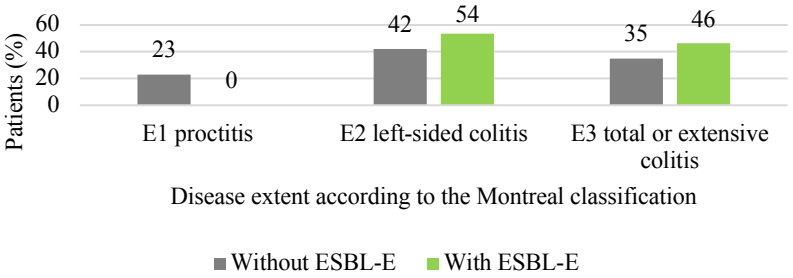

Figure 4.6. **Disease extent according to the Montreal classification in UC patients with (n = 13) and without (n = 109) ESBL-E presence in the gut (p = 0.042)**

Adapted Truelove and Witt's index

The presence of ESBL-E in the gut showed a significant effect on the clinical disease activity as measured by the adapted Truelove and Witt's index. X2 (2, n = 122) = 26.73 $p < 0.0001$, $\phi_C = 0.55$ (See Figure 4.7). Moderate to severe disease activity was predominantly found in UC patients with ESBL-E in their gut, while mild disease activity was predominantly found in UC patients without ESBL-E in their gut.

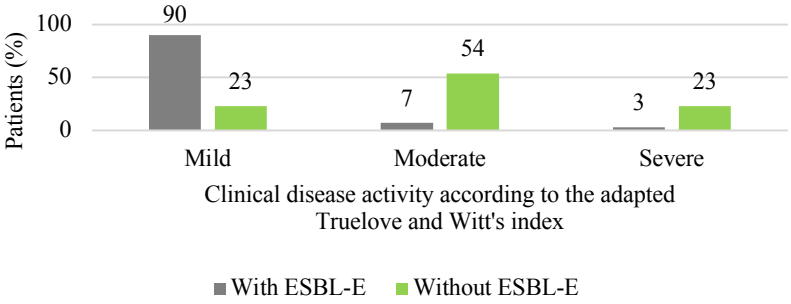

Figure 4.7. **Clinical disease activity according to the adapted Truelove and Witt's index in UC patients with (n = 13) and without (n = 109) ESBL-E presence in the gut ($p < 0.0001$)**

4.3.2. Crohn's disease

Crohn's disease activity index (CDAI)

The presence of ESBL-E in the gut had a significant effect on the clinical disease activity of CD patients, as assessed by the CDAI. X 2 (2, n = 55) = 7.14 $p = 0.028$, $\phi_C = 0.44$ (See Figure 4.8). Moderate disease activity was predominantly observed in CD patients with ESBL-E in their gut, whereas remission and mild disease activity was predominantly observed in CD patients

without ESBL-E in their gut. None of the analyzed CD patients had severe clinical disease activity (CDAI > 450) according to the CDAI.

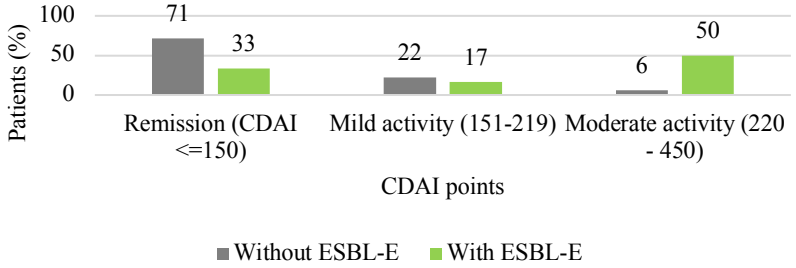

Figure 4.8. **Clinical disease activity according to the CDAI in CD patients with (n = 6) and without (n = 49) ESBL-E presence in the gut (p = 0.028)**

Also when comparing CDAI points in previosly hospitalised CD patients with and without ESBL-E presence in their gut, moderate disease activity (Mdn = 219.53, IQR = 281.71) was observed in CD patients with ESBL-E presence in their gut, whereas remission (Mdn = 103.99, IQR = 119.01) was observed in previosuly hospitalised CD patients without ESBL-E in their gut (U = 64, p = 0.023) (See Figure 4.9).

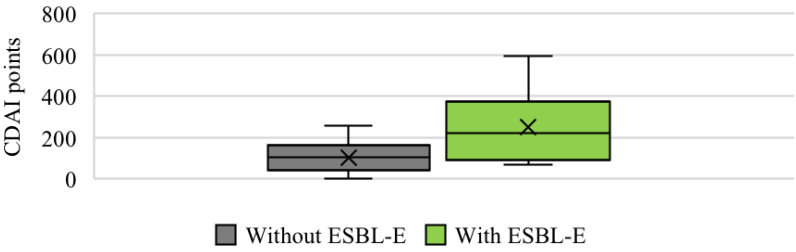

Figure 4.9. **Clinical disease activity points according to the CDAI in CD patients with (n = 6) and without (n = 49) ESBL-E presence in the gut (p = 0.023)**

Harvey-Bradshaw index (HBI)

The presence of ESBL-E in the gut of CD patients had a significant effect on the clinical disease activity, as assessed by the HBI. X2 (2, n = 55) = 9.58 p = 0.008, ϕ_C = 0.48 (See Figure 4.10). Moderate disease activity was predominantly observed in CD patients with ESBL-E in their gut, whereas remission was predominantly seen in CD patients without ESBL-E in their gut.

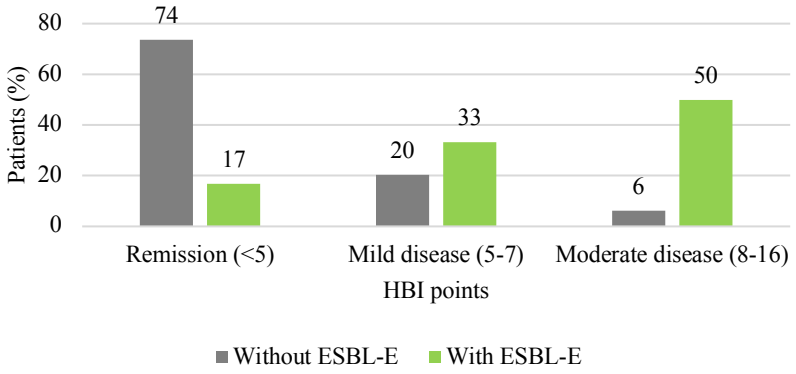

Figure 4.10. **Clinical disease activity according to the HBI in CD patients with (n = 6) and without (n = 49) ESBL-E presence in the gut (p = 0.008)**

Also when comparing HBI points, CD patients with ESBL-E in their gut had moderate disease activity (Mdn = 7, IQR = 8), whereas CD patients without ESBL-E in their gut were in remission (Mdn = 3, IQR = 5), U = 54, p = 0.01 (See Figure 4.11).

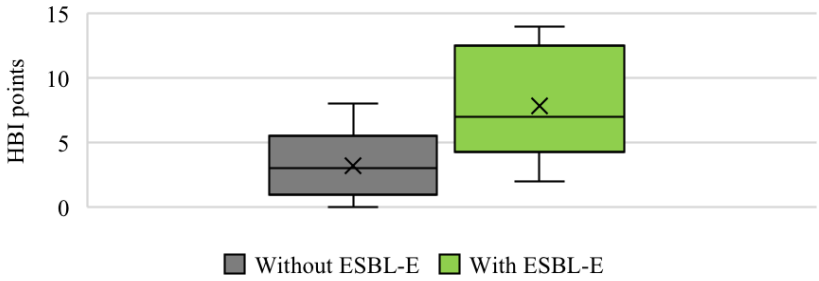

Figure 4.11. **Clinical disease activity points according to the HBI in CD patients with (n = 6) and without (n = 49) ESBL-E presence in the gut (p = 0.01)**

Montreal classification

No statistically significant differences were found between CD patients with and without the presence of ESBL-E their gut according to the Montreal classification – age of CD diagnosis (p = 0.4), clinical behavior of the disease (p = 0.54) and the disease localisation (p = 0.49).

5. DISCUSSION

5.1. Previous studies describing ESBL-E in IBD patients

Up untill now there have been three studies (Leung et al., 2012; Li et al., 2015; Vaisman et al., 2013) describing ESBL-E in IBD patients. All studies have been carried out between 2012 and 2015. These studies have looked at prevalence, incidence and risk factors for gut colonisation with ESBL-E and ESBL-E species. None of these studies have previously analysed whether there is a difference in UC and CD clinical disease activity between patients with and without ESBL-E presence in their gut. Also bacterial plasmid genes that determine ESBL production have never been described in IBD patient population before. Previous studies have been conducted in North America (Canada and the USA) (Leung et al., 2012; Vaisman et al., 2013) and Asia (China) (Li et al., 2015), but ESBL-E prevalence has never been described in IBD patient population in Europe before.

This study is providing additional information to the results of the previous studies – giving insight into frequency of ESBL-E presnce in the gut, as well as bacterial plasmid genes that determine ESBL-E production in previously hospitalised UC and CD patients in Europe. Also this study looks at ESBL-E in IBD patients from a completely new perspective – analyses whether there is a difference in IBD clinical activity in previously hospitalised UC and CD patients with and without ESBL-E presence in their gut. However, when interpreting the results of this study, the limitations of the study should be taken into consideration (see Section 5.4).

5.2. Comparison of ESBL-E parameters to the data described in previous studies

The frequency of ESBL-E presence in the gut of previously hospitalised UC and CD patients that was found in this study (11 %) is considered to be high when compared to frequency of presence reported in other studies in IBD patients, where it ranges from 4 % to 11 % (Leung et al., 2012; Vaisman et al., 2013). The frequency of ESBL-E presence in the gut of previously hospitalised UC and CD patients found in this study was almost three times higher than the prevalence of ESBL-E presence in the gut in the general population (4 %) in the region where this study was conducted (Karanika et al., 2016). However, when comparing the results, the patient sample used in this study and other study limitations should be taken into consideration (see Section 5.4).

Data found in this study concerning ESBL-E species and bacterial plasmid genes that determine ESBL production correspond to the data described in the previous studies – both in the general population and in IBD patient population. *E. coli* (Li et al., 2015) is the most common ESBL-E and CTX-M encoded by the *bla*CTX-M bacterial plasmid gene is the most common type of ESBL in Europe and worldwide (Shaikh et al., 2015). As mentioned above, when interpreting and comparing the results of this study to other studies, the limitations of the study (see Section 5.4) and the IBD patient cohort used in this study should be taken into account. The previosuly hospitalised IBD patients used in this study may reflect a specific IBD patient cohort.

5.3. Differences in IBD clinical activity in previously hospitalised UC and CD patients with and without ESBL-E presence in their gut

In this study, higher IBD clinical activity was observed in both previously hospitalised UC and CD patients in cases when ESBL-E were detected in patients' gut. The IBD clinical activity was higher according to several scores, indices and classifications that are commonly used in clinical practice and clinical trials. In addition, previously hospitalised UC patients with ESBL-E presence in their gut had more extensive inflammation in the colon, according to the Montreal classification. The differences found in the grading of IBD clinical activity (clinical remission and mild activity vs. moderate activity) could also be of a potential clinical significance, since the grading of clinical disease activity (mild, moderate and severe) determines not only different prognosis, complication and oncological disease development, but it is also related to the different medication use, costs, quality of life and mortality rates – the higher the IBD clinical activity, the worse the prognosis (Gomollón et al., 2017; Magro et al., 2017). However, the limitations of the study should be taken into account when interpreting the results of this study (see Section 5.4).

A higher IBD clinical activity in UC and CD patients with ESBL-E presence in their gut has not been previously described, but there are several studies (Nakai et al., 2016; Leung et al., 2012; Vaisman et al., 2013; McLaughlin et al., 2010 Arcilla et al., 2017) suggesting similar differences: 1) both colonization and infection with ESBL-E are more commonly observed in severely ill patients and in patients with chronic medical conditions (Nakai et al., 2016); 2) severity and chronic course of IBD is cited as a hypothetical reason why IBD patients might be colonised with ESBL-E more often than the general population (Leung et al., 2012; Vaisman et al., 2013); 3) presence of ESBL-E in the gut is associated not only with inflammation of the intestinal wall in case of pouchitis, but also with pre-pouch ileitis (McLaughlin et al., 2010); 4) pre-

existing bowel disease has been identified as a risk factor for gut colonisation with ESBL-E in the general population (Arcilla et al., 2017); 5) pathological abnormalities in gut microbiota (a composition of all intestinal bacteria, viruses and fungi) – dysbiosis. Dysbiosis plays an important role in etiology and pathogenesis of IBD (Halfvarson et al., 2017; Li et al., 2015; Rutgeerts et al., 1991; O'Brian & Downward, 2017). Dysbiosis is seen in exacerbations of IBD and higher disease activity (Halfvarson et al., 2017). One of the characteristics of dysbiosis in the case of IBD is the increased proportion of *Enterobacteriaceae* and specifically *E. coli* in the gut microbiota compostion (Imhann et al., 2016; Nagy-Szakal et al., 2017; Ochoa-Repára et al., 2017). It is also known that in case of dysbiosis, the gut is more frequently colonised with MDRM, including ESBL-E (Buelow et al., 2017). These might be the possible reasons why ESBL-E are more prevalent in the IBD patient population compared to the general population. Also, these could be the reasons for more frequent ESBL-E presence in the gut in IBD patients with higher IBD clinical activity.

However, this study only indicates the differences in IBD clinical activity in cases with and without ESBL-E presence in the gut, and further studies are needed to discover if there is an association between ESBL-E presence in the gut and higher IBD activity.

5.4. Limitations of the study

When interpreting the results of this study, the differences in IBD clinical activity found between patients with and without ESBL-E presence in their gut cannot be directly applied to the entire IBD patient population, because IBD outpatients who have never been hospitalised before were not analysed in this study. The IBD patient cohort analysed in this study (previously hospitalised IBD patients) may reflect a specific group of IBD patients, who might have previously

been in contact with the medical system more often, might have previously received a more intensive treatment, might possess a more severe underlying disease and more likely might get colonised with ESBL-E than patients who have never been hospitalised before. This study also does not include currently hospitalised IBD inpatients who often have a more severe IBD clinical activity. Moreover, IBD patients are selected from the two largest tertiary medical centers in Latvia – RAKUS and PSKUS; therefore, the hospitalisation tendencies of these hospitals could influence the patient selection. Also, the frequency of ESBL-E presence in the gut, ESBL-E species and bacterial palsmid gene profiles could be affected by the local infection control practices applied in these medical centers.

The distribution of the patients within the ESBL-E positive (patients with ESBL-E presence in their gut) versus ESBL-E negative (patients without ESBL-E presenec in their gut) groups should also be taken into consideration. The number of the ESBL-E positive patients is significantly lower, compared to the ESBL-E negative patients in both the UC and CD patient groups. Particular attention should be paid to the rather small and heterogeneous CD patient group. This fact could affect the ability to replicate the results of this study. It would be more appropriate to compare the ESBL-E positive and ESBL-E negative patients while taking into consideration other relevant factors that could influence the IBD activity.

A control group is needed to correctly interpert the significance of ESBL-E presence in the gut in IBD patient population. The control group could be the general population, also other chronic and autoimmune disease patient populations.

Despite the widly used indices, scoring systems and classifications also used in this study, these scoring systems are often not validated and criticised as subjective (Spekhorst et al., 2014; Walsh et al., 2016; ECCO, 2016). Further

studies should use more objective criteria for IBD activity determination, such as faecal calprotectin, centrally read endoscopy data, standardised laboratory and morphological results or magnetic resonance imaging data in case of CD.

Endoscopy, histology and laboratory data were not obtained primarily for this study. Medical records with data from examinations conducted in the previous month at various medical facilities and at different time points relative to the study visit were used in this study. This could affect the quality of objective data and could affect both the results of this study and the ability to reproduce the results of this study in the future.

Potential confounding factors that might affect the IBD clinical activity as well as ESBL-E presence in the gut have not been analysed in this study, such as composition of the gut microbiota, the treatment methods and medications used, the course and the nature of IBD, etc. These factors could influence the frequency of ESBL-E presence in the gut, the ESBL-E species and bacterial plasmid gene data as well as be the possible reasons for a higher IBD clinical activity in patients with ESBL-E presence in the gut.

5.5. Possible reasons for the differences in IBD clinical activity in previously hospitalised UC and CD patients with and without the presence of ESBL-E in their gut and possible directions for further development of the study

Based on the evidence of the previous studies (see Section 5.3), the presence of ESBL-E in IBD patients' gut may seem self-explanatory. At a time when so many associations can be explained by the changes in the entire gut microbiota, the likelihood that a single microorganism or a group of microorganisms (ESBL-E) may play a vital role in the etiology or pathogenesis of a disease (UC and CD) seems less likely. However, there is some evidence for this hypothesis as well: microorganism-related intestinal infections, such as

Salmonella spp., Shigella spp., Yersinia spp., Campylobacter spp., E. coli O157:H7, C. difficile, CMV infections and instestinal tuberculosis, are both differential diagnoses of IBD, as well as potential triggers of IBD onset or more severe inflammation (Gomollón et al., 2017; Magro et al., 2017; Rahier et al., 2014); it is also known that higher IBD activity is associated with the presence of adherent invasive *E. coli* (AIEC) in the gut (Palmela et al., 2018). The presence of ESBL-E in IBD patients' gut with moderate to severe IBD clinical activity could play a similar role as these pathogens. However, further studies are needed to research this possibility.

To analyze the association between ESBL-E presence in the gut and higher IBD activity, disease activity should be measured over time (at least in IBD exacerbation and remission) by controlling the possible confounding factors that could affect both the IBD activity and the presence of ESBL-E in patients' gut. Interventions should be undertaken to reduce the presence of ESBL-E in patients' gut, such as the correction of dysbiosis or eradication of ESBL-E, in parallel with exploring the possibility of a spontaneous gut decolonisation. Future research should address the issue whether the presence of ESBL-E in patients with IBD is responsible not only for a more severe disease activity, but also for a more severe disease course – more frequent hospitalisations, more intensive use of medications and the need for IBD surgery. The limitations of the study mentioned above (see Section 5.4) should also be taken into account and corrected in the future studies.

Even if in the further studies it turns out that ESBL-E do not determine a higher IBD activity and only colonises IBD patients' gut in cases of a higher IBD clinical activity, ESBL-E also have their own epidemiological and clinical value in IBD patients involving infection control and treatment strageries. This allows to further investigate whether IBD patients with moderate to severe IBD clinical activity are reservoirs for further ESBL-E spread. A potential area of research for

ESBL-E spread would be screening and following-up IBD patients' household and medical contacts. It provides an opportunity to investigate the need and the cost-effectiveness of ESBL-E screening in specific IBD cohorts, for example in prevously hospitalised IBD patients with moderate to severe IBD clinical activity. Also further studies could be carried out to find out whether IBD patients aquire ESBL-E in the outpatient or inpatient setting, what are the risk factors for acquiring ESBL-E in IBD patient population, how long ESBL-E are present in the gut, whether it is necessary and what are the most successful and cost-effective strategies to reduce the presence of ESBL-E in the gut.

6. CONCLUSIONS

1. The presence of extended spectrum beta-lactamase producing Enterobacteriaceae (ESBL-E) was found in the gut of previously hospitalized patients with ulcerative colitis (UC) and Crohn's disease (CD) in 11% of the cases. ESBL producing E. coli containing *bla*CTX-M bacterial plasmid gene were the most frequently detected ESBL-E.

2. Clinical remission, mild clinical disease activity and left-sided colitis was most frequently observed in previously hospitalized UC patients, while clinical remission and inflammation in ileum was most frequently observed in previously hospitalized CD patients.

3. Higher clinical inflammatory bowel disease (IBD) activity was observed in previously hospitalised UC and CD patients with ESBL-E presence in their gut. More extensive impairment in colon was observed in previously hospitalised UC patients with ESBL-E presence in their gut.

7. PRACTICAL RECOMMENDATIONS AND SUGGESTIONS

1. Further studies, including IBD outpatients who have never been hospitalised before and currenty hospitalised IBD inpatients, are needed to ascertain whether the same differences found in this study are also true in these IBD patient cohorts. More objective and standardised criteria should be used to determine the IBD activity.

2. Further studies are needed to determine the importance of ESBL-E presence in the gut in association with the IBD activity, to further investigate the need of ESBL-E eradication in order to reduce IBD activity.

3. Further studies should look at the need and the cost-effectiveness of screening for ESBL-E in previously hospitalised IBD patients with moderate to severe clinical disease activity and more extensive disease, in order to apply infection control measures to prevent a further spread of ESBL-E within the medical system.

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1. **V. Skuja**, A. Derovs, K. Pekarska, D. Rudzīte, E. Lavrinoviča, L. Piekuse, I. Kempa, Z. Straume, E. Eglīte, A. Krūmiņa, A. Lejnieks, R. Eliakim. Gut colonization with ESBL producing *Enterobacteriaceae* may increase disease activity in biologic-naïve out-patients with ulcerative colitis: an interim analysis. European Journal of Gastroenterology and Hepatology, Volume 30 (1), January, 2018, 92-100. ISSN:0954-691X; Online ISSN: 1473-5687 (PubMed)
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Awards

1. 25th United European Gastroenterology Week – National Scholar Award 2018 – Barcelona, Spain, October 2017
2. RSU Gada doktorants 2017 (PhD student of the year) – Riga, Latvia, April, 2017
3. 24th United European Gastroenterology Week – National Scholar Award 2017 – Vienna, Austria, October 2016

APPENDICES

Ulcerative colitis classifications

Ulcerative colitis disease extent according to the Montreal classification

	Disease extent	Description
E1	Ulcerative proctitis	Involvement limited to the rectum (proximal extent of inflammation is distal to the rectosigmoid junction)
E2	Left-sided UC (distal UC)	Involvement limited to a portion of the colorectum distal to the splenic flexure
E3	Extensive/total UC (pancolitis)	Involvement extends proximal to the splenic flexure

(ECCO, 2016; ECCO, Montreal Classification; A. Dignass et al., 2012)

Ulcerative colitis disease activity according to the Montreal classification

	Disease activity	Description
S0	Clinical remission	Asymptomatic
S1	Mild ulcerative colitis	Stool frequency <= 4 x/day (with or without blood), no systemic symptoms and normal inflammatory markers (ESR)
S2	Moderate ulcerative colitis	Stool frequency >4 x/day, but with minimal systemic symptoms
S3	Severe ulcerative colitis	Stool frequency at least 6 x/day with blood, pulse rate at least 90 x/min, body temperature at least 37.5 °C, hemoglobin < 10.5 g/100 ml and ESR at least 30 mm/h.

(ECCO, 2016; ECCO, Montreal Classification; A. Dignass et al., 2012)

Full Mayo score components and points

Parameter	Clinical evaluation (points)
1. Stool frequency (per day)	1. Normal (0) 2. 1–2 x/day > normal (1) 3. 3–4 x/day > normal (2) 4. > 4 x/day > normal (3)
2. Rectal bleeding (indicating the most severe bleeding of the day)	1. None (0) 2. Streaks of blood with stool in less then half of the cases (1) 3. Obvious blood with stools in most cases (2) 4. Blood alone passes (3)
3. Endoscopic findings	1. Normal or inactive disease (0) – normal mucosa or inactive disease 2. Mild disease (1) – erythema, reduced vascular pattern, mild mucosal friability

	3. Moderate disease (2) – noticeable erythema, lack of vascular pattern, moderate mucosal friability, erosions, 4. Severe disease (3) – spontanoues blooding, exudation, ulcers
4. Physcian's global assessment	1. Normal (0) 2. Mild (1) 3. Moderate (2) 4. Severe (3)

(ECCO, 2016; ECCO, Montreal Classification; A. Dignass et al., 2012)

Full Mayo score interpretation

Points	Interpretation
0–2	remission (if no individual parameter subscore is higher than 1)
3–5	mild activity
6–10	moderate activity
> 10	severe activity

(ECCO, 2016; ECCO, Montreal Classification; A. Dignass et al., 2012)

Partial Mayo score interpretation

Points	Interpretation
< 2	resmission
2–4	mild activity
5–7	moderate activity
> 7	severe activity

(ECCO, 2016; ECCO, Montreal Classification; A. Dignass et al., 2012)

Adapted Truelove and Witt's index in patients with ulcerative colitis

Activity	Bloody stools per day	Pulse	Body temperature	Hgb	ESR (or CRP)	CRP (or ESR)
Mild	< 4	< 90 x/min	< 37.5 °C	> 11.5 g/dl	< 20 mm/h	Normal
Moderate	4 and more, if:	≤ 90 x/min	≤ 37.8 °C	≥ 10.5 g/dl	≤ 30 mm/h	≤ 30 mg/l
Severe	≥ 6 and:	> 90 x/min or	> 37.8 °C	< 10.5 g/dl or	> 30 mm/h	> 30 mg/l

(ECCO, Truelove & Witt's Score; ECCO, 2016)

Crohn's disease classifications

Montreal classification in patients with Crohn's disease

Age at diagnosis	A1	< 16 years
	A2	17–40 years
	A3	> 40 years
Localisation of the disease	L1	ileum
	L2	colon
	L3	ileum and colon
	L4*	only the upper gastrointestinal tract*
Clinical disease behaviour	B1	does not form strictures or penterations
	B2	Stricturising disease
	B3	Penetrating disease
	p	is added to B1-B3, if the patient has perianal CD

*L4 is also used as a modifier, that can be added to L1-L3, if the patient has Crohn's disease in the upper gastrointestinal tract.

(ECCO, Truelove & Witt's Score; ECCO, 2016)

Crohn's disease activity index (CDAI)

Criteria	**Descroption**	**Coefficient and calculation**
A. Liquid stools	Number of liquid or soft stools (sum of the previous 7 days)	Sum x2
B. Abdominal pain	Sum of the previous 7 days: No (0) Mild (1) Moderate (2) Severe (3)	Sum x5
C. General well-being	Sum of the previus 7 days: Generally well (0) Slightly below par (1) Poor (2) Very poor (3) Terrible (4)	Sum x7
D. Extra-intestinal manifestations	No (0) Arthritis/arthralgia (1) Iritis/uveitis (1) *Erythema nodosum* (1) *Pyoderma gangrenosum* (1)	Points x20

	Aphtous stomatitis (1) Anal fissures/fistulas/abscesses (1) Fever > 37.8 °C (1)	
E. Anti-diarrhea drug use	For reducing the symptoms of diarrhea No (0) Yes (1)	Value x30
F. Abdominal mass	On palpation of the abdomen No (0) Questionable (2) Definite (5)	Value x10
G. Hematocrit	From a recent laboratory test Male (47 – hematocrit) Female (42 – hematocrit)	Value x6
H. Weight	Current weight of the patient Ideal (standard) weight for females Ideal (standard) weight for males	100 x (1 – (current/standard)

(ECCO, CDAI; IG-IBD Scores, CDAI)

Crohn's disease clinical disease activity grading according to CDAI

Points	**Interpretation**
<=150	remission
151–219	mild activity
220–450	moderate activity
> 450	severe activity

(ECCO, CDAI; IG-IBD Scores, CDAI)

Description of Crohn's disease activity according to CDAI

Mild	**Moderate**	**Severe**
CDAI 150–220	**CDAI 220–450**	**CDAI > 450**
Outpatient, the patient is eating, drinking, weight loss < 10 %, no signs of obstruction, no fever, no dehydration, no palpable abdominal masses/ formations or tenderness. CRO is usually raised above the upper limit of the normal.	Intermittent vomiting or weight loss > 10 %. Ineffective treatment of mild disease or sensitive abdominal masses. There is no obvious sign of obstruction. CRO is increased above the upper limit of normal.	Cachexia (BMI < 18 kg/m2) or evidence of obstruction or abscess. Persistent symptoms despite intensive treatment. Increased CRO above the upper limit of normal.

(ECCO, CDAI; IG-IBD Scores, CDAI)

Harvey-Bradshaw index (HBI)

Parameter	Points
1. General well-being (the previous day)	Very well (0), Slightly below par (1), Poor (2), Very poor (3), Terrible (4)
2. Abdominal pain (the previous day)	No (0), Mild (1), Moderate (2), Severe (3)
3. Number of liquid or soft stools (the previous day)	The number usually ranges between 1–25
4. Abdominal mass	No (0), Questionable (1), Definite (2), Definite and painful (3)
5. Complications	No (0), Arthralgia (1), Uveitis (1), *Erythema nodosum* (1), *Pyoderma gangrenosum* (1), Aphtous ulcer (1), Anal fissures (1), Abscess (1), Appearance of a new fistula (1)

(Harvey & Bradshaw, 1980; ECCO, HBI; Alberta Health Services, Harvey Bradshaw Index, 2016)

Crohn's disease activity according to Harvey-Bradshaw index (HBI)

Points	Interpretation
< 5	resmission
5–7	mild activity
8–16	moderate activity
> 16	severe activity

(Harvey & Bradshaw, 1980; ECCO, HBI; Alberta Health Services, Harvey Bradshaw Index, 2016)