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RELIABILITY OF ELISA TEST IN THERAPEUTIC DRUG MONITORING OF ADALIMUMAB AND INFLIXIMAB IN PEDIATRIC INFLAMMATORY BOWEL DISEASE

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Inflammatory bowel disease (IBD) is a significant cause of morbidity and disability in pediatric patients, characterized by chronic intestinal inflammation. Biologic therapies, such as adalimumab and infliximab, are widely used for induction and maintenance of remission. Therapeutic drug monitoring (TDM) enables individualized treatment by optimizing drug dosage based on serum concentrations. The aim of this study was to evaluate the reliability of the enzyme-linked immunosorbent assay (ELISA) for determining serum levels of adalimumab and infliximab in pediatric patients with IBD. This retrospective study analyzed serum samples of 40 pediatric patients, divided into two groups: 20 receiving adalimumab or infliximab therapy and 20 controls. Serum levels were measured using the RIDASCREEN ELISA kits (R-Biopharm AG) and the Dynex DS2 analyzer. Method reliability was assessed through precision, accuracy, sensitivity, and specificity. Calibration curves demonstrated high reliability, with coefficients of determination (R^2) of 0.998 (adalimumab) and 0.999 (infliximab). Precision, indicated by coefficients of variation, was 4.3% for adalimumab and 4.1% for infliximab. Accuracy, measured by bias, was 3.75% for adalimumab and 0.05% for infliximab. Sensitivity and specificity were both 100%, confirming the test's ability to accurately detect or exclude drug presence in serum samples. R-Biopharm AG ELISA-based TDM provides reliable, precise, and accurate results for monitoring adalimumab and infliximab levels in pediatric patients. These findings support its use as a gold standard for individualized treatment optimization in IBD.

Keywords: inflammatory bowel disease, biologic therapies, adalimumab, infliximab, therapeutic drug monitoring, ELISA

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INTRODUCTION

Inflammatory bowel disease (IBD) is a group of chronic, relapsing-remitting inflammatory conditions affecting the gastrointestinal tract, primarily encompassing Crohn's disease (CD) and ulcerative colitis (UC). The prevalence of pediatric IBD onset has been increasing globally, particularly in developed countries, suggesting a strong interplay between genetic predisposition and environmental factors. Although the precise etiology remains unknown, IBD is believed to result from an inappropriate immune response to intestinal microbiota in genetically susceptible individuals (1, 2).

The incidence of pediatric IBD varies across different geographic regions. In Europe, the incidence of IBD in children is estimated at 23 per 100,000, whereas in North America, it is approximately 15 per 100,000. The rising prevalence in developing countries suggests that environmental factors such as diet, hygiene, and antibiotic use play a crucial role in disease pathogenesis (1-4).

Genetic factors also contribute significantly to IBD susceptibility. Genome-wide association studies have identified multiple risk loci, including genes involved in immune regulation, epithelial barrier integrity, and microbial interactions. However, environmental factors such as early antibiotic exposure, a diet rich in processed foods, and reduced microbial diversity due to improved hygiene are believed to modulate disease expression.

IBD pathogenesis involves a complex interplay of immune dysregulation, genetic susceptibility, and environmental triggers. The disruption of the gut barrier function allows microbial antigens to activate immune cells, leading to chronic intestinal inflammation. Key cytokines involved in this inflammatory cascade include tumor necrosis factor-alpha (TNF-α), interleukin-12 (IL-12), and interleukin-23 (IL-23) (5,6).

Clinically, pediatric IBD presents with a wide spectrum of symptoms. CD can affect any segment of the gastrointestinal tract and is characterized by transmural inflammation, leading to complications such as strictures and fistulae. In contrast, UC primarily involves the colon and is limited to the mucosal layer, often presenting with bloody diarrhea and urgency. Systemic manifestations, including growth retardation, weight loss, and extraintestinal manifestations (e.g., arthritis, uveitis, and erythema nodosum), are common in pediatric patients (7-10).

The diagnosis of IBD relies on a combination of clinical assessment, laboratory tests, imaging, and endoscopic evaluation with histopathological confirmation. Laboratory markers such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and fecal calprotectin are useful in assessing disease activity. Endoscopic findings, including skip lesions in CD and continuous colonic involvement in UC, aid in differentiation (11-16).

The therapeutic approach to pediatric IBD has evolved significantly with the introduction of biologic therapies targeting specific inflammatory pathways. TNF-α inhibitors such as infliximab and adalimumab have demonstrated efficacy in inducing and maintaining remission in moderate-to-severe cases. The optimization of biologic therapy through therapeutic drug monitoring (TDM) ensures adequate drug exposure while minimizing immunogenicity and adverse effects (17-20).

Due to the central role of TNF-α in the pathogenesis of IBD, it has become a key target for biological therapy. Biological drugs such as infliximab and adalimumab are monoclonal antibodies that specifically inhibit TNF-α activity, preventing its binding to receptors, thereby reducing inflammation. This allows for healing of the intestinal mucosa, reduction of symptoms, and long-term maintenance of remission in patients with IBD (21-27).

The quality of life in children with IBD varies depending on several factors, including the severity of the disease, response to therapy, presence of complications, and the child's overall health condition. CD and UC are chronic and often unpredictable diseases, carrying a certain degree of disability. However, with timely diagnosis, appropriate treatment, and adequate psychological support, long periods of remission and good quality of life can be achieved (28,29).

The enzyme-linked immunosorbent assay (ELISA) is widely utilized for measuring serum drug levels and detecting anti-drug antibodies in IBD patients receiving biologic therapy. ELISA is based on antigen-antibody interactions and provides a cost-effective and reliable method for quantifying therapeutic agents such as adalimumab and infliximab. The application of ELISA in TDM allows clinicians to adjust drug dosing based on serum concentrations, thereby optimizing treatment outcomes (30).

Recent studies have highlighted the clinical benefits of proactive TDM using ELISA. Maintaining optimal drug levels is associated with better mucosal healing rates, prolonged remission, and reduced need for corticosteroids. Furthermore, early detection of antidrug antibodies enables timely intervention, such as dose escalation or switching to alternative therapies (31,32).

Given the increasing reliance on biologic therapies in pediatric IBD, accurate and reliable methods for serum drug monitoring are essential. The aim of this study was to assess the reliability of an ELISA-based method for determining adalimumab and infliximab serum concentrations in pediatric patients. By evaluating calibration, precision, accuracy, sensitivity, and specificity, we aimed to validate the clinical utility of ELISA in therapeutic drug monitoring.

METHODS

Study design and participants

This retrospective study was conducted at the Institute for Health Protection of Children and Youth of Vojvodina. It included 40 pediatric patients, of whom 20 were receiving adalimumab or infliximab therapy, while the remaining 20 served as controls. Inclusion criteria were pediatric patients (<18 years) with confirmed IBD treated with adalimumab or infliximab, with at least one ELISA-based serum drug level available, complete medical records, and documented clinical data; control patients were age-matched, not receiving biologic therapy, and without inflammatory or autoimmune diseases. Exclusion criteria included incomplete documentation, missing serum levels, use of other biologics, known immunodeficiencies or severe comorbidities, and, for controls, presence of IBD or chronic inflammatory conditions.

Sample collection and processing

Serum samples were collected using vacuum tubes with clot activators (Becton Dickinson, New Jersey, USA). The samples were centrifuged at 4000 rpm for 5 minutes (Rotofix 32A, Hettich, Tuttlingen, Germany), and the serum was separated and stored at -20°C until analysis, with a maximum storage period of three months.

ELISA analysis

Serum concentrations of adalimumab and infliximab were determined using RIDASCREEN ADM Monitoring and RIDASCREEN IFX Monitoring kits (R-Biopharm AG, Darmstadt, Germany) on a DYNEX DS2 automated ELISA analyzer (Dynex Technologies, Chantilly, Virginia, USA). The method was based on a sandwich ELISA technique where TNF-α molecules were immobilized on microtiter wells. The analysis included the following steps:

1. Incubation of serum samples (100 µL per well) at 37°C for 1 hour, allowing the drug to bind to TNF-α.
2. Washing and addition of enzyme-conjugated antibody (100 µL per well) followed by incubation at 37°C for 30 minutes.
3. Addition of substrate solution (hydrogen peroxide) and incubation for 10 minutes, producing a color reaction.
4. Stopping the reaction with sulfuric acid (50 µL per well), followed by spectrophotometric measurement at 450 nm with a 620 nm reference filter.

Calibration and quality control

Calibration was performed using six standard solutions to generate a sigmoidal calibration curve. The accuracy of the calibration was assessed using the coefficient of determination (R2). Quality control was conducted using control sera with predefined low (8–16 µg/mL) and high (20–40 µg/mL) concentration ranges.

Statistical analysis

Method reliability was assessed through precision, accuracy, sensitivity, and specificity. Precision was expressed as the coefficient of variation (CV), accuracy as bias percentage, and diagnostic performance via sensitivity and specificity calculations. Statistical analysis was performed using Microsoft Excel 2021 Professional Plus.

Ethical approval

This study was approved by the Ethics Committee of the Institute for Health Protection of Children and Youth of Vojvodina (Approval No. 3987-8, August 15, 2024). All procedures were conducted in accordance with the Declaration of Helsinki.

RESULTS

The reliability of the method for determining serum concentrations of adalimumab and infliximab was examined through precision, accuracy, sensitivity, and specificity, after calibration and quality control were performed.

Calibration

Calibration curves for determining the serum concentrations of adalimumab and infliximab were successfully created, with a coefficient of determination of 0.998 for adalimumab (Figure 1) and 0.999 for infliximab (Figure 2).

Quality control

The obtained concentrations of adalimumab and infliximab in control sera are shown in Table 1.

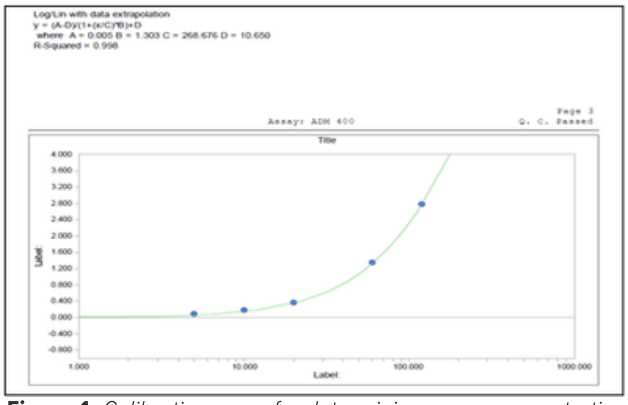

Figure 1. *Calibration curve for determining serum concentration of adalimumab*

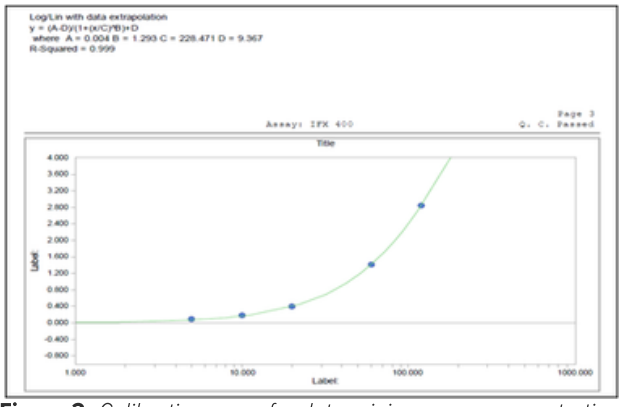

Figure 2. *Calibration curve for determining serum concentration of infliximab*

Precision

The coefficient of variation for determining serum concentration of adalimumab using the ELISA method in the Laboratory Diagnostics Department of the Institute for Health Care of Children and Youth Vojvodina was 4.3% (x = 13.229, SD = 0.572).

The coefficient of variation for determining serum concentration of infliximab using the ELISA method in the Laboratory Diagnostics Department of the Institute for Health Care of Children and Youth Vojvodina was 4.1% (x = 22.165, SD = 0.904).

Accuracy

The inaccuracy, or dispersion (d), in determining serum concentration of adalimumab using the ELISA method in the Laboratory Diagnostics Department of the Institute for Health Care of Children and Youth Vojvodina was 3.75%.

The inaccuracy, or dispersion (d), in determining serum concentration of infliximab using the ELISA method in the Laboratory Diagnostics Department of the Institute for Health Care of Children and Youth Vojvodina was 0.05%.

Sensitivity

In all samples from children receiving adalimumab, the drug was successfully detected (with no false negative results), confirming the test's sensitivity of 100%.

In all samples from children receiving infliximab, the drug was successfully detected (with no false negative results), confirming the test's sensitivity of 100%.

Specificity

In no sample from children not receiving adalimumab was the drug detected (with no false positive results), confirming the test's specificity of 100%.

In no sample from children not receiving infliximab was the drug detected (with no false positive results), confirming the test's specificity of 100%.

DISCUSSION

The determination of serum concentrations of adalimumab and infliximab plays a crucial role in the individualization of therapy for patients with inflammatory bowel diseases.

The metabolism of adalimumab and infliximab primarily occurs in the liver through the reticuloendothelial system, but a significant portion of these drugs may be lost in the stool, especially in patients with active inflammatory bowel disease. This loss of the drug through stool represents one of the most important factors contributing to subtherapeutic drug levels in serum, which directly impacts the effectiveness of therapy. Such losses can be attributed to increased intestinal permeability and augmented protein turnover due to active inflammation, which necessitates adjustments to the therapeutic regimen.

According to recommendations, drug concentration should be measured in the following scenarios:

•After induction therapy: This measurement helps assess the primary response to therapy and identify patients who do not respond to treatment.

Table 1. *Control serum concentrations for adalimumab and infliximab*

Control	Obtained value (μg/mL)	Target value (μg/mL)	Range (μg/mL)
Adalimumab - low control	11.137	12	8-16
Adalimumab - high control	27.786	28	20-40
Infliximab - low control	11.745	12	8-16
Infliximab - high control	26.275	28	20-40

•Before maintenance therapy: This measurement enables dose adjustments before transitioning to long-term therapy, ensuring optimal drug levels.
•At any point when secondary loss of response occurs: Secondary loss of response may arise from the development of antibodies against the drug or due to reduced drug concentrations in serum.
Interpretation of serum drug and antibody levels is as follows:
•High drug level / Low antibody level: The drug is present in adequate concentrations with no significant development of antibodies. Optimization may involve switching to a drug outside the anti-TNF class if necessary.
•Low drug level / High antibody level: This indicates immune resistance. Switching to another anti-TNF drug within the same class is recommended.
•Low drug level / Low antibody level: This suggests an insufficient dose, prompting an increase in dosage or shortening the dosing interval.
The goal of therapy optimization is to achieve maximum therapeutic results without losing the response to treatment. This can be achieved through careful dosing that avoids episodic administration, which could lead to the development of antibodies. Additionally, combining with immunomodulators like azathioprine or methotrexate may reduce the risk of antibody development and enhance therapeutic effectiveness. Eliminating other negative factors, such as smoking, may further improve therapeutic outcomes.
Proactive monitoring through regular assessment of drug levels in serum (trough levels) enables early dose optimization, prevents biological relapses, and minimizes the development of immunogenicity, thus ensuring long-term improved therapeutic outcomes. This approach significantly reduces the risk of therapeutic failure and enhances the quality of life for patients (33-37).
Thanks to its simplicity and reliability, ELISA testing has become the gold standard for monitoring the levels of biological drugs. The aim of this study was to evaluate the reliability of the method for determining serum concentrations of adalimumab and infliximab in terms of precision, accuracy, sensitivity, and specificity at the Laboratory Diagnostics Department of the Institute for Health Care of Children and Youth of Vojvodina in Novi Sad.

Calibration curves for determining serum concentrations of adalimumab and infliximab showed extremely high precision, with a coefficient of determination of 0.998 for adalimumab and 0.999 for infliximab. The concentrations of the tested drugs in control sera were within the expected ranges, with minimal deviations from target values.
The precision of the ELISA test, measured by the coefficient of variation, was 4.3% for adalimumab and 4.1% for infliximab. These values indicate high consistency in repeated measurements, which is particularly important in clinical practice where tests are expected to be reliable and reproducible. Compared to other studies where the coefficient of variation for ELISA tests typically ranges from 5% to 10%, our results demonstrate superior precision in measurements, a critical factor for clinical decision-making.
The accuracy of measurements, expressed by a dispersion of 3.75% for adalimumab and 0.05% for infliximab, is of a high standard. This level of accuracy defines our tests as ideal choices for routine monitoring of serum concentrations of adalimumab and infliximab.
The obtained sensitivity of 100% indicates the excellent ability of the ELISA test to detect adalimumab and infliximab in all serum samples from children undergoing therapy. This result is crucial for avoiding false-negative results and ensuring accurate clinical decisions.
The specificity of the test was also 100%, indicating no false-positive results in children not receiving therapy, which further confirms the reliability of the ELISA method. Specificity is one of the most important aspects of diagnostic tests, and our results demonstrate that the tested ELISA assays can precisely differentiate between patients receiving therapy and those not receiving it, thus minimizing the risk of incorrect treatment (38-40).
Proactive monitoring of serum concentrations of adalimumab and infliximab plays a pivotal role in the personalized treatment approach for patients with inflammatory bowel diseases (IBD). The results of this study demonstrate that the method for determining serum concentrations of these drugs using the investigated ELISA test is highly reliable, as confirmed by the parameters of precision, accuracy, sensitivity, and specificity. This underscores the utility of the ELISA method as an effective tool for optimizing therapy and ensuring the best possible outcomes in IBD management.

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Authors' Contributions

Conceptualization, J.K. and D.D.; Methodology, J.K. and D.D.; Investigation, J.K., D.D. and M.S.; Formal Analysis, J.K., D.D. and M.S.; Writing – original draft, J.K.; Writing – review & editing, D.D. and M.S. All authors have read and approve the published version of the manuscript.

Statement of Ethics

This study was approved by the Ethics Committee of the Institute for Health Protection of Children and Youth of Vojvodina (Approval No. 3987-8, August 15, 2024). All procedures were conducted in accordance with the Declaration of Helsinki.

Statement of Competing Interest

The authors declare no relevant financial or non-financial conflicts of interest.

Statement of Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Statement of Generative AI Use

The authors declare that no generative artificial intelligence tools were used in the writing or preparation of this manuscript.

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