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LABORATORY TESTING OF ANTI-DRUG ANTIBODIES IN BIOLOGICAL THERAPY OF INFLAMMATORY BOWEL DISEASE

PhD thesis

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LIST OF ABBREVIATIONS

5-ASA – 5-aminosalicylates

AADA – Anti-Adalimumab Antibody

ADA – Adalimumab

AIFX – Anti-Infliximab Antibody

ANCA – Anti-neutrophil Cytoplasmic Antibody

ASCA – Anti-Saccharomyces Cerevisiae Antibody

AUC – Area Under Curve

AUST – Anti-Ustekinumab Antibody

AVDZ – Anti-Vedolizumab Antibody

CD – Crohn's Disease

CRP – C-reactive Protein

ELISA – Enzyme-linked Immunosorbent Assay

ESPGHAN – European Society for Paediatric Gastroenterology Hepatology and Nutrition

ESR – Erythrocyte Sedimentation Rate

IBD – Inflammatory Bowel Disease

IBD-U – Inflammatory Bowel Disease - Unclassified

IFX – Infliximab

Ig - Immunglobulin

LoD – Limit of Detection

LOR – Loss of Response

NAb – Neutralizing Antibodies

OR – Odds Ratios

RES – Reticulo-Endothelial System

TDM – Therapeutic Drug Monitoring

TNF-α – Tumor Necrosis Factor-alpha

TRL – Trough Level

UC – Ulcerative Colitis

UST – Ustekinumab

VDZ – Vedolizumab

1. INTRODUCTION

Inflammatory bowel diseases (IBD) are affecting more and more people both worldwide and in Hungary. The age of IBD patients (Crohn's disease (CD) and ulcerative colitis (UC) patients) is also decreasing: approximately 15-25% of patients are diagnosed with the disease before the age of 20 [1; 2].

1.1 Overview

The two best-known types of IBD are CD and UC, but there is also a group that does not clearly belong to either form: IBD-U (**Table 1**) [3; 4].

1. **Table** Types of IBD and characteristics of the disease

Type	Affected area	Nature of inflammation	Depth	Common symptoms
Crohn's disease	Anywhere in the digestive tract	Intermittent, patchy	Deeper layers	Abdominal pain, diarrhea, weight loss
Ulcerative colitis	Only the large intestine	Continuous	Mucous membrane	Bloody diarrhea, cramps
Inflammatory bowel disease unclassified	Unclear	Mixed	Mixed	Transient symptoms

1.1.1. Pathophysiology

The pathogenesis of IBD is extremely complex and involves the interaction of numerous factors. Its development is multifactorial: in addition to genetic and environmental influences and nutrition, it is caused by immune system dysregulation and intestinal

microbiome disbiosis. In all cases, the diseases are caused by increased activity of the intestinal immune system, which results in tissue damage [5; 6; 7; 8; 9].

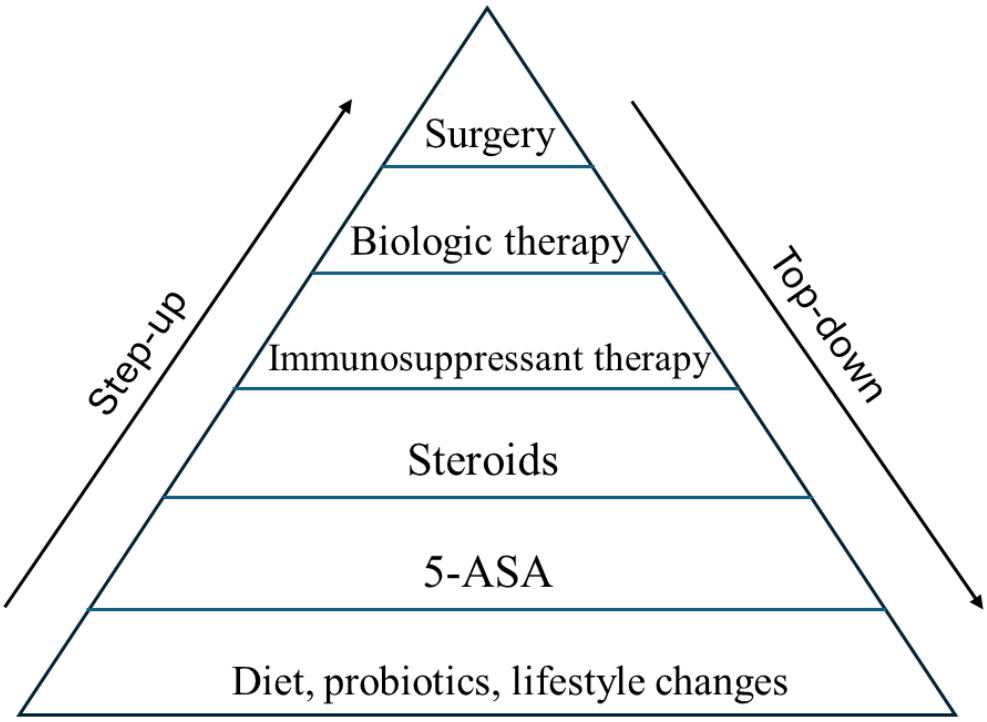
1.1.2. Diagnosis and Clinical Picture

The diagnosis of inflammatory bowel disease (IBD) is based on a combination of clinical[10] presentation, endoscopic findings, histological examination, and imaging modalities. Colonoscopy with ileal intubation represents a key diagnostic tool, particularly in ulcerative colitis, but it is not sufficient as a standalone gold standard in all cases, especially in Crohn's disease, where upper endoscopy is also mandatory [11; 12; 13]. Endoscopic evaluation allows assessment of the location, extent, and severity of mucosal inflammation, while targeted biopsies provide histopathological confirmation and help to differentiate between IBD subtypes. In Crohn's disease, cross-sectional imaging techniques such as magnetic resonance enterography [14] or computed tomography are often required to evaluate transmural inflammation and small bowel involvement [15; 16]. Laboratory markers such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) [17], as well as measurement of albumin levels as a negative acute phase protein [18], can help monitor the activity of inflammation. Fecal biomarkers (calprotectin, lactoferrin, M_2pyruvate kinase, and immunological tests like perinuclear anti-neutrophil cytoplasmic antibody (pANCA), anti-Saccharomyces cerevisiae antibody (ASCA) are helpful in differential diagnosis [19]. The clinical picture is diverse, with patients experiencing diarrhea, abdominal pain, bleeding, and weight loss.

1.1.3. Therapies

Pharmacological treatment: Pharmacotherapy used in the treatment of inflammatory bowel disease (IBD) includes aminosalicylates, corticosteroids, immunomodulators, and biological therapies (**Figure 1**). The treatment of moderate to severe IBD is based on anti-TNF-α (tumor necrosis factor) [20] preparations IFX, ADA (infliximab, adalimumab), and second- and third-line biologics, such as the integrin inhibitor VDZ (vedolizumab) and the direct anti-IL-12/23 UST (ustekinumab), which specifically reduce intestinal inflammation [21; 22]. Key factors in achieving and maintaining clinical and mucosal remission include pharmacokinetic variability, patient- and disease-specific characteristics, and regular and targeted monitoring of immunogenicity (anti-drug antibodies) [23; 24].

Surgical intervention: If drug treatment does not lead to in adequate clinical improvement requires surgical management. Surgery is intended to remove sections of the intestine or to treat intestinal perforations. Approximately 20–30% of patients with IBD will require surgical intervention at some point during their lifetime [25; 26].



1. **Figure.** The higher we go up the pyramid, the more targeted the therapy, but along with this, adverse effect risk also increases. The treatment strategy can range from mild to severe or early targeted therapy to prevent complications [27]. (Abbreviations: 5-ASA: 5-aminosalicylates).

1.2. Mechanism and clinical role of biological therapies

1.2.1. Mechanism of action

Infliximab, a chimeric IgG1 (Immunoglobulin) monoclonal antibody, and adalimumab, a completely human IgG1 antibody (**Figure 2**), neutralize tumor necrosis factor-α (TNF-α), thereby inhibiting the downstream inflammatory cascade, reducing the expression of endothelial adhesion molecules, and suppressing the activity of effector immune cells [17; 28; 29].

Vedolizumab is mainly used as a second- or third-line treatment for moderate to severe Crohn's disease and ulcerative colitis. Vedolizumab is an anti-α4β7 integrin monoclonal antibody that inhibits the entry of lymphocytes into the intestinal wall, thereby selectively reducing inflammation in the intestine. As a result, it has a good safety profile and a lower risk of systemic infection, but the clinical effect is often slower to develop [30; 31; 32].

Ustekinumab inhibits the p40 subunit of the cytokines IL-12 and IL-23, thereby exerting a systemic anti-inflammatory effect by reducing the Th1 and Th17 immune responses. It is effective in both Crohn's disease and ulcerative colitis, and may also be beneficial in extraintestinal manifestations. It is often used after TNF inhibitor therapy and is generally characterized by a faster clinical response than vedolizumab [33].

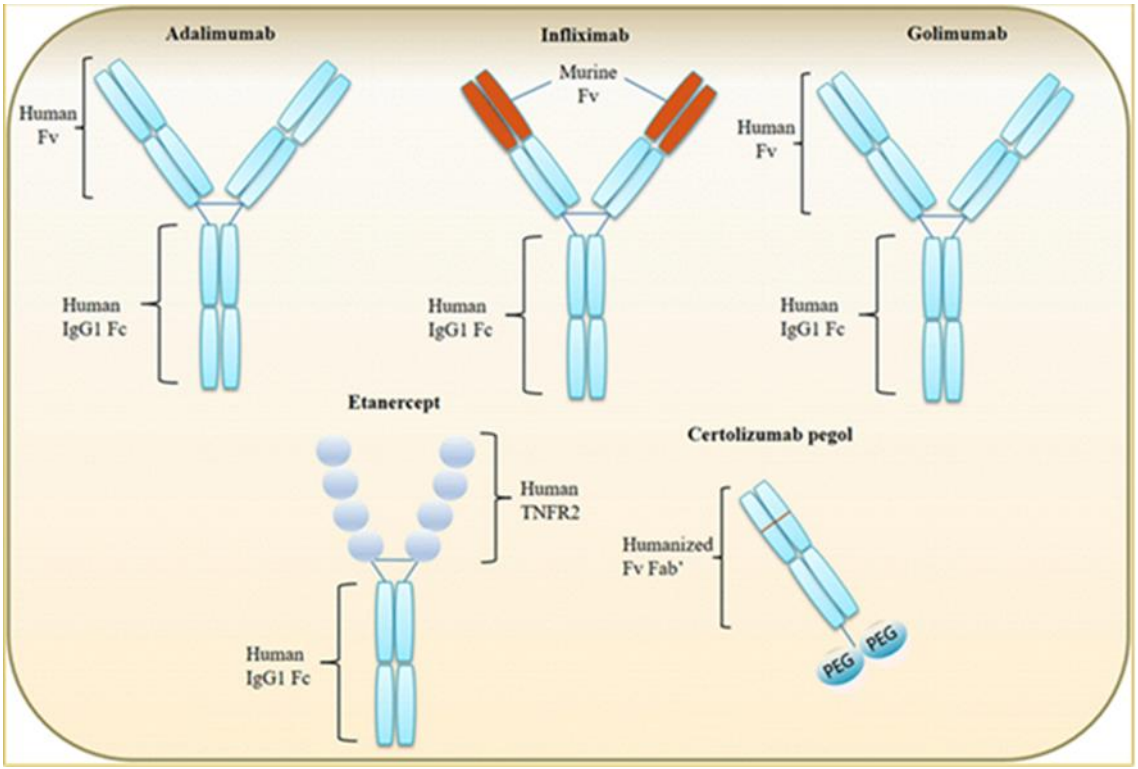


Figure 2. Immune structure of various biological therapeutic drugs [34]. (Abbreviations: TNFR-Tumornecrosis Factor Alpha Receptor, Fv fragment variable, Fc-fragment constant)

1.2.2. Clinical indication

The use of anti-TNF-alpha drugs is recommended for achieving and maintaining remission in Crohn's disease and ulcerative colitis, with the goal of achieving and sustaining mucosal healing. In pediatric patients, effective anti-TNF therapy is of particular importance for preserving normal growth and development. Vedolizumab and ustekinumab are both indicated for the therapy of moderate to severe Crohn’s disease and ulcerative colitis in adults who have an inadequate response, lost response, or intolerance to conventional therapies or TNF inhibitors (see **Table 2**) [35; 22; 36].

1.2.3. Pharmacokinetics of Infliximab

Clearance of infliximab is influenced by multiple factors: markers of disease severity and inflammation, including serum albumin levels and body weight, concomitant immunosuppressive therapy, immunogenicity (anti-drug antibodies), and route of administration (intravenous vs. subcutaneous) [37; 38].

1.2.4. Pharmacokinetics of Adalimumab

After subcutaneous administration, adalimumab is slowly absorbed, with an absolute bioavailability of approximately 60–65%, and peak serum concentrations typically reached within 5–7 days. Its distribution is largely confined to the intravascular and interstitial spaces, with a small apparent volume of distribution (~5–6 L), consistent with its monoclonal antibody structure. Adalimumab is not metabolized by cytochrome P450 enzymes but is degraded via nonspecific proteolytic catabolism into small peptides and amino acids. The terminal elimination half-life is about 14 days, supporting dosing every other week; clearance may be influenced by body weight, concomitant immunosuppressive therapy, and the presence of anti-adalimumab antibodies [39].

1.2.5. Pharmacokinetics of Vedolizumab

Vedolizumab exhibits linear pharmacokinetics over the therapeutic dose range. Following intravenous administration, its distribution occurs mainly in the

vascular and interstitial spaces, with a small volume of distribution (~4.8 L). It is degraded via nonspecific proteolytic catabolism rather than hepatic metabolism. The terminal half-life averages 25 days, allowing dosing every 4–8 weeks. Clearance may be affected by the presence of anti-vedolizumab antibodies and patient body weight, but it is generally low and consistent with other monoclonal antibodies [40].

1.2.6. Pharmacokinetics of Ustekinumab

Ustekinumab exhibits linear pharmacokinetics across therapeutic doses. Following intravenous administration, it distributes predominantly in blood vessels and interstitial spaces with a small volume of distribution (~5–6 L). Metabolism occurs through nonspecific proteolytic degradation to peptides and amino acids rather than hepatic enzymatic pathways. Its terminal half-life is approximately 21 days, supporting dosing intervals ranging from every 8 to 12 weeks. Clearance can be influenced by body weight and the development of anti-ustekinumab antibodies, although immunogenicity is generally low [36].

Table 2. Summary table of the applied biological therapies. Abbreviations: IBD: inflammatory bowel disease; CD: Crohn's disease; UC: ulcerative colitis; TNF: tumor necrosis factor alpha; IL: interleukin. Based on publicly available data in the drug registry [41; 42].

Active Ingredient (Brand)	Target	Key Study / Efficacy	Typical Dosing	Approx. Cost
Infliximab (Remicade; biosimilars: Zessly, Inflectra, Remsima, Flixabi)	TNF-α, chimeric IgG1	REACH [43] (CD): wk10 – 84% response, 59% remission	5 mg/kg IV wk0,2,6; then q8w	100 mg vial: 97,000–154,000 HUF
Adalimumab (Humira; biosimilars: Hyrimoz, Yuflyma, Amgevita, Hulio, Idacio, Hukyndra)	TNF-α, fully human IgG1	RESEAT [44](CD): wk10 – 82% response; IMAGINE [45] (CD): wk26 – 34% remission	40 mg SC q2wks (induction: double dose for first 1–2 injections)	40 mg prefilled pen: 174,000–275,000 HUF/2
Vedolizumab (Entyvio)	α4β7 integrin, human IgG1	GEMINI [46]: CD wk6 – 15% vs 7%; UC wk6 – 47% vs 26%; peds wk14 – UC 37–76%, CD 14–42%	300 mg IV wk0,2,6; then q8w	300 mg vial: 702,000 HUF
Ustekinumab (Stelara; biosimilars: Yesintek, Imuldosa, Pyzchiva, Steqeyma, Uzpruvo, Wezenla)	IL-12/23, human IgG1	UNITI [40] (CD): wk6 – TNF-experienced 34% vs 22%, TNF-naïve 52% vs 29%; peds CD approved	IV 6 mg/kg wk0 (≤55 kg 260 mg, 55–85 kg 390 mg, >85 kg 520 mg), then SC 90 mg wk8 and q12w	Vials: 45–130 mg 823,000–852,000 HUF; 90 mg SC 1,849,000 HUF

1.3. Antibody formation: immunogenicity, types, and determining factors

Antibodies play a particularly key role in the treatment of IBD, as biological treatments such as TNF-α inhibitors (e.g., IFX, ADA) or VDZ and UST work by using different types of antibodies. These biological therapeutic drugs behave as antigens, so they can trigger antibody formation, which can affect the effectiveness of treatment and the occurrence of side effects [47].

1.3.1. Antibody formation during biological treatments

The process occurs in the following steps:

Antigen recognition and B-cell activation: A biological drug such as IFX, which is a chimeric (half human, half animal) monoclonal antibody, can act as an antigen when it enters the body. The antigen activates B cells, which produce antibodies (counter-substances) against the drug [48].

Immunological reactions: The development of anti-drug antibodies can not only reduce the effectiveness of the drug, but also cause side effects such as allergic reactions. The development of antibodies can worsen the patient's condition, as the reduced effectiveness of the drug can cause IBD symptoms to flare up again [49].

IFX and ADA can effectively reduce inflammation in the therapy of IBD, but antibody responses to the drugs can affect treatment efficacy. Excessive antibody responses can cause patients to not respond adequately to treatment, and symptoms may return [50]. The frequency of drug use, dosage, and individual genetic factors influence the extent to which antibodies develop. As antibodies develop, drug levels may decrease, requiring dose adjustment or review of therapy [51]. Newer biologic drugs, such as VDZ or UST, which target the intestinal tract more specifically, may reduce the likelihood of antibody formation [22].

1.4. Monitoring treatment efficacy: measuring drug levels and antibodies

Measuring drug levels and any antibodies that may form during biological therapy plays a key role in monitoring treatment effectiveness. Blood tests to measure antibodies to drugs can help determine whether the patient has responded to treatment with antibodies;

if the effect of the therapy is less than expected, the treating physician may decide to change the drug [52].

1.4.1. Types of antibodies

1. Neutralizing antibodies (NAb): These antibodies attach directly to the active site (Fab region) of the drug-antibody complex, preventing the drug from binding to the target molecule (e.g., TNF-α, α4β7 integrin). This directly neutralizes the therapeutic effect.
2. Non-neutralizing antibodies (Non-neutralizing/Binding Antibodies): These antibodies are capable of binding to other parts of the drug. Although they do not directly inhibit binding, they generate immune complexes that are removed more rapidly via the reticuloendothelial system (liver, spleen). This can result in a reduction in the half-life of the drug and a drop in serum levels (accelerated clearance), but in some cases there is no effect [53].

1.5. Therapeutic drug monitoring (TDM) during biological therapy: principles and practice

When to measure? According to the reactive TDM approach, when the clinical picture deteriorates, in cases of partial drug response, or infusion/injection reactions. According to the proactive TDM approach, at the end of the induction phase and in the early maintenance phase, especially in pediatrics, in order to increase long-term remission and durability [54; 55].

1.5.1. Therapeutic drug level monitoring: reactive and proactive approaches

Therapeutic drug monitoring has become one of the most important tools in the therapy of inflammatory bowel disease during the optimization of biological therapies [56].

Reactive therapeutic drug monitoring is a strategy used in cases of loss of response or the appearance of symptoms [57]. It helps to distinguish between the causes of loss of efficacy:

• Pharmacokinetic cause: Low drug levels, which may be immunogenic (high antibody titer) or non-immunogenic (no antibodies, but rapid clearance).

• Pharmacodynamic cause: The disease is active even at adequate drug levels (mechanistic failure, e.g., non-TNF-mediated inflammation).

• Immunogenicity: Formation of NAbs leads to loss of drug effectiveness. During proactive therapeutic drug monitoring, drug levels and antibodies must be measured at regular intervals, regardless of the patient's clinical condition, in order to maintain the drug level within a specified therapeutic window. In the case of IFX and ADA, a growing body of data, including the results of the PAILOT and PANTS studies, suggest that proactive therapeutic drug monitoring correlates with improved maintenance outcomes. The PAILOT study in children confirmed that proactive monitoring significantly increased the chance of steroid-free remission. A proactive approach can prevent low drug levels, which are one of the main causes of immunogenicity ("trough predicts antibodies") [58; 59].

1.6. Measurement methods

1.6.1. Drug-tolerant antibody tests

Fewer false-negative antibody detections at high drug levels; validated positivity thresholds for clinical interpretation.

1.6.2. Chemiluminescent immunoassays

More recently used methods. Their advantage is the speed of measurement and the ability to test patient samples as single tests.

1.6.3. ELISA tests

Standardized, quantitative measurements of drug and ADA levels, separate protocols for induction and maintenance phases; broad device platform compatibility (DAS APE ELITE, DAS APEX). The LISA-TRACKER portfolio offers drug+antibody parallel measurement and WHO calibration for several biotherapies. ELISA tests are the gold standard method for testing biological therapeutic agents [60].

1.7. Applicability of decision support algorithms based on TDM

Based on international consensus, there are guidelines that provide recommendations based on measured drug and antibody levels (**Table 3**). The recommendations take into account the types of biological therapies, the drug levels measured in patient samples, any antibodies that may appear, and recommend decision support steps [24; 61; 62].

Table 3. Drug and antibody formation strategy during first-line treatments [63].

Drug	**Phase**			
	Induction		**Post induction**	**Maintenance**
Infliximab	Week 2nd 20-25 µg/ml	Week 6th 15-20 µg/ml	Week 14th 7-10 µg/ml	5-10 µg/ml
Adalimumab	Week 4th 8-12 µg/ml		Week 12th 8-12 µg/ml	8-12 µg/ml

1.8. Second- and third-line therapies

Regarding vedolizumab therapy, the primary cause of loss of clinical response is not antibody formation, but low serum drug levels. In such cases, dose intensification based on TDM - primarily decreasing the dosing interval from 8 weeks to 4-6 weeks - has proven to be an effective strategy [22]. Trough levels of ≥20-25 µg/mL measured during the induction phase and ≥10-15 µg/mL achieved in the maintenance period treatment are associated with better clinical and endoscopic outcomes [22; 64]. Due to its low immunogenicity, routine use of immunosuppressive combination therapy (e.g., thiopurine or methotrexate) is not justified during vedolizumab treatment. In cases of persistently high ADA levels and therapeutic failure with loss of clinical response, a change of biologic should be considered, typically to a product with a different mechanism of action [65; 66].

During ustekinumab treatment, clinical response is closely correlated with serum trough levels. Trough levels of ≥3-7 µg/mL measured during the induction phase (week 8) and

≥1-3 µg/mL measured during maintenance treatment [67; 68] are associated with favorable clinical response and remission. In case of loss of response associated with low drug levels, the primary therapeutic step is to shorten the dosing interval (from 12 weeks to 8 weeks, then to 4 weeks if necessary) (see **Table 4**) [69].

In cases of combined antibody positivity and low drug levels, dose intensification is primarily recommended, while immunosuppressive combination therapy should only be considered in exceptional cases. In case of therapeutic failure with high, persistent antibody titers and repeated dose escalation, transitioning to a therapy with a different mechanism of action is warranted [70].

Table 4. Drug and antibody formation strategy during second- and third-line treatments (Abreviations: VDZ-vedolizumab, UST-ustekinumab)

Drug	Phase	Trough level target (µg/mL)	Clinical interpretation	Recommended action in case of low levels	Significance of antibodies
VDZ	Induction (week 6)	≥20–25	Higher chance of clinical response	Shortening of dosing interval	Rarely clinically relevant
VDZ	Maintenance	≥10–15	Remission and mucosal healing	8 → 4–6 weeks	Mostly temporary
VDZ	Loss of response	<10	Subtherapeutic level	Interval shortening	Switching in case of high antibody
UST	Induction (Week 8)	≥3–7	Clinical response more likely	IV reinduction may be considered	Rare, but may be relevant
UST	Maintenance	≥1–3	Maintenance of remission	12 → 8 → 4 weeks	Persistent antibody rare
UST	Loss of response	<1	Subtherapeutic level	Dose intensification	Switching in case of high antibody

1.9. Dilemmas of combination therapy and discontinuation

The main advantage of combination therapies is that immunosuppressive combination therapy (thiopurine, methotrexate) can reduce the formation of antibodies and increase drug persistence, especially in the case of infliximab. The risk is that immunosuppression may increase the number of infections, hepatotoxicity, and lymphoproliferative risks, so

individual risk-benefit analysis is particularly important. Strategies for discontinuing medication: In stable remission, some protocols investigate the early tapering of combination immunosuppressive agents while continuing biological therapy. The decision should be considered based on TDM and objective activity [71].

2. OBJECTIVES

The aim of the thesis was to:

1. Obtain information on the therapeutic effects of IFX and ADA by collecting data from patients with inflammatory bowel disease (IBD) compared pediatric and adult patients.
2. Based on the data, to show what influences the development of anti-drug antibody in biologicalsand what kind of antibody response the drugs can trigger.
3. Compare the incidence of antibody formation in second- and third-line VDZ and UST treatments and first-line (ADA, IFX) therapies.

3. METHODS

3.1. Patient characteristics of first-, second-, and third-line therapies

In our study, we analyzed 153 pediatric and adult IBD patients who received first-line infliximab or adalimumab therapy and 183 patients who received second- or third-line ustekinumab or vedolizumab therapy. The patients were treated at the Semmelweis University Pediatric Center, Department of Surgery, Transplantation and Gastroenterology, and Department of Internal Medicine and Oncology between 2020 and 2025 [72]. The selection criteria were determined based on the diagnostic guidelines of the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) for children and the European Crohn's and Colitis Organization (ECCO) for adults. It was also important that the subjects had at least one therapeutic drug level monitoring result. During the analyses, we excluded cases where a patient had multiple measurement points, as further measurements confirmed the presence of antibodies but would have distorted the statistical analyses. [73].

3.2. Ethical considerations

All participants in the study were fully informed about the study protocol and provided written consent to participate in the study. All procedures were performed in accordance with ethical approval number 19048-Á2/2018/EKU issued by the Ethics Committee (Clinical Pharmacology Ethics Committee of the Health Sciences Council) [72]. Data anonymization ensured patient anonymity. All procedures performed in the study were in accordance with the 1964 Declaration of Helsinki and its later amendments [74].

3.3. Methods

Prior to administration of the next biological therapy dose, 6 ml of native blood sample was collected to measure the trough level (TRL) of various biological therapies. Serums as part of routine laboratory procedures were centrifuged (at room temperature, 2300×g, for 10 minutes) and stored at -20 °C until use.

LisaTracker Duo Infliximab, Duo Adalimumab, Duo Vedolizumab, and Duo Ustekinumab In Vitro Diagnostic ELISA kits (Biosynex Theradiag, Croissy Beaubourg, France) were used for measurement. The tests were performed using the DAS APE

ELITE ELISA device (DAS Instruments, Rome, Italy) [72]. The tests were used in accordance with the manufacturer's protocol following internal quality control alongside patient samples to ensure the validity and reproducibility of the procedure within the batch. The performance data for the various ELISA assays are summarized in **Table 5**.

Table 5. Detection limit (LoD) and test range data for the duo adalimumab and duo infliximab ELISA assays [75]. (Abbreviations: LoD: Limit of detection) [72]

ELISA test name	Limit of detection (LoD)	Assay range
Adalimumab/Infliximab	0.3 mg/L (above the 95th percentile)	0.3 mg/L - 20 mg/L
anti-Adalimumab/anti-Infliximab	10 µg/L (above the 95th percentile)	10 µg/L - 160 µg/L /10 µg/L - 200 µg/L
Ustekinumab	0.4 mg/L (above the 95th percentile)	0.4 mg/L - 10 mg/L
anti-Ustekinumab	3 AU/L (above the 95th percentile)	3 AU/L - 100 AU/L
Vedolizumab	2 mg/L (above the 95th percentile)	2 mg/L - 60 mg/L
anti-Vedolizumab	35 µg/L (above the 95th percentile)	35 µg/L – 500 µg/L

Throughout induction phase, the target trough level value was set at a minimum of 15 mg/L for IFX and a minimum of 10 µg/mL for ADA at 6 and 4 weeks, respectively. Throughout maintenance phase the target trough level range is between 3 and 7 mg/L for

IFX and between 5 and 10 mg/L for ADA. For AIFX and AADA, the thresholds are 9 μg/L and 4 μg/L, respectively, as recommended by ESPGHAN [72; 76].

For the patients selected for the study, there were no selection criteria based on the subtype of disease (CD vs. UC), the type of treatment, location or the stage of the IBD. However, we consider these disease characteristics to be important to take into account during the evaluation. The various patient characteristics are summarized in **Tables 6** and **7**.

Table 6. Patient characteristics. Clinical data were normalized to the number of patients, excluding multiple measurement time points from the same individual. Comparisons were performed between Infliximab and Adalimumab treatment groups in the Crohn's disease and Colitis ulcerosa cohorts. Abbreviations: 5-ASA, 5-aminosalicylates; IBD, inflammatory bowel disease. Disease localization: L1, terminal ileum; L2, colon; L3, ileocolon; L4, upper gastrointestinal tract (modifier of L1–L3); E1, proctitis; E2, left-sided colitis (up to the splenic flexure); E3, extensive colitis (beyond the splenic flexure). Disease course: B1, non-stricturing, non-penetrating; B2, stricturing; B3, penetrating. Disease severity: S0, clinical remission; S1, mild UC (≤4 bloody stools/day, no systemic toxicity); S2, moderate Colitis ulcerosa (above 4-5 stools/day, minor systemic toxicity) (see on the next page). [72].

	Crohn's disease		Ulcerative colitis		Total
	Infliximab	**Adalimumab**	**Infliximab**	**Adalimumab**	
Number of patients (m/f)	50 (27/23)	59 (23/36)	13 (6/7)	31 (14/17)	**153 (70/83)**
Children	10 (6%)	18 (12%)	5 (3%)	7 (5%)	**40 (26%)**
Adult	40 (26%)	41 (27%)	8 (5%)	24 (16%)	**113 (74%)**
Onset in childhood	24 (16%)	37 (24%)	5 (3%)	10 (7%)	**76 (50%)**
Age at disease onset (years)	19.1 [11.3-25	15.3 [12.5-21.8	22.5 [15.7-32.9]	24.1 [14.8-40.8]	**18.6 [12.2-27.7]**
Time from disease onset to initiation of biologic therapy (year)	5.09 [3.6-12.9]	3.92 [1.4-11.5]	4.16 [1.2-8.9]	3.59 [1.5-10.4]	**4.42 [1.7-11.3**
Antibody against IFX or ADA:					
Yes	16 (11%)	5 (3%)	5 (3%)	6 (4%)	**32 (21%)**
No	34 (22%)	54 (36%)	8 (5%)	25 (16%)	**121 (79%)**
Sampling age (year)	31.9 [23.4-41	26.6 [17.8-38.8]	32.5 [17.2-45.1]	33.9 [18.6-53]	**30.7 [18-41.7]**
Localization:					
L1/E1	7 (4%)	11 (7%)	1 (1%)	-	**19 (12%)**
L2/E2	16 (10%)	14 (9%)	6 (4%)	12 (8%)	**48 (31%)**
L3-4/E3-4	21 (14%)	29 (19%)	5 (3%)	18 (12%)	**73 (48%)**
N/A	6 (4%)	5 (3%)	1 (1%)	1 (1%)	**13 (9%)**
Attitude:					
B1/S0	26 (17%)	25 (16%)	5 (3%)	14 (9%)	**68 (45%)**
B2/B3/S1	21 (14%)	30 (19%)	7 (5%)	17 (11%)	**75 (49%)**
N/A	3 (2%)	4 (3%)	1 (1%)	-	**10 (6%)**
Immunosuppression:					
Yes	21 (14%)	33 (22%)	6 (4%)	13 (8%)	**73 (48%)**
No	16 (10%)	20 (13%)	4 (3%)	11 (7%)	**51 (33%)**
N/A	13 (8%)	6 (4%)	3 (2%)	7 (5%)	**29 (19%)**
Steroid:					
Yes	9 (6%)	8 (5%)	3 (2%)	6 (4%)	**26 (17%)**
No	27 (17%)	44 (29%)	7 (5%)	18 (12%)	**96 (63%)**
N/A	14 (9%)	7 (5%)	3 (1%)	7 (5%)	**31 (20%)**
5-ASA:					
Yes	9 (6%)	21 (14%)	7 (5%)	18 (12%)	**55 (36%)**
No	7 (5%)	20 (13%)	2 (1%)	3 (2%)	**32 (21%)**
N/A	34 (22%)	18 (12%)	4 (3%)	10 (6%)	**66 (43%)**
Intensification:					
Yes	11 (7%)	13 (8%)	4 (3%)	8 (5%)	**36 (23%)**
No	35 (23%)	44 (29%)	7 (5%)	22 (14%)	**108 (71%)**
N/A	4 (3%)	2 (1%)	2 (1%)	1 (1%)	**9 (6%)**

Table 7. Frequency of biological therapeutic antibody formation in patients with inflammatory bowel disease treated with TNF and non-TNF inhibitors, Crohn's disease and ulcerative colitis [77].

Anti-TNF (Infliximab, Adalimumab)					
	Crohn's disease		**Ulcerative colitis**		**Total**
	Infliximab	**Adalimumab**	**Infliximab**	**Adalimumab**	
Patients number (male/female)	50 (27/23)	59 (23/36)	13 (6/7)	31 (14/17)	153 (70/83)
Children	10 (6%)	18 (12%)	5 (3%)	7 (5%)	40 (26%)
Adults	40 (26%)	41 (27%)	8 (5%)	24 (16%)	113 (74%)
Imm. supp. therapy	19 (38%)	32 (54%)	7 (54%)	14 (45%)	72 (47%)
Antibody formation against IFX/ADA	16 (32%)	5 (8%)	5 (38%)	6 (19%)	32 (21%)
Ustekinumab–Vedolizumab					
	Crohn's disease		**Ulcerative colitis**		**Total**
	Ustekinumab	**Vedolizumab**	**Ustekinumab**	**Vedolizumab**	
Number of patients (men/women)	83 (43/40)	24 (15/9)	18 (8/10)	56 (26/30)	181 (84/97)
Children	40 (22%)	7 (4%)	9 (50%)	20 (36%)	76 (42%)
Adults	43 (24%)	17 (9%)	9 (50%)	36 (64%)	105 (58%)
Imm. supp. therapy	3 (4%)	1 (4%)	0	1 (2%)	4 (2%)
Antibody formation against UST/VDZ	13 (16%)	8 (33%)	2 (11%)	14 (25%)	37 (20%)

3.4. Statistical analysis

In the first part of our study, we performed hierarchical logistic regression analysis to identify clinical variables associated with drug antibody positivity, using binary variables (Yes/No for the presence of AIFX/AADA). Continuous predictors (age at disease onset and duration of treatment) were standardized using logarithmic transformation. Nominal predictors composed of gender, IBD subtype (UC or CD), immunosuppressive therapy status, type of biologic therapy (ADA or IFX), and dose intensification [72].

The hierarchical model consisted of four steps: 1. The basis was a reference model. 2. In step 2, biological sex, age at disease onset, localization, and IBD subtype were added. Then, in step 3, treatment duration, immunosuppressive therapy, type of biological therapy, and dose intensification were added to the system [72]. Finally, in step 4, two- and three-way interaction terms were fitted into the model. Collinearity among predictors, including higher-order interaction terms, was assessed using the Variance Inflation Factor (VIF). In the final model, the total VIF value of the predictors was below the critical threshold of 5.0 (maximum VIF = 2.85), confirming the acceptable stability of the model [72].

We sought to determine the incremental variance (ΔR 2) explained by treatment-related factors and higher-order interactions over established, unmodifiable patient characteristics. Progressive block design allows for a systematic evaluation of whether the addition of complex interactions significantly improves model fit compared to simpler additive models, thereby confirming the presumed synergistic nature of immunogenicity risk (see **Table 8**) [72].

Table 8. Fit of statistical analysis results and degree of fit in the final model

Diagnostic test	Value	Explanation
Hosmer–Lemeshow goodness-of-fit test (p-value)	0.518	Confirms appropriate model calibration ($p > 0.05$).
Maximum VIF (Variance Inflation Factor)	2.85	Indicates minimal multicollinearity
Model performance (Nagelkerke R^2)	0.287	Indicates partial variance explanation

3.5. Second- and third-line therapies

Clinical severity was evaluated using CDAI for adult Crohn's disease, pMayo for adult ulcerative colitis, and PCDAI or PUCAI for paediatric patients. Remission was defined as CDAI <150, pMayo <3, or PCDAI/PUCAI <10. Vedolizumab was administered intravenously at weeks 0, 2nd, and 6th, followed by 300 mg at 8-week intervals, with dose intensification to every 4th weeks if required. Ustekinumab was given as intravenous induction (weight-based), followed by 90 mg subcutaneous maintenance every 8–12th weeks, with interval adjustment to every 4 weeks if necessary [77].

3.6. Therapeutic drug monitoring and statistical analysis

Therapeutic drug monitoring (TDM) was performed using ELISA to assess pre-dose drug concentrations and anti-drug antibodies, applying reactive or proactive strategies. Target trough levels were above 3.5 µg/mL (induction) and above 1 µg/mL (maintenance) for ustekinumab, and above 15 µg/mL (induction) and above 12 µg/mL (maintenance) for vedolizumab.

In this part of the study, the prevalence of antibody formation was compared between first-line and second-/third-line ustekinumab and/or vedolizumab therapies using chi-square (χ^2) test or Fisher's exact test in a multivariate analysis [77].

4. RESULTS

4.1. First-line treatments

We performed hierarchical logistic regression to identify factors associated with AIFX/AADA positivity. The basic analysis consisted of five models (M0–M5), each of which contained variable blocks based on thematic relevance [72].

The base model (M0) was completely empty, containing no variables, and was used as a reference for comparison with subsequent models. In this model, we estimate the frequency of antibody formation based on which is more prevalent in the data set (antibody present: 15.78%, antibody absent: 84.21%) [72].

Model M1: this model included the biological sex of the patients (we had to exclude the age of the patients, as age was strongly correlated with the time of onset of the disease). **Table 9** shows that model M1 was not significantly different from the empty (M0) model. Therefore, it cannot be stated that considering the sex of patients as an independent factor provides a better estimate of antibody formation [72].

Table 9. Significance level of models M0 and M1 in relation to each other.

Model	Deviance	AIC	BIC	df	ΔX^2	p
M_0	198.890	200.890	204.319	227		
M_1	198.743	202.743	209.602	226	0.147	0.701

The second model (M2) introduced biological sex and age at onset, location, and type of IBD, but these additions did not significantly improve the fit of the model (**Table 10**).

Table 10. Introduction of the next (M2) model into the system.

Model	Deviance	AIC	BIC	df	ΔX^2	p
M_0	198.890	200.890	204.319	227		
M_1	198.743	202.743	209.602	226	0.147	0.701
M_2	193.128	203.128	220.275	223	5.615	0.132

The third model (M3), which included treatment-related factors (treatment duration, immunosuppressive therapy, type of biological therapy, and intensification), showed a

significant improvement in performance compared to the previous models (p = 0.002) (**Table 11**).

Table 11. Introduction of the next (M3) model into the system.

Model	Deviance	AIC	BIC	df	ΔX^2	p
M_0	198.890	200.890	204.319	227		
M_1	198.743	202.743	209.602	226	0.147	0.701
M_2	193.128	203.128	220.275	223	5.615	0.132
M_3	176.666	194.666	225.530	219	16.463	0.002

Model M4: In this model, we initially considered CRP as a laboratory parameter with predictive value. **Table 12** shows that the inclusion of CRP also improved the performance of the model.

Table 12. Introduction of the M4 model into the system.

Model	Deviance	AIC	BIC	df	ΔX^2	p
M_0	198.890	200.890	204.319	227		
M_1	198.743	202.743	209.602	226	0.147	0.701
M_2	193.128	203.128	220.275	223	5.615	0.132
M_3	176.666	194.666	225.530	219	16.463	0.002
M_4	169.865	189.865	224.158	218	6.801	0.009

The final model (M5), which also included interaction terms, further improved the model fit (p = 0.047). The Nagelkerke R^2value of M4 was 0.287, indicating that our selected clinical variables explain 28.7% of the variance in antibody positivity. This indicates that most of the variance (more than 70%) is not explained by the clinical parameters used here. The predictive power of the model was confirmed by an AUC of 0.806, a sensitivity of 71.0%, and a specificity of 77.6% at the optimal cutoff point (**Tables 13** and **14**).

Table 13. Introduction of the M5 model into the system.

Model	Deviance	AIC	BIC	df	ΔX^2	p
M_0	198.890	200.890	204.319	227		
M_1	198.743	202.743	209.602	226	0.147	0.701
M_2	193.128	203.128	220.275	223	5.615	0.132
M_3	176.666	194.666	225.530	219	16.463	0.002
M_4	169.865	189.865	224.158	218	6.801	0.009
M_5	147.779	189.779	261.795	207	22.086	0.024

Table 14. Details of the final model (Estimate coefficient). A positive value correlates with antibody formation, while a negative value indicates no correlation.

Coefficients

Model		Estimate	p
M_5	(Intercept)	−5.349	3.451e−5
	Std log(Age at onset)	−0.840	0.073
	Localisation	0.685	0.098
	Std log(Treatment time)	0.992	0.017
	Std log(CRP)	0.705	0.003
	Gender (Female)	0.178	0.696
	IBD (UC)	0.840	0.406
	Immunotherapy (Yes)	0.596	0.230
	Biological (IFX)	1.917	0.006
	Intensification (Yes)	−0.563	0.317
	Std log(Age at onset) * Std log(Treatment time)	0.216	0.633
	Std log(Age at onset) * Biological (IFX)	0.975	0.082
	Std log(Age at onset) * IBD (UC)	1.795	0.059
	Std log(Treatment time) * Biological (IFX)	−1.757	0.002
	Std log(Treatment time) * IBD (UC)	−1.332	0.142
	IBD (UC) * Biological (IFX)	2.761	0.241
	Std log(Age at onset) * Std log(Treatment time) * Biological (IFX)	0.534	0.349
	Std log(Age at onset) * Std log(Treatment time) * IBD (UC)	−0.080	0.928
	Std log(Age at onset) * IBD (UC) * Biological (IFX)	−5.206	0.022
	Std log(Treatment time) * IBD (UC) * Biological (IFX)	4.790	0.045
	Std log(Age at onset) * Std log(Treatment time) * IBD (UC) * Biological (IFX)	−3.649	0.097

The CRP laboratory parameter introduced in the M4 model as an interaction term predicting antibody formation is a well-known and well-studied factor in the literature. Therefore, we did not take this model into account in our further investigations. We inserted our final interaction model (M5) into the hierarchical logistic regression system and will refer to it as model M4 in the following (**Table 15**).

Table 15. Summary of hierarchical model. The table shows the p-values and goodness-of-fit of the hierarchically nested models (from M0 to M4) based on Nagelkerke R^2values [72]

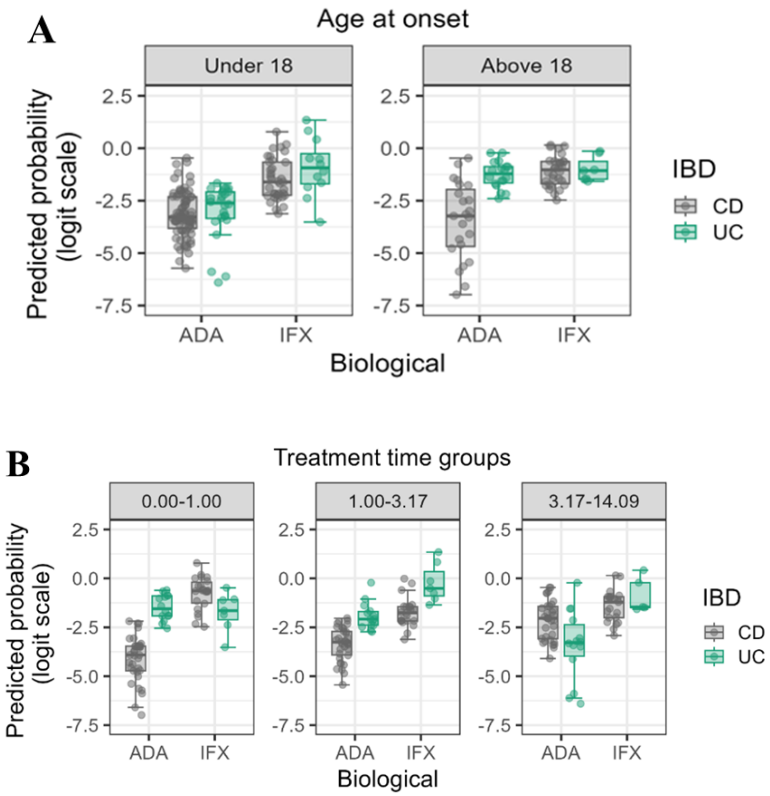
Model	*p*	R^2	Variables
M0			
M_1	0.701	0.001	Gender
M_2	0.132	0.025	M1 + Onset age, Localisation and the IBD type
M_3	0.002	0.107	M2 Treatment Time, Imm. suppr. therapy, Type of biological therapy and Intensification
M_4	0.043	0.291	M3 Interaction terms

The M_4model identified several significant main effects and interaction factors. IFX therapy was a strong predictor of antibody positivity, with patients receiving infliximab nearly seven times more likely to have detectable antibodies than those receiving adalimumab (OR = 6.940, p = 0.004). The duration of treatment also significantly influenced antibody formation: longer treatment duration increased the likelihood of antibody positivity (OR = 2.505, p = 0.029). Localization was also a significant factor (OR=2.216, p=0.049), suggesting that specific anatomical locations influence AIFX/AADA antibody positivity [72].

I also found synergistic interactions in the M_4model. The interaction between treatment duration and type of biological therapy (OR=0.220, p=0.005) indicates that longer treatment duration increases the risk of antibody formation, which was not apparent in the case of infliximab. The interaction between age at disease onset and type of IBD (OR = 8.023, p = 0.031) suggests that UC patients, regardless of the type of biological therapy, were more likely to be antibody-positive if their IBD began in childhood. However, when examining a three-way interaction (age at onset × type of IBD × type of biological therapy (OR = 0.019, p = 0.042)) showed that the combined effect of childhood onset and IBD

type (UC or CD) on AIFX/AADA positivity was significantly influenced by the type of anti-TNF agent. In patients whose disease began in childhood, IFX therapy was associated with a higher risk of e antibody positivity than adalimumab therapy. This effect was more pronounced in UC. In contrast, in patients with adult-onset IBD, the difference in antibody positivity prevalence between ADA and IFX therapy is reduced in the UC group [72].

A significant three-way interaction between antibody positivity and treatment duration × IBD type × type of biological therapy (OR = 82.745, p = 0.042) indicates a complex relationship between the variables. Longer treatment durations with IFX significantly predispose patients with both CD and UC to a higher likelihood of antibody positivity compared with ADA therapy (**Figure 3A** and **B**) [72].



3. Figure

A: Probabilities of antibody positivity by initial age, biologic therapy, and type of IBD. The y-axis shows the predicted log probabilities, and biologic therapies (ADA and IFX) are shown on the x-axis. Gray (CD) and green (UC) dots represent an individual data points.

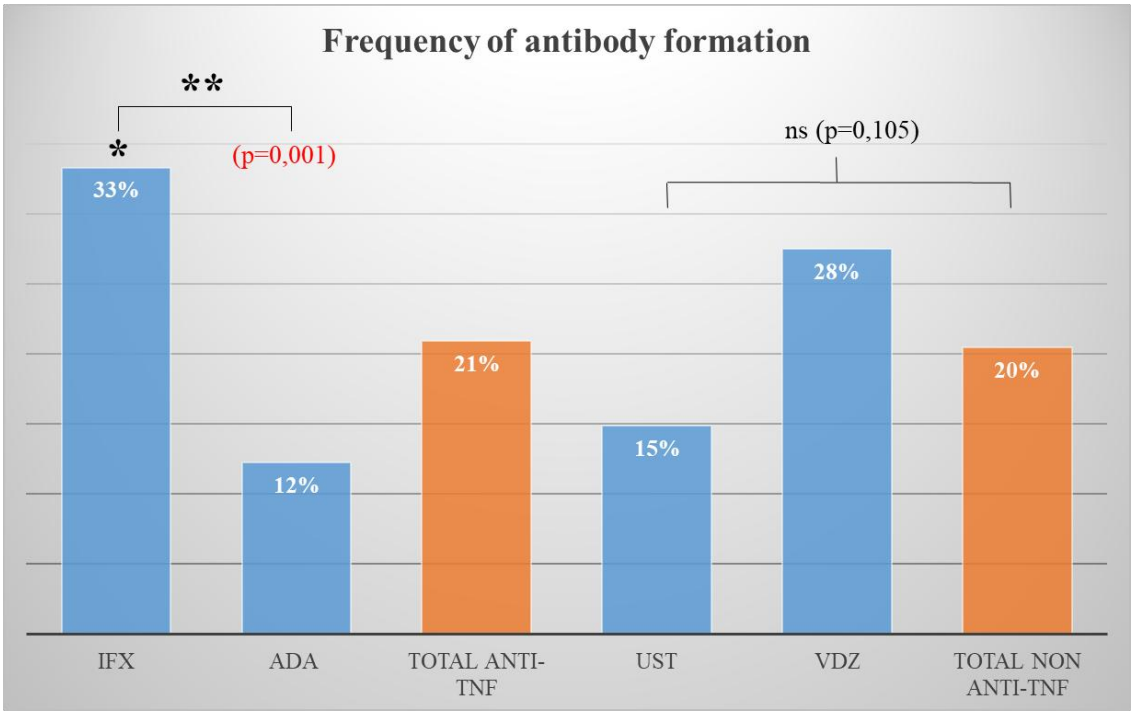
B: Antibody positivity probabilities by treatment duration, anti-TNF-α therapy, and IBD status. The panels depict three treatment groups duration quartiles: (0.00–1.00), (1.00–3.17), and (3.17–14.09) years. The y-axis shows the predicted log probabilities, and biological drugs (ADA and IFX) are plotted on the x-axis. Individual data points are represented by gray (CD) and green (UC) dots [72].

4.2. Second- and third-line preparations

In our further investigations, we compared the first-line therapeutic results (ADA/IFX) with the antibody formation rates observed in second-line biological therapies (VDZ/UST) and the effect of immunosuppressive treatments on them [77].

The patient composition of the two databases differed slightly. In the population receiving TNF-α inhibitors, the proportion of children was 26%, while in the USTE/VDZ group it was significantly higher, at 42%. In the entire patient population, the proportion of Crohn's disease was 57–60%, while that of ulcerative colitis was 40–43%. The incidence of antibody formation in the two databases was 21% and 20%, respectively (IFX/ADA: 32/153; UST/VDZ: 37/181). No statistically significant difference was observed between the two groups (χ^2=0.0006; p=0.98). No difference was observed between the patient groups in terms of IBD type: the same immunogenicity rate was observed in CD (p=0.92) and UC (p=0.21) [77].

The comparison between biological therapies showed significant differences. In the case of IFX, the frequency of antibody formation was 33%, while in the case of ADA it was only 12%, which showed a significant difference (χ^2=10.8; p=0.001). In second-line treatments, the UST antibody formation rate was 15%, while that of VDZ-treated patients was 28%, but the difference between the two groups did not reach statistical significance (χ^2=2.62; p=0.105), but a tendency toward higher immunogenicity was observed with VDZ therapy. It should be noted that a significant proportion of the antibodies detected for vedolizumab and ustekinumab did not cause clinical loss of efficacy, suggesting that some of these antibodies are low-titer, non-neutralizing, or transient antibodies. Notably that the increased analytical sensitivity of the drug tolerance measurement method used may have contributed to the more frequent detection of antibody positivity, but this does not necessarily indicate clinically relevant immunogenicity (**Figure 4**) [77].



4. **Figure.** Frequency of antibody formation in biological therapy for IBD in case of TNF and non-TNF inhibitors. (Abbreviations: IFX: Infliximab, ADA: Adalimumab, TNF: Tumor Necrosis Factor, UST: Ustekinumab, VDZ: Vedolizumab) [77]

4.3. Combination therapy

The simultaneous use of immunosuppressive agents occurred significantly more frequently in patients receiving anti-TNF-α treatment than in those receiving UST or VDZ therapy. In the anti-TNF group, 47% of patients received immunosuppressive treatment, compared with only 2% in the UST/VDZ group. The Fisher's exact test performed showed an extremely significant difference between the two therapeutic groups ($p=1.33\times10^{-24}$), with an OR (odds ratio) was 39.3, indicating that anti-TNF-α treatment is associated with a 39-fold higher chance of concomitant use of immunosuppressive agents than VDZ or UST. This difference is statistically and clinically significant, but it is consistent with the pharmacological profile of biological agents and current therapeutic recommendations. According to the literature, anti-TNF agents have higher immunogenicity, therefore it is often necessary to use immunosuppressive combinations to prevent or reduce antibody formation. In contrast, UST and VDZ have low immunogenicity, and combination therapy is not part of the standard treatment practice which was also confirmed in the study

population. In a combined multivariate analysis, neither age (child–adult division), biological sex, nor inflammatory bowel disease diagnosis significantly influenced the occurrence of antibody formation in any of the patient populations we examined [77].

5. DISCUSSION

5.1. First-line therapies

In our publication at the end of 2025, we highlighted that the long-term effectiveness of anti-TNF-α therapy (TNF-α) therapies used as biological therapies, such as infliximab (IFX) and adalimumab (ADA), is often affected by the development of antibodies against the drug. Several individual factors contributing to their immunogenicity are known, but the complex, interacting effects of various clinical variables in real-world clinical practice not completely clear yet [72].

The main finding of our studies is the identification of complex interactions between clinical indicators that contribute to antibody formation in a heterogeneous patient population over the course of their lives. The introduction of a hierarchical logistic regression model has made it possible to gain a more accurate understanding of these relationships, which, based on the literature, have not been studied before.

In our research, we analyzed and identified several factors associated with the occurrence of anti-TNF-α antibodies in patients with UC and CD. The model used in this study supports the information found in the literature that the duration of treatment and the type of biological therapy (IFX vs. ADA) are key predictors of TNF antibody formation [78] and that IFX has a more pronounced immunological effect than ADA [72].

Earlier studies reported that childhood-onset IBD is associated with altered immune regulation, which may be linked to an increased risk of drug-induced antibody formation [79; 80]. My results support this and add that this risk is further influenced by the specific anti-TNF agent used and the subtype of IBD. In patients with childhood-onset UC treated with infliximab, the particularly high tendency to form antibodies is consistent with the known higher immunogenicity of the chimeric IFX molecule compared to the humanized ADA molecule. This suggests that the immature and developing immune system of young patients with ulcerative colitis may respond more strongly to non-human epitopes present in infliximab. Overall, these findings highlight the importance of carefully tailoring biological therapy in childhood-onset IBD, particularly in ulcerative colitis, where infliximab is frequently used as first-line treatment [81].

The different antibody formation risks between IFX and ADA and their association with longer treatment duration emphasize the importance of long-term TDM and antibody testing. This confirms observations in the literature that longer treatment periods may result in a stronger immune response. [82].

The model we used showed statistically significant predictive performance, as evidenced by an AUC value of 0.806. However, Nagelkerke $R^2 = 0.287$ suggests that predictions based solely on clinical factors have limited explanatory power. In contrast, prediction models that also integrate critical pharmacokinetic variables, such as initial drug levels or genetic markers related to drug metabolism, typically show higher predictive power, often with AUC values above 0.85 [72].

The primary goal of our study is to provide an easily accessible clinical framework for early antibody formation risk assessment. This allows for the identification of patients who can benefit most from proactive therapeutic drug monitoring (TDM) interventions [83]. The beneficial effects of TDM have been confirmed by several studies, and according to the latest data, it is a recommended and widely used procedure for optimizing anti-TNF treatments in pediatric IBD patients [84].

5.2. Second- and third-line therapies

In the further part of our investigations, we compared the immunogenicity of first-, second- and third-line preparations. In our paper accepted for publication in Orvosi Hetilap in 2026, we pointed out that although the average immunogenicity of biological agents may be similar, there are significant differences between individual molecules. The high immunogenicity of IFX, compared to the lower value of ADA, justifies close proactive monitoring and combination therapy. In the case of VDZ and UST, the results require a more nuanced approach, taking into account the possible presence of transient antibodies [77].

The immunological response to the different agents was approximately the same in the entire patient population, with a total antibody formation rate of 21% and 20% in the two groups, respectively. This indicates that differences in the composition of patient populations (e.g., child-adult ratio or differences in diagnosis distribution) do not significantly affect the immune response. There was a significant difference in the

comparison between therapies. IFX treatment was associated with the highest immunogenicity, which was more than twice the rate observed with ADA treatment. This is consistent with previous international observations (e.g., the PANTS study), which found that chimeric IFX is more immunogenic than fully human ADA [85].

Among the second-line drugs, UST showed the lowest antibody formation rate, which is consistent with the international literature, where long-term studies (e.g., IM-UNITI LTE) have also confirmed immunogenicity below 5% [22]. VDZ is known to have low immunogenicity, but a higher rate of antibody positivity can be detected in certain groups, as confirmed by my own studies. The 28% anti-vedolizumab positivity I measured is higher than the 4% measured in the GEMINI studies [86]. This difference can probably be explained by the measurement methodology: modern drug-tolerant assays are able to detect antibodies even when the drug is present in the serum, thus revealing "hidden" immunogenicity. Drug-tolerant assays may reveal more frequent antibody positivity, but their clinical relevance is not always clear, as in many cases the antibodies are non-neutralizing or transient [87].

Our studies also included a comparison between IBD types. Here, no difference in the frequency of antibody formation was detected in either therapeutic group when comparing Crohn's disease and ulcerative colitis. We also did not identify any association between antibody formation and gender or age. This confirms the assumption that immunogenicity is determined by the type of biological agent, its protein structure, and its pharmacokinetics [53].

5.3. Limitations

During our investigations, we had to contend with a number of methodological and practical limitations that affected both the model construction and the interpretability of the results. The most important limitation was the retrospective and cross-sectional nature of the study, as the single-time point sampling of the does not allow for the examination of the temporal dynamics of antibody formation. As a result, transient and persistent antibodies cannot be distinguished, even though, according to the literature, transient antibodies often disappear spontaneously and have less influence on long-term clinical outcomes, while persistent antibodies are clearly associated with loss of efficacy and a

sustained decrease in drug levels. Therefore, based on a single measurement, the degree of clinically relevant immunogenicity may be overestimated.

Due to the cross-sectional design, causal relationships cannot be clearly established. It cannot be determined with certainty whether low drug levels led to immunization or whether the appearance of antibodies caused the decrease in drug levels; at most, it can be said that these differences are associated. Therefore, the results primarily reflect associations, and prospective validation is necessary in order to draw causal conclusions and formulate clinical guidelines.

Another significant limitation was the relatively small sample size (n=153/n=183), which led to low statistical power and wide confidence intervals, especially when examining higher-order, three-variable interactions. This suggests instability and inaccuracy in the models, so my results are more hypothesis-generating in nature and need to be confirmed in larger, well-defined, independent groups. For several variables, such as 5-ASA status or immunosuppressive treatment, a significant proportion of data were missing, which I treated as an "N/A" category. This may have caused bias and contributed to the relatively low explanatory power of the model (Nagelkerke $R^2 = 0.287$).

The interpretation of the results may also have been influenced by the heterogeneity of the patient population, as the sample included both pediatric and adult patients, different disease phenotypes, and both bio-naive and previously biologically treated patients. Although the statistical analysis did not show significant differences along these factors, these differences could potentially affect immunogenicity. Finally, the results are assay-specific: the sensitivity and drug tolerance of the LisaTracker ELISA kits used may have influenced the measurement outcomes, limiting direct comparison with studies using different methods, such as the homogeneous mobility shift assay (HMSA) [88].

Overall, it is important to consider the limitations arising from the cross-sectional design, sample size, data loss, and methodological characteristics when interpreting the study [72; 77].

6. CONCLUSIONS

The long-term efficacy of first-line TNF-α inhibitors (IFX, ADA) and second- and third-line agents (VDZ, UST) commonly used in the treatment of IBD is significantly influenced by the development of antibodies against them. Although several factors are known to contribute to immunogenicity, the complex, interacting roles of routinely available clinical variables have been little studied in real-world patient populations.

Our studies confirm that the formation of anti-TNF-α antibodies represents one of the most significant and critical challenges in the biological treatment of IBD. Based on our results, infliximab treatment and duration of therapy are key predictive factors that are closely correlated with an elevated risk of immunogenicity. However, the most important novelty of this work is not the identification of a single isolated factor, but the discovery of complex, synergistic interactions between routinely available clinical variables. These results indicate that the combination of patient demographics, disease subtype, and therapeutic parameters creates predictive patterns that are moderate in themselves but have statistically significant predictive value. This systems-level approach opens up new perspectives in the assessment of immunogenicity risk [72].

The hierarchical modeling strategy used in the evaluation of first-line therapies allows risk assessment in clinical practice to be based not only on individual parameters but also on higher-order relationships between factors, thereby contributing to the refinement of personalized monitoring and therapeutic decision-making [72].

Based on our further results, the frequency of antibody formation does not depend on the patient population, but primarily on the biological therapy used. There are significant differences in immunogenicity between different agents, which justifies the differentiated use of therapeutic drug level monitoring. Close, regular monitoring is recommended during IFX treatment, while in the case of UST and ADA, less frequent monitoring may be sufficient due to their lower immunogenicity risk. Therapeutic drug monitoring plays a crucial role in the early detection of antibody formation and timely intervention, which fundamentally influence long-term clinical outcomes. Proactive monitoring and wider use

of targeted combination therapies are expected to contribute to maintaining the effectiveness of biological treatments in both pediatric and adult IBD [72; 77].

7. SUMMARY

Overall, the novelty of our findings is as follows:

1. In a real-world pediatric and adult IBD patient population, we demonstrated that a combination of routinely available clinical variables can predict antibody formation associated with biologic therapies to a moderate but clinically relevant extent.
2. Using a hierarchical logistic regression model, we demonstrated that infliximab treatment is an independent, significant risk factor for the development of immunogenicity, and that the duration of treatment further increases this risk.
3. We were the first to identify that three-way interactions between age at disease onset, IBD subtype, and type of therapy significantly modify the risk of immunogenicity.
4. We confirmed that the probability of antibody formation is significantly higher in childhood-onset ulcerative colitis treated with infliximab.
5. We demonstrated that prolonged exposure to infliximab further increases the risk of immunogenicity in ulcerative colitis.
6. In patients receiving second- and third-line biological therapies, antibody formation was determined predominantly by the immunogenicity of the administered biologic rather than by patient-related characteristics, including age, disease type, or prior biologic exposure. This finding supports the use of biologic-specific TDM to guide therapy optimization.

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Article

Interaction of Clinical Factors Modestly Predict Anti-TNF-Alpha Antibody Formation in a Real-World Cohort of Inflammatory Bowel Disease Patients

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Abstract

Background: Biological therapy is frequently used for the treatment of inflammatory bowel disease (IBD); however, the long-term efficacy of anti-tumor necrosis factor-alpha (TNF-α) therapies, such as infliximab (IFX) and adalimumab (ADA), is often compromised by the development of antidrug antibodies (AIFX and AADA, respectively). While several individual factors are known to contribute to immunogenicity, the complex, interactive effects of various clinical variables have not been fully elucidated in a real-world setting. **Methods:** We conducted a hierarchical logistic regression analysis on a retrospective cohort of 153 pediatric and adult IBD patients receiving IFX or ADA therapy to identify clinical factors and their interactions associated with AIFX/AADA positivity. The analysis progressively incorporated demographic, disease-related, and treatment-related variables, culminating in a model that included two- and three-way interaction terms. **Results:** Our final model demonstrated modest predictive power, with a Nagelkerke R^2 of 0.287, explaining less than 30% of the variance in antibody positivity using readily available clinical data (AUC of 0.806, 71.0% sensitivity and 77.6% specificity). Key predictors included the type of biological therapy (IFX vs. ADA) and the duration of treatment, with IFX therapy being a significant independent predictor (OR = 6.940, $p = 0.004$) for antibody positivity. Importantly, we identified novel three-way interactions, revealing that the combined effect of age at disease onset, IBD subtype, and biological therapy type significantly influences antibody formation ($p = 0.042$), particularly in childhood-onset ulcerative colitis patients treated with IFX. A similar interaction was found for treatment duration, IBD subtype, and therapy type ($p = 0.042$), where the risk of antibody positivity with IFX increased significantly with treatment length, particularly in UC patients. **Conclusions:** This study highlights that the combination of routine clinical variables in IBD offers a data-driven, mechanistically insightful framework, supporting the prediction of AIFX/AADA positivity to a modest extent. This framework requires prospective and external validation before clinical implementation.

Keywords: infliximab; adalimumab; immunogenicity; inflammatory bowel disease; Crohn's disease; ulcerative Colitis; antidrug antibodies; ELISA

1. Introduction

Inflammatory bowel disease (IBD), comprising Crohn's disease (CD) and ulcerative colitis (UC), is a group of chronic inflammatory conditions of the gastrointestinal tract. The pathophysiology of IBD is multifaceted and involves an abnormal immune response in the gut, leading to chronic inflammation. Tumor necrosis factor-alpha (TNF−α) plays a central role in the inflammatory cascade, and anti-TNF−α therapies, such as infliximab (IFX) and adalimumab (ADA), have revolutionized the management of IBD [1].

The effectiveness of these agents, however, is often limited by the development of antidrug antibodies (ADAs), which can reduce drug concentration and lead to secondary loss of response (sLOR) [2]. The presence of anti-infliximab antibodies (AIFX) is particularly concerning, as it has been linked to higher rates of treatment discontinuation and an increased risk of infusion-related adverse events [3,4]. While IFX is known to have a higher immunogenicity due to its chimeric structure, the formation of anti-adalimumab antibodies (AADAs) also remains a significant clinical concern [5,6].

The ability to identify patients at an increased risk of developing ADAs is crucial for optimizing treatment strategies and improving long-term outcomes. In a cohort study, patients who developed AIFX experienced a higher rate of treatment discontinuation due to loss of response compared to those without AIFX [7,8]. This report highlights the clinical importance of early detection of AIFX to make appropriate decisions on therapy modification.

ADA, which is another biologic against TNF-α, offers an alternative to IFX, but the presence of AADA has been correlated with decreased serum adalimumab concentrations and reduced clinical response rates. In the DIAMOND trial, a subanalysis revealed that patients with detectable AADA had significantly lower trough levels of ADA and diminished clinical remission rates [8]. Furthermore, a study demonstrated that higher ADA trough levels were associated with sustained clinical remission, whereas the presence of AADA predicted treatment failure [9]. This reinforces the utility of AADA detection in guiding therapeutic decisions.

There is a clear clinical need to acknowledge patient-specific factors that predispose to antibody development [10]. This would help the identification of patients who are at an increased risk of having AIFX and AADA. Despite the wealth of literature on individual risk factors, the complex interplay and synergistic effects between clinical variables have not been adequately explored in heterogeneous, real-world patient populations. Traditional multivariate methods often struggle to systematically assess the incremental value of adding high-order interaction terms informed by clinical theory. To address this gap, we hypothesized that the risk of immunogenicity is not merely an additive effect of known factors but is modulated by complex, synergistic high-order interactions among patient demographics, disease characteristics, and treatment regimens. We applied a hierarchical statistical modeling approach to systematically analyze the individual and interactive effects of various clinical parameters on the development of anti-TNF-alpha antibodies in a diverse cohort of pediatric and adult IBD patients.

2. Patients and Methods

2.1. Patients' Enrollment

A cross-sectional study was conducted between June 2020 and December 2021, enrolling 40 children and 113 adults with IBD from the Paediatric Centre, Department

of Surgery, Transplantation and Gastroenterology, and Department of Internal Medicine and Oncology at Semmelweis University. The inclusion criteria were a diagnosis of IBD based on the revised Porto criteria of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) for children and the diagnostic recommendations of the European Crohn's and Colitis Organisation (ECCO) for adults, and were performed using a consecutive method [11,12]. The inclusion of both pediatric (n = 40) and adult (n = 113) patients reflects the spectrum of IBD care managed in a high-volume tertiary setting. Recognizing the critical difference in immunological development between these groups, the study design explicitly incorporated Age at Onset as a major predictor, specifically utilizing its interaction terms to model and account for potential age-related confounding effects on immunogenicity risk.

2.2. Ethical Statement

Data were collected for routine clinical care, and no additional blood samples were taken for the purpose of this study. Informed consent was obtained from all participants in this study, and all procedures were approved as declared in our ethical permission issued by the ethics committee (Medical Research Council, Ministry of Interior) with No. 19048-Á2/2018/EKU. The de-identification of data ensured the anonymity of the patients. All procedures performed in this study were in accordance with the 1964 Helsinki Declaration and its later amendments.

2.3. Methods

Before the receipt of the next IFX or ADA dose, 6 mL native blood samples were taken to measure trough IFX and ADA levels (TRL). Sera for IFX, ADA and antibody level detection were centrifuged (room temperature, 2300× *g*, 10 min). Samples were stored at −20 °C and used for ADA, AADA, IFX, and AIFX concentration measurements. For this purpose, LisaTracker Duo Infliximab and Duo Adalimumab In Vitro Diagnostic ELISA kits (Biosynex Theradiag, Croissy Beaubourg, France) were used. The assays were performed with the DAS APE ELITE ELISA instrument (DAS Instruments, Rome, Italy). The assays were performed according to the manufacturer's specifications with internal quality controls run alongside patient samples to ensure procedural validity and reproducibility within the batch. Assay performance data are summarized in Table 1.

Table 1. Limit of detection (LoD) and assay range data of duo adalimumab and duo infliximab ELISA assays [13].

Limit of detection (LoD)	Adalimumab/Infliximab 0.3 mg/L (>95th percentile)	anti-Adalimumab/anti-Infliximab 10 µg/L (>95th percentile)
Assay range	Adalimumab/Infliximab 0.3 mg/L–20 mg/L	anti-Adalimumab/anti-Infliximab 10 µg/L–160 µg/L/10 µg/L–200 µg/L

During induction therapy, target TRL were defined at least IFX 15 mg/L and at least ADA 7.5 µg/mL at 6 and 4 weeks, respectively. During maintenance therapy, target TRL ranges are 3–7 mg/L and 5–10 mg/L for IFX and ADA, respectively. For AIFX and AADA the cut-off levels are 9 µg/L and 4 µg/L, respectively, as proposed by ESPGHAN [14].

2.4. Statistical Analysis

A hierarchical logistic regression analysis was performed to identify clinical variables associated with anti-drug antibody positivity. The dependent variable was binary (Yes/No for AIFX/AADA presence). Continuous predictors (Age at Onset and Treatment Time) were standardized using a logarithmic transformation. Categorical predictors included Gender,

IBD subtype (UC or CD), immunosuppressive therapy status, type of biological therapy (ADA or IFX), and dose intensification. This approach was utilized not for prediction optimization (as in stepwise regression) but to test a theory-driven, confirmatory hypothesis. Specifically, we aimed to determine the incremental variance (ΔR^2) explained by treatment-related factors (M_3) and high-order interactions (M_4) over established, non-modifiable patient characteristics (M_1 and M_2). The progressive block design allows for a systematic assessment of whether the addition of complex interactions significantly improves model fit compared to simpler additive models, thereby confirming the hypothesized synergistic nature of immunogenicity risk.

The hierarchical model consisted of four steps: a reference model (M_0), followed by M_1 (adding Gender), M_2 (adding Age at Onset, Localization, and IBD subtype), M_3 (adding Treatment Time, immunosuppressive therapy, type of biological therapy, and dose intensification), and finally, M_4, which incorporated two- and three-way interaction terms. Collinearity among predictors, including high-order interaction terms, was assessed using the Variance Inflation Factor (VIF). All VIF values for the predictors in the final model (M_4) were below the critical threshold of 5.0 (Maximum VIF = 2.85), confirming acceptable model stability, see Table 2.

Table 2. Model Diagnostic Metrics for the Final Logistic Regression Model (M_4).

Diagnostic Test	Value	Interpretation
Hosmer–Lemeshow Goodness-of-Fit Test (*p*-value)	0.518	Confirms adequate model calibration ($p > 0.05$).
Maximum VIF (Variance Inflation Factor)	2.85	Indicates minimal multicollinearity.
Model Performance (Nagelkerke R^2)	0.287	Indicates partial variance explanation.

The analysis included several categorical variables (e.g., Localization, Immunosuppression status) containing notable proportions of missing data ("N/A"), as detailed in Table 3. To avoid listwise deletion, which would reduce the effective sample size and introduce selection bias, these "N/A" values were treated as a distinct, third categorical level within the respective predictor variables (e.g., Immunosuppression: Yes, No, N/A). We recognize that this approach may introduce limitations, which are discussed subsequently. Model comparisons were based on likelihood-ratio tests, and explanatory power was assessed using the Nagelkerke R^2 value. The optimal cut-off value for the final model was determined using the Youden method to calculate sensitivity, specificity, and the area under the curve (AUC). All analyses were conducted in JASP (0.19.3) with a level of significance of 0.05.

Table 3. Patients' characteristics. Clinical data normalized to the number of patients (excluding multiple measurement points from one patient): IFX vs. ADA in CD vs. UC sample. Abbreviations: 5-ASA: 5-aminosalicylates; ADA: adalimumab; CD: Crohn's disease; IBD: inflammatory bowel disease; IFX: infliximab; UC: ulcerative colitis; Localization (CD): L1: Terminal ileum; L2: Colon; L3: Ileocolon; L4: Upper gastrointestinal (modifier for L1; L2; or L3); (UC): E1: Ulcerative proctitis (rectum only); E2: Left-sided colitis (up to splenic flexure); E3: Extensive colitis (beyond splenic flexure). Attitude: B1: Non-stricturing; non-penetrating; B2: Stricturing; B3: Penetrating. Severity: S0: Clinical remission; S1: Mild UC ($\leq$4 bloody stools daily; no systemic toxicity); S2: Moderate UC (>4–5 stools daily; minimal systemic toxicity).

	CD		UC		Total
	IFX	ADA	IFX	ADA	
Number of patients (m/f)	50 (27/23)	59 (23/36)	13 (6/7)	(14/17)	153 (70/83)
Children	10 (6%)	18 (12%)	5 (3%)	7 (5%)	40 (26%)
Adult	40 (26%)	41 (27%)	8 (5%)	24 (16%)	113 (74%)
Childhood onset	24 (16%)	37 (24%)	5 (3%)	10 (7%)	76 (50%)
Disease initial age (year)	19.1 [11.3–25]	15.3 [12.5–21.8]	22.5 [15.7–32.9]	24.1 [14.8–40.8]	18.6 [12.2–27.7]

Table 3. *Cont.*

	CD		UC		Total
	IFX	**ADA**	**IFX**	**ADA**	
Time from the onset of the disease to biological therapy (year)	5.09 [3.6–12.9]	3.92 [1.4–11.5]	4.16 [1.2–8.9]	3.59 [1.5–10.4]	4.42 [1.7–11.3]
Antibody against IFX or ADA:					
Yes	16 (11%)	5 (3%)	5 (3%)	6 (4%)	32 (21%)
No	34 (22%)	54 (36%)	8 (5%)	25 (16%)	121 (79%)
Age at sampling (year)	31.9 [23.4–41]	26.6 [17.8–38.8]	32.5 [17.2–45.1]	33.98 [18.6–53]	30.7 [18–41.7]
Localization:					
L1/E1	7 (4%)	11 (7%)	1 (1%)	-	19 (12%)
L2/E2	16 (10%)	14 (9%)	6(4%)	12 (8%)	48 (31%)
L3–4/E3–4	21 (14%)	29 (19%)	5 (3%)	18 (12%)	73 (48%)
N/A	6 (4%)	5 (3%)	1 (1%)	1 (1%)	13 (9%)
Attitude:					
B1/S0	26 (17%)	25 (16%)	5 (3%)	14 (9%)	68 (45%)
B2/B3/S1	21 (14%)	30 (19%)	7 (5%)	17 (11%)	75 (49%)
N/A	3 (2%)	4 (3%)	1 (1%)	-	10 (6%)
Immunosuppression:					
Yes	21 (14%)	33 (22%)	6 (4%)	13 (8%)	73 (48%)
No	16 (10%)	20 (13%)	4 (3%)	11 (7%)	51 (33%)
N/A	13 (8%)	6 (4%)	3 (2%)	7 (5%)	29 (19%)
Steroid:					
Yes	9 (6%)	8 (5%)	3 (2%)	6 (4%)	26 (17%)
No	27 (17%)	44 (29%)	7 (5%)	18 (12%)	96 (63%)
N/A	14 (9%)	7 (5%)	3 (1%)	7 (5%)	31 (20%)
5-ASA:					
Yes	9 (6%)	21 (14%)	7 (5%)	18 (12%)	55 (36%)
No	7 (5%)	20 (13%)	2 (1%)	3 (2%)	32 (21%)
N/A	34 (22%)	18 (12%)	4 (3%)	10 (6%)	66 (43%)
Intensification:					
Yes	11 (7%)	13 (8%)	4 (3%)	8 (5%)	36 (23%)
No	35 (23%)	44 (29%)	7 (5%)	22 (14%)	108 (71%)
N/A	4 (3%)	2 (1%)	2 (1%)	1 (1%)	9 (6%)

3. Results

3.1. Patients' Characteristics and Clinical Data

A total of 153 patients were included in the analysis, of whom 40 were children and 113 were adults. The clinical characteristics of the cohort are detailed in Table 3. Overall, 21% of patients (32/153) were positive for ADAs.

3.2. Model Fit and Hierarchical Structure

A hierarchical logistic regression was conducted to identify factors associated with AIFX/AADA positivity. The analysis comprised four models (M_0–M_4), each incorporating blocks of variables based on thematic relevance. For a detailed description of the performance of the models created in the block design, see Table 4.

The baseline model (M_0) was completely empty and was used as a reference for comparing subsequent models. The first and second models (M_1 and M_2) introduced the Gender and Age at Onset, Localization, and IBD type, respectively, but these additions did not improve the model fitness.

The third model (M_3), which incorporated treatment-related factors (Treatment Time, Immune suppression therapy, Type of Biological Therapy, and Intensification), showed a significant improvement in performance over the previous models ($p = 0.002$).

The final model (M_4), which included interaction terms, further improved the model fit ($p = 0.047$). The Nagelkerke R^2 value for M_4 was 0.287, indicating that our selected clinical variables explain a partial 28.7% of the variance in antibody positivity. This indicates that a large portion of the variance (over 70%) remains unexplained by the clinical parameters utilized here. The model's predictive power was confirmed by an AUC of 0.806, with a sensitivity of 71.0% and a specificity of 77.6% at the optimal cut-off value.

Table 4. Hierarchical Model Structure. The table shows the *p*-value and the efficiency of the fit of the hierarchically constructed models (M_0–M_4) via Nagelkerke R^2 values. The *p* values in the table indicate whether the model's performance significantly improved in comparison with the previous model.

Model	*p*	R^2	Variables
M0			
M_1	0.701	0.001	Gender
M_2	0.132	0.025	M1 + Age at onset, Localization, IBD type
M_3	0.002	0.107	M2 + Treatment Time, Immune suppression therapy, Type of Biological Therapy, Intensification
M_4	0.043	0.291	M3 + Interaction terms

3.3. *Significant Main Effects and Interactions*

The final model identified several significant main effects and interaction terms, see Table 5. IFX therapy was a powerful predictor of antibody positivity, with patients receiving IFX being nearly seven times more likely to have detectable antibodies than those on ADA (OR = 6.940, $p = 0.004$). The duration of treatment was also a significant predictor, with a longer treatment period increasing the likelihood of antibody positivity (OR = 2.505, $p = 0.029$). Localization was also a significant factor (OR = 2.216, $p = 0.049$), suggesting that specific anatomical localizations are associated with AIFX/AADA antibody positivity.

Table 5. Significant factors in the final logistic regression model (M_4).

Variable	Odds Ratio (OR)	95% Confidence Interval	*p*-Value
Type of Biological Therapy (IFX vs. ADA)	6.940	1.890–25.467	0.004
Treatment Time	2.505	1.107–5.672	0.029
Localization	2.216	1.002–4.902	0.049
Interaction: Treatment Time × Type of Biological Therapy	0.220	0.076–0.638	0.005
Interaction: Age at Onset × IBD Type	8.023	1.222–52.684	0.031
Interaction: Age at Onset × IBD Type × Type of Biological Therapy	0.019	0.001–0.725	0.042
Interaction: Treatment Time × IBD Type × Type of Biological Therapy	82.745	1.250–5473.497	0.042

In addition to the main effects, the final model included a number of interaction terms to explore possible synergistic effects between predictors, and four interactions reached statistical significance. The Treatment Time and Type of Biological Therapy interaction (OR = 0.220, $p = 0.005$) indicates that longer treatment appears to increase the risk of AADA, but not for AIFX. The interaction Age at Onset p × IBD type (OR = 8.023, $p = 0.031$) suggests that UC patients, variations in age at onset affect AIFX/AADA positivity, as AIFX/AADA positivity was more probable when IBD started in childhood.

The significance of three-way interaction, Age at Onset × IBD type × Type of Biological Therapy (OR = 0.019, $p = 0.042$), highlights the possibility of a complex relationship between age at onset, IBD type, and type of biologics used. This interaction demonstrates that the combined effect of childhood during onset and IBD type (UC or CD) on AIFX/AADA positivity is significantly influenced by the type of anti-TNF agent. However, it should be noted that the interpretation of the three-way interactions should be interpreted with caution due to the marginal *p* values ($p = 0.042$) and the limited sample size. For patients who were children at onset, IFX therapy was associated with a greater risk of AIFX/AADA positivity compared to ADA therapy. This effect is more significant in UC. To the contrary, in patients with adult-onset IBD, the difference in antibody positivity prevalence between ADA and IFX therapy diminishes in the UC group (Figure 1).

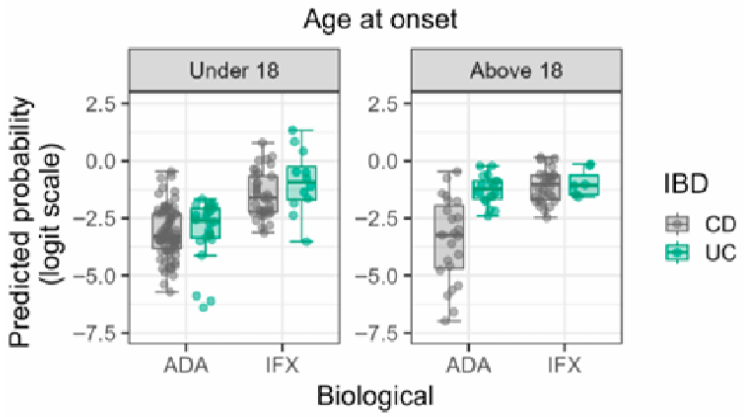

Figure 1. Logit predicted probabilities of antibody positivity stratified by age at onset, biological therapy, and IBD type. Predicted log probabilities are plotted on the *y*-axis, with biological therapies (ADA and IFX) on the *x*-axis. Gray dots represent individual Crohn's disease (CD) patients and green dots represent individual Ulcerative Colitis (UC) patients. The plot illustrates the complex three-way interaction, particularly the increased risk associated with IFX in childhood-onset UC.

The significant three-way interaction between AIFX/AADA positivity and Treatment Time × IBD type × Type of Biological Therapy (OR = 82.745, $p = 0.042$) highlights the complex relationship between these variables. This suggests that longer treatment durations with IFX significantly predispose both CD and UC patients to a higher prevalence of antibody positivity compared to ADA therapy (Figure 2). Furthermore, it is essential to note that the three-way interaction involving Treatment Time exhibited a highly volatile odds ratio (OR = 82.745) with an extremely wide confidence interval (95% CI 1.250–5473.497). This wide interval strongly suggests limited precision and potential instability in modeling this highly specific subgroup, likely due to cell sparsity, and should be interpreted as purely exploratory rather than confirmatory.

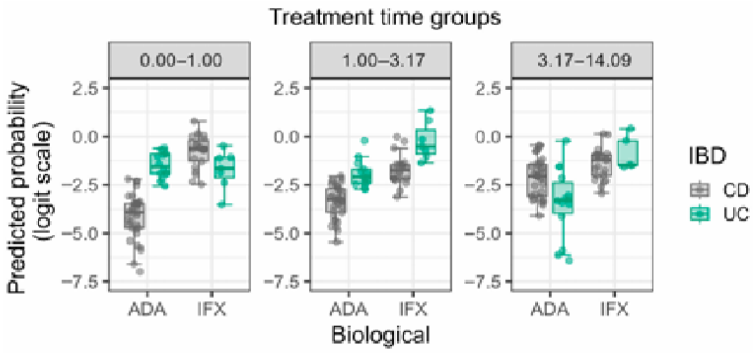

Figure 2. Logit predicted probabilities of antibody positivity stratified by treatment time, anti-TNF-alpha therapy, and IBD status. The panels represent three treatment time quartiles derived from the log-transformed data: Q1 (0.00–1.00 years), Q2 (1.00–3.17 years), and Q3 (3.17–14.09 years), respectively. Predicted log probabilities are plotted on the *y*-axis, with biological therapies (ADA and IFX) on the *x*-axis. Gray dots represent individual Crohn's disease (CD) patients and green dots represent individual Ulcerative Colitis (UC) patients.

4. Discussion

Our study reinforces the well-documented finding that IFX has a higher immunogenic potential compared to ADA. However, the primary value of our research lies in the identification of complex interactions between clinical factors that contribute to antibody formation in a real-world, heterogeneous patient population. Our hierarchical modeling approach allowed for a nuanced understanding of these relationships, which has not been widely explored in the existing literature. In our cross-sectional study, we analyzed and identified some factors that are associated with the presence of anti-TNF antibodies in UC and CD subjects. The hierarchical logistic regression model in the present study supports that treatment duration and type of biological therapy (IFX vs. ADA) are key predictors of anti-TNF antibody formation, which is consistent with previous studies published in the literature [15].

The finding of a significant three-way interaction involving age at onset, IBD subtype, and biological therapy is particularly notable. Previous research has suggested that IBD starting in childhood is associated with altered immune regulation, potentially leading to a heightened risk of anti-drug antibody formation [4,10]. Our findings expand on this by showing that this risk is further modulated by the specific anti-TNF agent used and the IBD subtype. The particularly high risk observed in childhood-onset UC patients on IFX aligns with the known higher immunogenicity of the chimeric IFX molecule compared to the humanized ADA molecule, suggesting that the naive, developing immune system in young UC patients may be highly reactive to the non-human epitopes present in IFX. This differential response underscores the need for personalized selection of biological agents in pediatric cohorts, particularly for UC, where IFX is often a frequent first-line choice [14].

The significant interaction between treatment duration and type of therapy also provides critical clinical insights. The increasing divergence in antibody prevalence between IFX and ADA with longer treatment duration underscores the importance of long-term Therapeutic Drug Monitoring (TDM) and antibody testing to preemptively address potential treatment failure [14]. This association is consistent with previous studies that demonstrate longer therapy periods enhance the immune response. These findings also support the importance of early detection of AIFX/AADA positivity and corresponding modification of therapy to prevent treatment failure in the long-term [4,5].

While our model achieved a statistically significant AUC of 0.806, the Nagelkerke R^2 of 0.287 confirms that clinical factors alone provide only partial prediction. Dedicated prediction models incorporating critical pharmacokinetic variables—such as initial drug trough levels or genetic markers associated with drug metabolism—typically achieve higher predictive capability (e.g., AUCs exceeding 0.85). The key implication of our study, therefore, is not to replace these dedicated biomarker panels but to provide a readily accessible, clinical framework for early risk stratification, identifying patients who may benefit most from proactive TDM interventions [16–19]. The advantage of TDM has been proposed by many researchers, and according to the latest surveys, it is the pediatric IBD population in which is recommended and widely used for the optimization of therapy in the case of anti-TNF treatments [20,21].

The study's design allowed us to enroll IBD patients of different ages and stages on ADA or IFX. Therefore, the results are characteristic of the population treated at a tertiary-care setting, where biological therapy treatment is provided for IBD, and they can be considered as "real-life" observations.

5. Study Limitations

This study is subject to several limitations inherent to its retrospective and cross-sectional design. First, the observed associations between clinical factors and antibody presence do not establish causality; thus, we cannot infer that longer treatment duration

causes antibody formation, only that longer duration is predicted by or associated with antibody presence at the time of sampling. Prospective validation is essential before any causal inference can be made or clinical guidelines derived. Second, despite recruiting from a tertiary center, the overall cohort size (N = 153) remains modest, especially when exploring high-order, three-way interaction terms. This limited power manifests in the extremely wide confidence intervals observed for some interaction effects (Table 5), indicating instability and limited precision in modeling these specific, sparse subgroups. The results derived from these complex interactions must therefore be treated as strictly hypothesis-generating, requiring confirmation in larger, well-powered, and externally validated cohorts. Third, the reliance on retrospective clinical data introduced notable proportions of missing data ("N/A") for several variables (e.g., 5-ASA status, Immunosuppression status). Although these were handled by treating "N/A" as a distinct category, this approach risks introducing bias associated with missingness, which may further contribute to the unexplained variance (71.3%) in the model.

6. Conclusions

In conclusion, our study confirms that anti-TNF-alpha antibody formation is a major challenge in IBD management, with IFX therapy and treatment duration being key predictors. The most significant finding, however, is the discovery of complex, synergistic interactions between easily accessible clinical factors, which provide a significant, albeit modest, predictive value. Our hierarchical modeling approach offers a framework for integrating multiple patient-specific variables to better assess the risk of immunogenicity. This framework requires prospective and external validation before it can be effectively integrated into routine clinical support systems for optimizing patient monitoring and preventing therapeutic failure in IBD care.

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Abbreviations

AADA	Anti-Adalimumab Antibody
ADA	Adalimumab
AIFX	Anti-Infliximab Antibody
AUC	Area Under Curve
CD	Crohn's Disease
ELISA	Enzyme-linked Immunosorbent Assay

ESPGHAN	European Society for Paediatric Gastroenterology Hepatology and Nutrition
IBD	Inflammatory Bowel Disease
IFX	Infliximab
LoD	Limit of Detection
LOR	Loss of Response
ORs	Odds Ratios
TDM	Therapeutic Drug Monitoring
TNF-α	Tumor Necrosis Factor Alpha
TRL	Trough Level
UC	Ulcerative Colitis

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Az antitestképződés gyakorisága biológiai terápiák során gyulladásos bélbetegségekben

Saját adatokkal illusztrálva

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A gyulladásos bélbetegségek kezelésében a biológiai terápiák forradalmi változást hoztak, lehetővé téve a nyálkahártyaszintű gyógyulást és a tartós remissziót. E készítmények hatékonyságát azonban jelentősen korlátozza az immunogenitás, vagyis a gyógyszerellenes antitestek képződése, amely hatásveszteséshez vezethet. Vizsgálatunk célja a hazai mérési eredmények áttekintése volt tumornekrózisfaktor-gátló infliximab (IFX)- és adalimumab (ADA)-, valamint másodvonalbeli vedolizumab (VDZ)- és ustekinumab (UST)-terápia mellett. A 2020 és 2025 között a Semmelweis Egyetemen gondozott 153 (IFX/ADA), valamint 183 (UST/VDZ) gyermek és felnőtt, gyulladásos bélbetegségben szenvedő beteg adatait elemeztük a két terápiás csoportban. Az elemzés során a keresztmetszeti adatgyűjtés módszerét alkalmaztuk, kiegészítve a legújabb immunoassay-technikák által szolgáltatott adatokkal. A teljes populációban az antitestképződés aránya a két kezelési csoportban 21% és 20% volt (IFX/ADA: 32/153; USTE/VDZ: 37/181; p = 0,98). A terápiák közötti összehasonlításban az IFX mellett nagyobb immunogenitás volt megfigyelhető (33,0%), amely meghaladta az ADA esetében tapasztalt értéket (12,0%; p = 0,001). Az antitest-pozitivitás UST mellett alacsony (15,0%), míg VDZ mellett nem szignifikánsan, de nagyobb arány volt mérhető (28,0%; p = 0,105). A gyulladásos bélbetegség típusa, az életkor és a nem szerint egyik terápiacsoportban sem volt kimutatható különbség az antitestképződés gyakoriságában. Eredményeink rámutatnak, hogy bár a biológiai szerek átlagos immunogenitása hasonló lehet, az egyes molekulák között jelentős eltérések vannak. Az IFX nagy immunogenitása, szemben az ADA alacsonyabb értékével, indokolja a szoros proaktív monitorozást és a kombinációs kezelést. A VDZ és az UST esetében az eredmények árnyaltabb megközelítést igényelnek, figyelembe véve az esetleges tranzitorikus antitestek jelenlétét. A vizsgálat alátámasztja a terápiás gyógyszerszint-monitorozás kiemelt szerepét a terápia optimalizálásában.
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Kulcsszavak: gyulladásos bélbetegség, biológiai terápia, immunogenitás, gyógyszerellenes antitest, terápiás gyógyszerszint-monitorozás

Frequency of antibody formation during biological therapies in inflammatory bowel diseases

Illustrated by original data

Biological therapies have revolutionized the treatment of inflammatory bowel diseases enabling mucosal healing and sustained remission. However, their long-term effectiveness is substantially limited by immunogenicity, namely the development of anti-drug antibodies, which may lead to secondary loss of response. The aim of our study was to evaluate real-world immunogenicity data in Hungary among patients treated with tumor necrosis factor inhibitors infliximab (IFX) and adalimumab (ADA), as well as second-line biological agents vedolizumab (VDZ) and ustekinu-

mab (UST). We performed a cross-sectional analysis of 153 inflammatory bowel disease patients receiving IFX or ADA and 183 patients treated with UST or VDZ, followed at Semmelweis University between 2020 and 2025. Both pediatric and adult patients were included. Immunogenicity data were assessed using modern immunoassay techniques, and results were analyzed according to treatment groups, disease characteristics, age, and sex. The overall prevalence of anti-drug antibodies was comparable between treatment groups (IFX/ADA: 21%, 32/153; UST/VDZ: 20%, 37/181; p = 0.98). In molecule-specific analyses, IFX was associated with significantly higher immunogenicity (33.0%) compared with ADA (12.0%; p = 0.001). Antibody positivity was low in patients treated with UST (15.0%), while a non-significantly higher rate was observed with VDZ (28.0%; p = 0.105). No significant differences in immunogenicity were detected according to inflammatory bowel diseases subtype, age, or sex. Although the overall immunogenicity of biological therapies may appear similar, substantial differences exist between individual agents. The higher immunogenicity of IFX compared with ADA supports the need for proactive therapeutic drug monitoring and, where appropriate, combination therapy. In the case of VDZ and UST, a more nuanced interpretation is required, considering the potential presence of transient antibodies. Our findings further emphasize the pivotal role of therapeutic drug monitoring in optimizing biological treatment strategies in inflammatory bowel diseases.

Keywords: inflammatory bowel disease, biological therapy, immunogenicity, anti-drug antibodies, therapeutic drug monitoring

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Rövidítések

AADA = antiadalimumab-antitest; ADA = adalimumab; AIFX = antiinfliximab-antitest; AUST = antiustekinumab-antitest; AVDZ = antivedolizumab-antitest; CD = (cluster of differentiation) differenciációs klaszter; ECCO = (European Crohn's and Colitis Organisation) Európai Crohn és Colitis Szervezet; ELISA = (enzyme-linked immunosorbent assay) enzimhez kapcsolt immunszorbens-vizsgálat; ESPGHAN = (European Society for Paediatric Gastroenterology Hepatology and Nutrition) Európai Gyermek-gasztroenterológiai, Hepatológiai és Táplálkozástudományi Társaság; HLA = humán leukocytaantigén; HMSA = (homogenous mobility shift assay) homogén mobilitási eltolódási vizsgálat; IFX = infliximab; OR = (odds ratio) esélyhányados; TNFα = tumornekrózisfaktor-alfa; UST = ustekinumab; VDZ = vedolizumab

A gyulladásos bélbetegségek, amelyek legfontosabb képviselői a Crohn-betegség és a colitis ulcerosa, a gastrointestinalis traktus krónikus, relapsusokkal és remissziókkal tarkított gyulladásos kórképei. E betegségek etiológiája multifaktoriális: a genetikai hajlam, a környezeti tényezők (mint például a táplálkozás és a dohányzás), valamint a bél mikrobiomjának dysbiosisa együttesen vezetnek az immunrendszer diszregulációjához és a bélfal autoimmun jellegű károsodásához. A betegségek növekvő incidenciája és prevalenciája világszerte, így hazánkban is, jelentős népegészségügyi kihívást jelent, különös tekintettel a fiatal életkorban történő manifesztációra, amely hosszú távú, élethosszig tartó orvosi ellátást és gondozást igényel [1].

Az elmúlt két évtizedben a gyulladásos bélbetegségek kezelése forradalmi változásokon ment keresztül. A hagyományos terápiás piramis – amely az aminoszalicilátoktól a kortikoszteroidokon át az immunszuppresszív szerekig (azatioprin, metotrexát) terjedt – csúcsán megjelentek a biológiai terápiák. A tumornekrózisfaktoralfa (TNFα)-gátlók, mint az infliximab (IFX) és az adalimumab (ADA) bevezetése drámaian javította a középsúlyos és súlyos betegek prognózisát, lehetővé téve nemcsak a klinikai tünetmentességet, hanem a szigorúbb végpontnak számító nyálkahártya-gyógyulást is. Ezt követően a terápiás paletta tovább bővült új hatásmechanizmusú szerekkel, mint az alfa4-béta7-integrin-gátló vedolizumab (VDZ) és az interleukin-12/23-gátló ustekinumab (UST), amelyek célzottabb hatást és kedvezőbb mellékhatásprofilt ígértek [2, 3].

Mindazonáltal a biológiai terápiák klinikai sikerét jelentősen korlátozza a hatásvesztés jelensége. A betegek jelentős része, mintegy 10–30%-a primer nonreszponder, azaz már a bevezető kezelésre sem reagál megfelelően. Még aggasztóbb a szekunder hatásvesztés aránya, amely a kezdetben jól reagáló betegek 20–50%-át érinti a fenntartó kezelés során. A hatásvesztés hátterében számos tényező állhat, de a legfontosabb és leginkább befolyásolható faktor a gyógyszer immunogenitása, vagyis a szervezet immunrendszerének reakciója a terápiás fehérjére, amely gyógyszerellenes antitestek termelésében nyilvánul meg [4].

Az immunogenitás nem csupán a gyógyszerszint csökkenését és a terápiás hatás elvesztését idézi elő, hanem növeli az infúziós reakciók és egyéb szisztémás mellékhatások kockázatát is. Az orvostudomány fejlődésével párhuzamosan megjelent a terápiás gyógyszerszint-monitorozás koncepciója, amely lehetővé teszi a gyógyszerszintek

és az antitestek pontos mérését, czáltal a terápia optimalizálását. Alkalmazása a reaktív (tünetvezérelt) megközelítésről főleg gyermekkorban és a TNF-gátlóknál egyre inkább a proaktív (megelőző) stratégia felé tolódik el, amelynek célja a tartós remisszió fenntartása és az immunogenitás megelőzése [5, 6].

A jelen közlemény célja, hogy áttekintést adjon az immunogenitás kialakulásának mechanizmusáról, az antitestképződés gyakoriságáról különböző biológiai szerek esetén, a klinikai következményekről, valamint részletesen tárgyalja a megelőzés és a kezelés bizonyítékokon alapuló lehetőségeit, különös tekintettel a kombinációs terápiára és a proaktív monitorozásra. A tanulmány emellett bemutatja a Semmelweis Egyetemen végzett keresztmetszeti vizsgálatunk eredményeit, összehasonlítva a hazai adatokat a nemzetközi irodalommal.

Az immunogenitás elméleti alapjai és mechanizmusa

A terápiás fehérjemolekulák immunrendszeri felismerése

A biológiai készítmények, lévén nagy molekulatömegű fehérjék, potenciálisan immungének a szervezet számára. Az immunrendszer a terápiás monoklonális antitesteket „idegenként" ismerheti fel, ami adaptív immunválaszt vált ki. Ez a folyamat a dendritikus sejtek általi antigénprezentációval kezdődik, amely aktiválja a CD4+ T-helper-sejteket. Ezek a sejtek stimulálják a B-sejteket, amelyek plazmasejtekké differenciálódva specifikus antitesteket termelnek a gyógyszermolekula ellen [1].

Az immunogenitás mértéke több tényezőtől függ:

- A gyógyszer szerkezete: A kiméra antitestek (mint az IFX, amely kb. 25%-ban egéreredetű fehérjeszekvenciát tartalmaz) immunogénebbek, mint a humanizált (például VDZ) vagy a teljesen humán (például ADA, UST) antitestek. Ennek ellenére a teljesen humán antitestek sem mentesek az immunogenitástól, mivel az idiotípusos régiók (a kötőhelyek) is tartalmazhatnak immunogén epitópokat.
- A gyógyszer tisztasága és formulációja: Az aggregátumok jelenléte növelheti az immunogenitást.
- Adagolási stratégia: Az epizodikus kezelés (csak panasz esetén történő adás) jelentősen növeli a szenzitizáció esélyét a rendszeres, fenntartó kezeléssel szemben.
- Betegspecifikus tényezők: A beteg immunstatusa és genetikai háttere meghatározó. A legújabb kutatások, mint például a PANTS-vizsgálat, azonosították a HLA-DQA1*05 allélt mint az immunogenitás független genetikai prediktorát. Azoknál a betegeknél, akik hordozzák ezt az allélt, nagyobb az anti-TNF-antitestek kialakulásának kockázata, függetlenül attól, hogy IFX-t vagy ADA-t kapnak [7].

Antitesttípusok és hatásmechanizmusok

Az antitestek két fő kategóriába sorolhatók a funkciójuk alapján:

- Neutralizáló antitestek (neutralizing antibodies): ezek az antitestek közvetlenül a gyógyszer aktív kötőhelyéhez (Fab-régió) kapcsolódnak, megakadályozva, hogy a gyógyszer a célmolekulához (például TNFα, α4β7-integrin) kötődjék. Ezzel közvetlenül semlegesítik a terápiás hatást.
- Nem neutralizáló antitestek (non-neutralizing/binding antibodies): ezek az antitestek a gyógyszermolekula egyéb részeihez kötődnek. Bár közvetlenül nem gátolják a kötődést, immunkomplexeket képeznek, amelyeket a reticuloendothelialis rendszer (máj, lép) gyorsabban eliminál. Ennek következménye a gyógyszer felezési idejének csökkenése és a szérumszint esése (accelerated clearance).

Klinikai szempontból mindkét típus releváns, mivel végső soron mindkettő a gyógyszerszint csökkenéséhez és a terápiás válaszvesztéshez vezet. A „clearing" (tisztító) hatás miatt még a nem neutralizáló antitestek is funkcionális elégtelenséget okoznak.

Az antitestképződés gyakorisága és jellemzői biológiai terápia szerint

A különböző biológiai terápiák eltérő immunogenitási profillal rendelkeznek, amelyet a klinikai vizsgálatok és a valós életbeli adatok is alátámasztanak [1].

Infliximab

Az IFX esetében az antitestképződés előfordulása a legnagyobb a biológiai szerek között. Számos vizsgálat 10–60% közötti gyakoriságról számol be, a mérési módszertől és a betegpopulációtól függően. A PANTS-vizsgálat adatai szerint a betegek jelentős részénél már a kezelés korai szakaszában (az indukciót követő hetekben) megjelennek az antitestek, ami a 14. heti alacsony gyógyszerszint egyik fő oka. Az antitestek jelenléte fokozza az akut és késői infúziós reakciók kockázatát, gyorsítja a készítmény eliminációját, és a terápiás válaszvesztés egyik legfőbb okozója [8].

Adalimumab

Az ADA esetében annak ellenére, hogy teljesen humán monoklonális antitest, az antitestképződés gyakorisága 5–30% közé tehető. A PAILOT-vizsgálat gyermekgyógyászati kohorszában igazolták, hogy az alacsony gyógyszerszintek hajlamosítanak az antitestek kialakulására, és a proaktív dózisoptimalizálás csökkentheti ezt a kockázatot. Alacsony titerű antitestképződés is elegendő lehet a gyógyszerszint jelentős csökkenéséhez, ami klinikai relapsushoz vezet [9].

Vedolizumab

A VDZ az egyik legalacsonyabb immunogenitású biológiai szer a gyulladásos bélbetegségek kezelésében. A GEMINI regisztrációs vizsgálatokban az antitestek előfordulása <5% volt, és ezek többsége tranzitorikus, klinikai következmény nélküli antitestnek bizonyult. Ugyanakkor újabb kutatások, amelyek érzékenyebb, gyógyszertoleráns assay-ket alkalmaztak, nagyobb immunogenitási rátákat is kimutattak, különösen azoknál a betegeknél, akiknél a kezelést megszakították, majd újraindították. *Williet és mtsai* rávilágítottak, hogy bár az antitestek jelenléte nem mindig jár azonnali hatásvesztéssel, a gyógyszerszintek csökkenését okozhatja. A vizsgálatunkban mért arány is erre a jelenségre, illetve az alkalmazott teszt érzékenységére utalhat [10, 11].

Ustekinumab

Az UST ellen kialakuló antitestek ritkák (3–5%), jellemzően alacsony titerűek, és a legtöbb vizsgálat nem mutatott ki klinikailag jelentős hatásromlást. Az IM-UNITI vizsgálat hosszú távú kiterjesztése során 5 éves utánkövetés mellett is alacsony maradt az immunogenitási ráta [12]. Ám *Roblin és mtsai* friss adatai szerint a gyógyszertoleráns assay-kkel detektált antitestek összefüggésbe hozhatók a terápiás válaszvesztéssel azoknál a betegeknél, akiknél alacsony gyógyszerszintet mértek [13].

Az immunogenitás klinikai következményei

Az immunogenitás klinikai manifesztációja sokrétű, és alapvetően befolyásolja a terápiás választ. A szekunder hatásvesztés a leggyakoribb következmény. Az antitestek felgyorsítják a gyógyszer eliminációját, így a szérumkoncentráció a terápiás küszöb alá esik. Ennek következtében a gyulladásos folyamatok újraaktiválódnak, a tünetek visszatérnek, és nő a szövődmények (sipolyok, szűkületek) kockázata [14].

Infúziós és injekciós reakciók is előfordulnak, az IFX esetében az antitestek (különösen a magas titerűek) erős korrelációt mutatnak az akut infúziós reakciókkal (anafilaxia, hipotenzió, dyspnoe). Emellett késői típusú, szérumbetegség-szerű reakciók (ízületi fájdalom, láz, kiütés) is előfordulhatnak az immunkomplexek lerakódása miatt. Az ADA-nál a reakciók általában lokálisak (bőrpír, fájdalom az injekció helyén), de a szisztémás hatás sem kizárt [15].

Keresztimmunogenitás hiánya esetében fontos megjegyezni, hogy az egyik szer elleni antitestképződés nem jelenti automatikusan azt, hogy a beteg más biológiai szerekre is antitestet fog termelni. *Costable és mtsai* igazolták, hogy a korábbi anti-TNF-terápia során kialakult immunogenitás nem növeli a VDZ vagy UST elleni antitestképződés kockázatát, így ezek a szerek biztonságos alternatívát jelentenek [5].

Az antitestek időbeli viselkedése is meghatározó. Beszélhetünk tranzitorikus (átmeneti) és perzisztens (tartós) antitestekről. A tranzitorikus antitestek gyakran alacsony titerben jelennek meg, majd spontán eltűnnek, és kevésbé befolyásolják a hosszú távú kimenetelt. Ezzel szemben a perzisztens antitestek tartós jelenléte szinte mindig a gyógyszerszint tartós csökkenéséhez és a kezelés végleges hatástalanságához vezet [16].

Terápiás gyógyszerszint-monitorozás: reaktív és proaktív megközelítések

A terápiás gyógyszerszint-monitorozás a gyulladásos bélbetegségek kezelésének egyik legfontosabb eszközévé vált a biológiai terápiák optimalizálása során [17].

Reaktív terápiás gyógyszerszint-monitorozás

A reaktív terápiás gyógyszerszint-monitorozás a válaszvesztés vagy tünetek megjelenése esetén alkalmazott stratégia. Segít megkülönböztetni a hatásvesztés okait:

- Farmakokinetikai ok: alacsony gyógyszerszint, amely lehet immunogén (magas antitesttiter) vagy nem immunogén (nincs antitest, de gyors a clearance) eredetű.
- Farmakodinámiás ok: megfelelő gyógyszerszint mellett is aktív a betegség (mechanisztikus elégtelenség, például nem TNF-vezérelt gyulladás).

Proaktív terápiás gyógyszerszint-monitorozás

A proaktív terápiás gyógyszerszint-monitorozás során a gyógyszerszinteket és az antitesteket rendszeres időközönként, a beteg klinikai állapotától függetlenül mérik, azzal a céllal, hogy a gyógyszerszintet egy meghatározott terápiás ablakban tartsák. IFX és ADA esetén egyre több adat, köztük a PAILOT és PANTS vizsgálatok eredményei utalnak arra, hogy a proaktív terápiás gyógyszerszint-monitorozás jobb fenntartó kimenetelekkel jár. A PAILOT-vizsgálat gyermekeknél igazolta, hogy a proaktív monitorozás szignifikánsan növelte a szteroidmentes remisszió arányát. A proaktív megközelítés révén megelőzhető az alacsony gyógyszerszint kialakulása, ami az immunogenitás egyik legfőbb kiváltó oka [18, 19].

Az immunogenitás megelőzésének és kezelésének lehetőségei

A biológiai terápiák tartósságának kulcsa az immunogenitás megelőzése. A szakirodalom és a klinikai tapasztalatok alapján több stratégia is rendelkezésre áll.

Kombinált immunszuppresszió

Ez az egyik legtöbbet alkalmazott preventív módszer, különösen az IFX esetében. A mérföldkőnek számító

SONIC-vizsgálat eredményei egyértelműen igazolták, hogy IFX és azatioprin kombinált alkalmazása szignifikánsan csökkentette az antitestek kialakulását a monoterápiához képest (0,9% *vs.* 14,6%), és ezzel párhuzamosan növelte a szteroidmentes remisszió arányát [20].

Az immunmodulátorok (tiopurinok vagy metotrexát) hatásmechanizmusa kettős: egyrészt gátolják az antitesttermelő B-sejtek proliferációját, másrészt farmakokinetikai interakció révén növelik a biológiai szer szérumszintjét. A PANTS-vizsgálat megerősítette, hogy ez a protektív hatás ADA esetén is érvényesül, bár kisebb mértékben, és különösen fontos lehet a HLA-DQA1*05 allélt hordozó, genetikailag veszélyeztetett betegeknél [7].

Proaktív gyógyszerszint-optimalizálás

Az alacsony gyógyszerszint az immunogenitás egyik legfőbb hajtóereje. Ha a gyógyszer koncentrációja tartósan alacsony, az immunrendszer könnyebben indít ellene választ. A proaktív terápiás gyógyszerszint-monitorozás segítségével biztosítható, hogy a gyógyszerszint mindig a „biztonságos", immuntoleranciát elősegítő tartományban maradjon. A megfelelő indukciós dózisok és a fenntartó kezelés során a dózisintenzifikáció (ha szükséges) megelőzheti az antitestek megjelenését [17].

Folyamatos kezelés

A korai tapasztalatok (főként IFX esetén) megmutatták, hogy az epizodikus („on-demand") kezelés, amikor a gyógyszert csak a tünetek visszatérésekor adják, drámaian megnöveli az immunogenitás kockázatát a folyamatos fenntartó kezeléshez képest. A modern terápiás protokollok ezért kizárólag a rendszeres adagolást javasolják [8].

Teendők antitestek megjelenése esetén

A kezelés során kialakult antitestek esetén a teendő az antitestek mennyiségétől és a gyógyszerszinttől függ:

- Alacsony titerű antitest + alacsony gyógyszerszint: a dózis emelése vagy az adagolási intervallum csökkentése (intenzifikáció) gyakran sikeres lehet. A nagyobb gyógyszerdózis képes „telíteni" és semlegesíteni az antitesteket, valamint visszaállítani a terápiás gyógyszerszintet. Ebben az esetben gyakran javasolt immunszuppresszáns (például azatioprin) hozzáadása is a „visszaszuppresszálás" érdekében.
- „Magas titerű antitest + alacsony/mérhetetlen gyógyszerszint: ez általában tartós hatásvesztést jelez. Ilyenkor a dózisemelés általában hatástalan és költséges, ezért osztályon belüli váltás (például IFX-ről ADA-ra) vagy osztályváltás (más hatásmechanizmusú szer, például VDZ, UST) javasolt [17].

Célkitűzés

Kutatásaink célja az volt, hogy az általunk vizsgált, különböző gyulladásos bélbetegségekben szenvedő betegektől származó minták alapján áttekintsük a hazai antitestmérések eredményeit a tumornekrózisfaktor-gátló IFX- és ADA-, valamint a másodvonalbeli VDZ- és UST-kezelések esetében, és összehasonlítsuk a három (ADA, IFX–UST–VDZ) eltérő hatásmechanizmusú készítmény antitestképződésének incidenciáját.

Betegek és módszerek

A betegek kiválasztása

Vizsgálatunk során 153, IFX- és ADA-, valamint 183, UST- és VDZ-terápiát kapó gyermek és felnőtt, gyulladásos bélbetegséggel kezelt beteget vizsgáltunk, akik a Semmelweis Egyetem Gyermekgyógyászati Klinikáján, Sebészeti, Transzplantációs és Gasztroenterológiai Klinikáján, illetve Belgyógyászati és Onkológiai Klinikáján álltak gondozásban 2020 és 2025 között. A beválasztási kritériumokat a gyermekek esetében az Európai Gyermek-gasztroenterológiai, Hepatológiai és Táplálkozási Társaság (ESPGHAN), felnőttek esetében pedig az Európai Crohn és Colitis Szervezet (ECCO) diagnosztikai ajánlásai alapján állapítottuk meg [21], illetve feltétel volt, hogy legyen legalább egy terápiás gyógyszerszint-monitorozási eredményük. Az elemzések során ugyanakkor kizártuk azokat a betegeredményeket, amelyek egy személy esetén több gyógyszerszintmérési pontot is tartalmaztak, illetve ezeknek a betegeknek csak az első mérési pontját vettük figyelembe.

Etikai engedélyek

A kutatásba beválogatott összes résztvevőt tájékoztattuk a vizsgálat elvégzéséről, annak menetéről, és mindenkitől írásbeli beleegyezést kértünk. Minden eljárást az etikai bizottság (az Egészségügyi Tudományos Tanács Klinikai Farmakológiai Etikai Bizottsága) által kiadott, 19048-Á2/2018/EKU számú etikai engedélyünkben foglaltak szerint végeztünk. Az adatokat anonimizáltuk, ezáltal biztosítva, hogy az eredmények a későbbiekben se legyenek összevethetők egyik, a vizsgálatban részt vevő pácienssel sem. A vizsgálatban végzett összes eljárás az 1964. évi Helsinki Nyilatkozattal és annak későbbi módosításaival összhangban történt.

Módszerek

Közvetlenül a soron következő gyógyszerbeadás előtt minden betegtől natív vérmintát vettünk. A mintákat centrifugálást (szobahőmérsékleten, 2300 × *g*, 10 percig) követően –20 °C-on tároltuk, és ADA-, AADA-, IFX- és AIFX-, UST- és AUST-, valamint VDZ- és AVDZ-koncentráció méréséhez használtuk. A méréseket

LisaTracker Duo Infliximab, Duo Adalimumab, Duo Ustekinumab, valamint Duo Vedolizumab In Vitro Diagnostic (IVD) minősítésű ELISA-kitekkel (Biosynex Theradiag, Croissy Beaubourg, Franciaország) végeztük. A méréseket a DAS-, APE-, ELITE-, ELISA-készülékkel (DAS Instruments, Róma, Olaszország) végeztük a gyártó előírásainak megfelelően [22].

Statisztikai elemzések

Vizsgálataink során először külön statisztikai elemzéseket végeztünk az első vonalbeli (IFX, ADA), majd pedig a másod- vagy harmadvonalbeli (UST, VDZ) biológiai terápiák során. Olyan modelleket igyekeztünk felállítani, amelyek előre jelezhetik a szuboptimális gyógyszerszintek kialakulását, valamint a terápiák során esetlegesen megjelenő antitestek képződésének rizikójáról is információt adnak. Jelen elemzésünk során azt vizsgáltuk, hogy mennyire hasonlít az első vonalbeli terápiák antitestképződési prevalenciája a második vonalban alkalmazott szerekéhez. Ehhez a két csoportban kapott eredményeket hasonlítottuk össze khi-négyzet (χ^2) statisztikai módszer segítségével.

Eredmények

Korábban már publikált első vonalbeli terápiás eredményeinket (n = 153; ADA/IFX) összehasonlítottuk a másodvonalbeli biológiai terápiák (n = 181; VDZ/UST) esetén tapasztalt antitestképződési rátákkal, valamint az immunszuppresszív kezelések azokra gyakorolt hatásával [23] *(1. táblázat, 1. ábra)*.

A két adatbázis betegösszetétele némileg eltért egymástól. A gyermekek aránya a TNF-gátlót kapó populációban 26% volt, míg az USTE/VDZ csoportban lényegesen nagyobb: 42%. A teljes beteganyagban a Crohn-betegségben szenvedők aránya 57–60%, míg a colitis ulcerosában szenvedőké 40–43% volt.

Az ellenanyag-képződés előfordulása a két adatbázisban egyezően 21%-nak, illetve 20%-nak bizonyult (IFX/ADA: 32/153; UST/VDZ: 37/181). A két csoport közötti különbség statisztikailag nem bizonyult szignifikánsnak (χ^2 = 0,0006; p = 0,98). A betegcsoportok a gyulladásos bélbetegség típusa szerinti bontásban sem mutattak eltérést: Chrohn-betegség esetén (p = 0,92) és colitis ulcerosa esetén (p = 0,21) is azonos immunogenitási arány volt megfigyelhető.

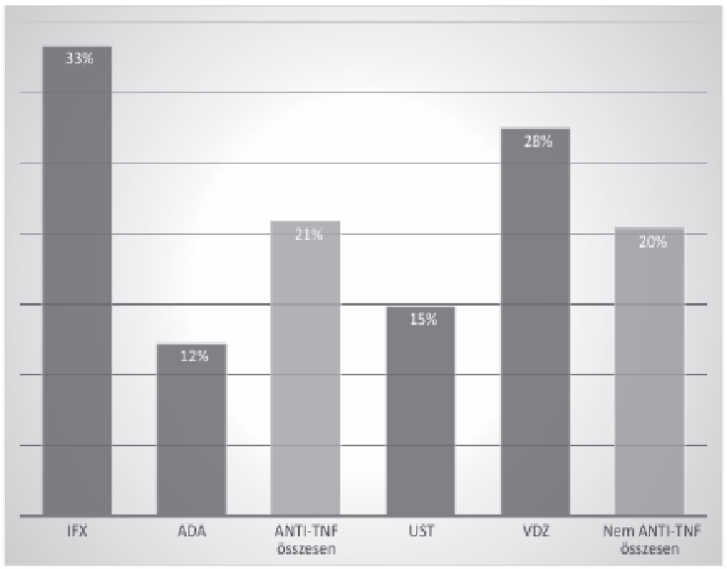
A biológiai terápiák közötti összehasonlítás ugyanakkor lényeges különbségeket mutatott. Az antitestképződés gyakorisága az IFX esetén 33% volt, míg az ADA esetében mindössze 12%, ami szignifikáns eltérést mutatott (χ^2 = 10,8; p = 0,001). A másodvonalbeli kezeléseknél az UST antitestképződési aránya 15%, míg a VDZ-kezelt betegeké 28% volt, a két csoport különbsége azonban nem érte el a statisztikai szignifikancia szintjét (χ^2 = 2,62; p = 0,105), noha tendenciájában nagyobb immunogenitás volt megfigyelhető a VDZ-terápia esetén. Fontos kiemelni, hogy a VDZ és az UST esetében kimutatott antitestek jelentős része nem társult klinikai hatásvesztéssel, ami arra utal, hogy ezen antitestek egy része alacsony titerű, nem neutralizáló vagy tranzitorikus

1. táblázat | Gyulladásos bélbetegek biológiai terápiás antitestképződési gyakorisága TNF- és nem TNF-gátlók, Crohn-betegség és colitis ulcerosa esetében

Anti-TNF (IFX, ADA)					
	Crohn-betegség		Colitis ulcerosa		Összesen
	IFX	ADA	IFX	ADA	
A betegek száma (ff/nő)	50 (27/23)	59 (23/36)	13 (6/7)	31 (14/17)	153 (70/83)
Gyermekek	10 (6%)	18 (12%)	5 (3%)	7 (5%)	40 (26%)
Felnőttek	40 (26%)	41 (27%)	8 (5%)	24 (16%)	113 (74%)
Immunszuppresszív terápia	19 (38%)	32 (54%)	7 (54%)	14 (45%)	72 (47%)
Antitestképződés IFX/ADA ellen	16 (32%)	5 (8%)	5 (38%)	6 (19%)	32 (21%)

UST-VDZ					
	Crohn-betegség		Colitis ulcerosa		Összesen
	UST	VDZ	UST	VDZ	
A betegek száma (ff/nő)	83 (43/40)	24 (15/9)	18 (8/10)	56 (26/30)	181 (84/97)
Gyermekek	40 (22%)	7 (4%)	9 (50%)	20 (36%)	76 (42%)
Felnőttek	43 (24%)	17 (9%)	9 (50%)	36 (64%)	105 (58%)
Immunszuppresszív terápia	3 (4%)	1 (4%)	0 (0%)	1 (2%)	4 (2%)
Antitestképződés UST/VDZ ellen	13 (16%)	8 (33%)	2 (11%)	14 (25%)	37 (20%)

ADA = adalimumab; IFX = infliximab; TNF = tumornekrózis-faktor; UST = ustekinumab; VDZ = vedolizumab



1. ábra Gyulladásos bélbetegek biológiai terápiás antitestképződési gyakorisága TNF- és nem TNF-gátlók esetében

ADA = adalimumab; IFX = infliximab; TNF = tumornekrózis-faktor; UST = ustekinumab; VDZ = vedolizumab

lehet. Az alkalmazott gyógyszertoleráns mérési módszer fokozott analitikai érzékenysége hozzájárulhatott az antitest-pozitivitás gyakoribb detektálásához, ugyanakkor ez nem feltétlenül jelent klinikailag releváns immunogenitást.

Az anti-TNF-kezelésben részesülő betegek körében lényegesen gyakoribb volt az immunszuppresszív szerek egyidejű alkalmazása, mint az UST- vagy VDZ-terápia esetén. Az anti-TNF-csoportban 47%, míg az UST/VDZ csoportban mindössze 2% kapott immunszuppresszív kezelést.

Az elvégzett Fisher-féle egzakt teszt extrém módon szignifikáns különbséget mutatott a két terápiacsoport között ($p = 1{,}33 \times 10^{-24}$), az OR (odds ratio) 39,3 volt, ami azt jelzi, hogy anti-TNF-kezelés mellett 39-szer nagyobb az esély immunszuppresszív szerek egyidejű alkalmazására, mint UST vagy VDZ mellett.

Ez a különbség mind statisztikailag, mind klinikai szempontból rendkívül jelentős, és összhangban áll a biológiai terápiák farmakológiai sajátosságaival és a jelenlegi terápiás irányelvekkel. Az anti-TNF-szerek immunogenitása nagyobb, ezért gyakran szükséges az immunszuppresszív kombináció alkalmazása az ellenanyag-képződés csökkentésére. Ezzel szemben az UST és a VDZ immunogenitása alacsony, a kombinált terápia alkalmazása nem része a standard kezelési gyakorlatnak, ami a vizsgált populációban is megfigyelhető volt.

Együttes, többváltozós elemzésben az antitestképződés előfordulását sem az életkor (gyermek/felnőtt felosztás), sem a nem, sem a gyulladásos bélbetegség diagnózisa nem befolyásolta érdemben egyik vizsgált populációban sem.

Megbeszélés

A vizsgálat eredményei alapján a biológiai terápiák immunogenitása a teljes beteganyagban megközelítőleg azonosnak bizonyult, a teljes antitestképződési arány a két csoportban 21%, illetve 20% volt. Ez arra utal, hogy a különböző betegpopulációk (gyermek/felnőtt arány, diagnózismegoszlás) eltérése ellenére az aggregált immunválasz mértéke stabilnak tekinthető.

Jelentős különbség ugyanakkor a terápiák közötti összehasonlításban mutatkozott: az IFX-kezeléshez társult a legnagyobb immunogenitás, amely több mint kétszerese volt az ADA mellett észlelt aránynak. Ez összhangban áll a korábbi nemzetközi megfigyelésekkel, amelyek szerint a kiméra szerkezetű IFX immunogénebb a teljesen humán ADA-hoz viszonyítva. A PANTS-vizsgálat eredményei is megerősítették, hogy az IFX-monoterápia mellett szignifikánsan gyakoribb az immunogenitás kialakulása [24].

A másodvonalbeli készítmények között az UST mutatta a legkisebb antitestképződési rátát, ami egyezik a nemzetközi irodalommal, ahol a hosszú távú vizsgálatok (például IM-UNITI LTE) is 5% alatti immunogenitást igazoltak [2]. Míg a VDZ-ról ismert, hogy bár immunogenitása általában alacsony, egyes kohorszokban a jelen

vizsgálattal összhangban nagyobb arányban lehet antitest-pozitivitást kimutatni [25]. A vizsgálatunkban mért 28%-os AVDZ-pozitivitás nagyobb a regisztrációs GEMINI-vizsgálatokban közölt 4%-nál [25]. Ez az eltérés valószínűleg a mérési metodikával magyarázható: a modern, gyógyszertoleráns assay-k képesek kimutatni az antitesteket akkor is, ha a gyógyszer jelen van a szérumban, így feltárják a „rejtett" immunogenitást. *Williet és mtsai* hasonlóan kimutatták, hogy a gyógyszertoleráns assay-kkel az antitest-pozitivitás gyakoribb lehet, de ezek klinikai relevanciája (hatásvesztés okozása) nem mindig egyértelmű, mivel sok esetben nem neutralizáló vagy átmeneti antitestekről van szó [10].

A különbség azonban ebben az elemzésben nem bizonyult statisztikailag szignifikánsnak, ami részben a kisebb alcsoportlétszámokkal magyarázható. A Crohn-betegség és a colitis ulcerosa összehasonlításában nem volt kimutatható különbség az antitestképződés gyakoriságában egyik terápiás csoportban sem. Ugyancsak nem azonosítottunk kapcsolatot az antitestképződés és a nem vagy az életkor között. Ez megerősíti azt a feltételezést, hogy az immunogenitást maga a biológiai szer típusa, annak fehérjestruktúrája és farmakokinetikája határozza meg [1].

Az immunogenitás klinikai jelentőségének vizsgálata kapcsán fontos kitérni a kis molekulatömegű, új típusú, gyulladásos bélbetegség elleni gyógyszerek lehetséges szerepére is. Ezek a készítmények (Janus-kináz-gátlók vagy a szfingozin-1-foszfát-receptor-modulátorok) nem fehérjetermészetűek, így alkalmazásuk során antitestképződés nem alakul ki, ami elméleti és gyakorlati előnyt jelenthet az immunogenitás okozta szekunder hatásvesztés elkerülésében.

Klinikailag ez különösen releváns lehet olyan betegek esetében, akiknél több, egymást követő biológiai terápia sikertelensége igazolható antitestképződés miatt. Ugyanakkor hangsúlyozandó, hogy e készítmények eltérő mellékhatásprofilja és hosszú távú biztonságossági adataik korlátozottabb volta miatt alkalmazásuk jelenleg elsősorban gondosan mérlegelt, individualizált döntést igényel. A jövőben a biológiai és kis molekulatömegű terápiák megfelelő sorrendjének és kombinációjának meghatározása kulcsszerepet játszhat a gyulladásos bélbetegségek személyre szabott kezelésében [26].

Limitációk

A vizsgálat értelmezésekor fontos figyelembe venni a módszertani korlátokat, különösen a vizsgálat keresztmetszeti jellegéből adódóakat.

- Keresztmetszeti jelleg: a legfontosabb korlát, hogy a betegektől egyetlen időpontban történt mintavétel. Ez a módszer nem teszi lehetővé az antitestképződés dinamikájának megfigyelését, így nem különíthetők el az átmeneti antitestek a tartós antitestektől. Az irodalmi adatok szerint az átmeneti antitestek gyakran spontán eltűnnek, és kevésbé befolyásolják a hosszú távú klinikai kimenetelt, míg a perzisztens antitestek egyértelműen a hatásvesztéshez és a gyógyszerszint tartós csökkenéséhez köthetők. Egyetlen mérés alapján a klinikailag releváns immunogenitás mértéke túlbecsülhető lehet [27].
- Ok-okozati összefüggés: a keresztmetszeti elrendezés miatt nem állapítható meg egyértelmű ok-okozati viszony. Nem dönthető el biztosan, hogy az alacsony gyógyszerszint vezetett-e az immunizációhoz, vagy az antitestek megjelenése okozta a gyógyszerszint csökkenését, bár a szakirodalom mindkét irányt alátámasztja.
- Betegpopuláció: a heterogén betegcsoport (gyermek és felnőtt, különböző betegségfenotípusok) és a változó előkezelés ('bio-naiv' vs. 'bio-experienced') befolyásolhatja az eredményeket, bár a statisztikai elemzés nem mutatott szignifikáns eltérést e faktorok mentén.
- Assay-specifikusság: az eredmények az alkalmazott ELISA-kitek (LisaTracker) érzékenységétől és gyógyszer-toleranciájától függenek, ami megnehezíti a más módszerekkel (például HMSA) végzett vizsgálatokkal való közvetlen összehasonlítást.

Következtetés

Összegzésként elmondható, hogy a két különböző betegpopulációt összehasonlítva az antitestképződési ráta független a kezeléscsoporttól, ugyanakkor terápiás szerek között jelentős különbségek vannak, amelyek a kezelési stratégia megválasztása és a terépiás gyógyszerszint-monitorozás gyakorisága szempontjából meghatározóak. A terépiás gyógyszerszint-monitorozás jelentőségét nem lehet elégszer hangsúlyozni, mivel az antitestképződés korai felismerése és megfelelő kezelése (intenzifikáció vagy terápiaváltás) alapvetően befolyásolja a hosszú távú klinikai kimeneteleket. A vizsgált populációban különösen az IFX esetén indokolt a szoros monitorozás, míg UST és ADA mellett a kisebb immunogenitási kockázat miatt ritkább vizsgálat is elegendő lehet. A jövőben a proaktív terápiás gyógyszerszint-monitorozási protokollok szélesebb körű bevezetése és a kombinációs terápiák célzott alkalmazása kulcsfontosságú lehet az immunogenitás megelőzésében és a biológiai terápiák hatékonyságának fenntartásában mind a gyermek, mind pedig a felnőtt gyulladásos bélbetegekben [28].

Anyagi támogatás: A dolgozat megírása nem részesült anyagi támogatásban.

Szerzői munkamegosztás: A szerzők egyenlő arányban és mértékben vettek részt az irodalomkutatásban és a kézirat elkészítésében. A közlemény végleges változatát valamennyi szerző elolvasta és jóváhagyta.

Érdekeltségek: A szerzőknek nincsenek érdekeltségeik.

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