Therapeutic drug monitoring to improve outcome of anti-TNF drugs in pediatric inflammatory bowel disease

Raffaella Franca[a], Debora Curci[b], Marianna Lucafò[c,d], Giuliana Decorti[a,d] and Gabriele Stocco[e]

[a]University of Trieste, Department of Medical, Surgical and Health Sciences, Trieste, Italy; [b]University of Trieste, PhD Course in Reproductive and Developmental Sciences, Trieste, Italy; [c]Centro di Riferimento Oncologico (CRO), Aviano, Italy; [d]Institute for Maternal and Child Health I.R.C.C.S. Burlo Garofolo, Trieste, Italy; [e]University of Trieste, Department of Life Sciences, Trieste, Italy

ABSTRACT

Introduction: Medical treatment of pediatric inflammatory bowel diseases (IBD) has been greatly changed by the introduction of a number of biologic agents that are able to target various players of the immune response. In particular, monoclonal antibodies against the pro-inflammatory cytokine TNF-alpha (TNF) such as infliximab, adalimumab, and golimumab are now in the clinics both in induction and maintenance therapy, and several efforts are currently ongoing to optimize the use of these drugs in children.

Areas covered: This review focuses on therapeutic drug monitoring (TDM) of anti-TNF levels and antidrug antibodies (ADAs), in IBD children. A revision of the analytical assays used for assessing anti-TNF plasma levels is also provided.

Expert opinion: Although there is a consensus across studies that higher anti-TNF trough levels are associated with a better clinical outcome, and that early anti-TNF serum measurements could be predictive of long-term response, it is still not clear what the best predictive time of sampling is and what the ideal target drug plasma concentration to achieve. Indeed, there are a number of published studies, particularly in pediatric cohorts, limited by the population size analyzed and more prospective large studies are needed to examine the value of these predictive markers.

1. Introduction

Due to the multifactorial nature of inflammatory bowel disease (IBD) pathogenesis, therapy is complex and mainly aimed at suppressing the inflammatory response; a number of drugs have been and are currently employed, with variable success, for controlling acute flares, maintaining remission, and preventing or treating complications [1]. Another goal of therapy, mucosal healing, has only recently become extremely important; in this context, in the last decades, medical treatment of IBD has been greatly changed by the introduction of a number of biologic agents, that are able to target various players of the immune response, in particular cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-12 and IL-23 and the $\alpha_4\beta_7$ integrin [2]. Monoclonal antibodies (mAb) directed against TNF-α have been the first biologics to be introduced in the therapy of IBD, and are still widely employed in moderate to severe disease; indeed, these agents are probably one of the most effective drugs available for this disease, also in cases resistant to other therapies [3]. Response to anti-TNF agents is however not uniform, as 10–30% of subjects do not respond to therapy, and are primary nonresponders [4]; moreover, up to 50% of primary responders lose the response at a later time [4]. In addition, these agents have multiple side effects and a high cost, hence optimization of therapy appears to be mandatory.

2. Inflammatory bowel diseases

IBD comprises two main diseases, ulcerative colitis (UC) and Crohn's disease (CD). These disorders have some common characteristics, but differ for others. In UC, mucosal inflammation involves the colonic mucosal layer, is confluent, starts in the rectum and extends proximally [5], while in CD, transmural inflammation can affect any part of the gastrointestinal tract, and the whole thickness of the gut wall; in addition, the inflammation often is not confluent, leaving areas of relatively normal mucosa. Due to the transmural nature of inflammation, complications such as fibrosis, strictures, and fistulas are frequent [6].

The pathogenesis of IBD is not yet clearly understood; however an altered response by the mucosal immune system to intestinal microbial flora, as well as environmental [7,8] and genetic factors, are believed to play a role in the disease [9,10]. Over 200 distinct susceptibility loci for IBD have been identified, and around 70% of genes are common to CD and UC, suggesting a high genetic overlap [11,12]. Unfortunately, no single genetic variant has proved to be clinically useful for the diagnosis or evolution of IBD.

The highest prevalence values of the disease, exceeding 0.3%, have been reported in Europe and North America [13]. Approximately 20% of patients with CD and 12% of patients with UC develop the disease before the age of 20 years [14], with a peak incidence in subjects between 15 and 30 years [15]. The incidence of IBD in the pediatric age has been

Article highlights

- IBD is common in the pediatric age. Clinical features are similar between children and adults but pediatric onset disease is often characterized by a particularly aggressive course and unique complications, such as growth failure and delayed puberty.
- Anti-TNF agents are now routinely used in pediatric IBD, both in induction and in maintenance therapy. However, their use is limited by primary and secondary failures, by the incidence of adverse effects and by elevated costs. TDM, meant as evaluation of anti-TNF trough levels and antidrug antibodies, could help physicians in overcoming these limits and optimizing drug management.
- Trough levels play the best role as predictors of clinical outcome. It is still an open question what are the ideal predictive sample timing and therapeutic range to achieve.
- Anti drug antibodies are generally investigated in case of loss of therapeutic response and are eventually used to guide patients to alternative anti-TNF agents.
- Several reliable analytical assays are now available. They present different advantages and disadvantages, and are chosen according to medical needs..

increasing [14,16–18], in particular in children under 10 years [19]; however, recently, a systematic review of population-based studies reported a stable or decreasing incidence in Europe and North America, with an increase in newly industrialized regions in Africa, Asia and South America [13].

IBD causes significant gastrointestinal symptoms that include diarrhea, abdominal pain, bleeding, anemia, and weight loss. Clinical features are similar between children and adults but some complication, such as growth failure and delayed puberty, are unique to the pediatric patients. In addition, pediatric onset disease is often characterized by a particularly aggressive course, with extensive intestinal involvement and rapid clinical progression [20,21].

3. Biologics for the treatment of IBD

A large number of drugs are employed in the treatment of IBD; however, there is still no truly curative therapy. The majority of currently employed drugs control inflammation and associated symptoms and should prevent the complications of the disease. As stated above, the pathogenesis of IBD remains not clearly understood; however, preclinical and clinical studies [9] have demonstrated that proinflammatory cytokines are important players of the disease. In particular, increased levels of IL-12, IL-23, interferon (IFN)-γ and TNF-α have been demonstrated and are believed to play a crucial role in the genesis of inflammatory processes that characterize this disease. In the light of these findings, biologics directed against various cytokines have been designed and introduced in the therapeutic armamentarium since the nineties for inducing and maintaining remission in refractory forms [22,23]. The mAbs infliximab, adalimumab, golimumab, and the humanized fragment antigen binding certolizumab-pegol are directed against TNF-α, but other targets have been identified. Among these, the α_4 integrin subunit is recognized by the humanized mAb vedolizumab, that inhibits the binding of the $\alpha_4\beta_1$ and $\alpha_4\beta_7$ to adressin on venular endothelial cells and prevents recruitment of lymphocytes to the intestinal mucosa [24]. Another mAb, ustekinumab, binds the P40 subunit in IL-12 and IL-23 [25]. Both drugs are approved for the treatment of IBD; however, they are not included in this review that will focus on anti-TNF agents.

Three full-length anti-TNF IgG1 mAbs, infliximab, adalimumab, and golimumab, and one antibody fragment certolizumab-pegol, are at present available in the clinics. Infliximab is a chimeric mAb with the variable regions of a mouse anti-human TNF mAb fused with the constant region of a human IgG1κ and was the first mAb to be approved for IBD therapy, also in children. Adalimumab, a humanized mAb is also approved for adult and pediatric IBD, while golimumab, a fully human antibody, has been recently included in European Crohn's and Colitis Organization (ECCO) and European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guidelines for use in UC in the pediatric population [26]; certolizumab-pegol is the antigen-binding portion of a humanized mAb, covalently conjugated to polyethylene glycol; conjugation with polyethylene glycol should reduce its immunogenicity and increase its half-life. In addition, for infliximab and adalimumab, biosimilars have been recently licenced for use.

Anti-TNF agents are effective in patients with moderate to severe IBD who do not adequately respond to glucocorticoids and immunosuppressants and are employed for the induction and maintenance of remission. These agents, and in particular infliximab, are often used in combination with an immunomodulator that increases their efficacy and reduces anti-drug antibody formation [23]. They have a rapid onset of effect, usually within 2 weeks after initiation of therapy, and have indeed revolutionized therapy in IBD. In addition to controlling inflammation and symptoms, biologics are also able to induce a complete deep remission, i.e. a combination of clinical remission, normalization of laboratory parameters and mucosal healing [27–30]

3.1. Anti-TNF agents mechanism of action

TNF-α is a proinflammatory cytokine produced in particular by activated macrophages, T cells, and monocytes, but also by other different cells following activation of toll-like receptor or cytokine receptor. TNF-α is expressed as a 26 kDa transmembrane protein (tmTNF); the cytoplasmic tail of the protein is cleaved by TNF-α converting enzyme (TACE) and soluble TNF (sTNF) is then released. sTNF and tmTNF, arranged in stable homotrimers, are biologically active and interact with TNF receptor (TNFR)1 and TNFR2 [31,32].

Anti-TNF agents bind with high affinity the sTNF and tmTNF forms and inhibit their binding with the receptors. They induce the apoptosis of lamina propria T cells, a direct antibody-dependent cytotoxicity induced by all antibody-derived anti-TNF agents. The full-length mAbs act also by binding tmTNF on activated T-cells and the Fc portion is recognized by Fc receptors expressed by monocytes, triggering their differentiation to wound-healing macrophages [33]. This mechanism is Fc-dependent and therefore is not involved in the effect of the antibody fragment certolizumab [34]. In inflamed tissues, these cellular effects can also restore the

balance between matrix metalloproteinases and tissue inhibitors of metalloproteinases [35].

3.2. Limits of anti-TNF therapies

About 10–30% of patients do not respond to initial therapy (primary failure) and a variable proportion of patients (up to 50%) loses the response over time (secondary failure) [4,27,36,37]. Such variability in response is related to several factors, including drug pharmacokinetic effects, disease-related features as well as the heterogeneous time-points used to define the primary resistance across studies (ranging from week 2 to week 14) and the heterogeneous definition given to secondary resistance, judged either by drug discontinuation alone or by both discontinuation and need for therapy intensification [38].

Furthermore, IBD patients could experience a wide range of adverse effects, which may also lead to therapy discontinuation: anti-TNF therapy is mainly associated with increased risk of infections (~36% of patients within 1 year), with infusion reactions in case of infliximab (3–17%) and skin reactions at the injection site in case of adalimumab (2–5%) [39]. Typically, infections are not serious and are easily treated: a systematic review and network meta-analysis of the existing IBD literature estimated odds of serious infection in adult IBD patients for all treatment strategies compared to placebo (i.e.: including anti-TNF), finding no statistically significant results [40]. Infusion reactions with infliximab are common and can be acute, occurring within 24 h, or delayed when developing between 1 and 14 days after the start of treatment. Acute reactions are the most common [41] and can be immunoglobulin E (IgE)-mediated anaphylactic reactions, with hypotension, bronchospasm, wheezing, and/or urticaria [42], or, more often, characterized by nonspecific symptoms, not mediated by IgE, and classified as anaphylactoid [43]. Delayed infusion reactions resemble serum sickness and are characterized by skin rash, diffuse joint pains, myalgias, fatigue, fever and may represent mild immune complex-mediated reactions. Overall, infusion reactions have a similar incidence between adults and children (~5–10%), whereas it is not clear if the rate of infusion reactions per individual varies across different age cohorts [44]. With subcutaneously administered agents, skin reactions characterized by itching, pain, redness, irritation, bruising, or swelling at the site of medication injection are common but usually minor problems [45], they occur during the first month of treatment and last for three to five days, with an incidence of 1–9% of patients depending on the drug [46–48].

The cost of biologics is another serious point. Humira (adalimumab), with its standout US$18 billions of sales in 2017, is a prime example [49]. Access to biologicals varies significantly between countries accordingly to budgetary constraints [50]. Considering the differences in anti-TNF therapy practice among IBD children internationally is important for the correct interpretation of treatment outcomes and safety data [51].

3.3. Anti drug antibodies

Several evidences suggest that the loss of treatment efficacy and drug adverse reactions in patients treated with anti-TNF may be in part the result of the formation of anti drug antibodies (ADAs) [52,53]. Despite the different level of humanization of infliximab, adalimumab, and golimumab, ADAs may be produced during treatment with all these agents although the highest percentage of patients developing ADAs is among those who received infliximab [54]. Indeed, the chimeric mAb infliximab is more immunogenic respect to the other anti-TNF antibodies, likely because of the variable regions derived from murine sequences [3]. Most of ADAs generated against anti-TNF antibodies neutralize their activity by binding to or near the active sites, accelerating drug clearance [55]; non-neutralizing ADAs can also contribute to a lack or loss of response by affecting the bioavailability of the therapeutic proteins [56].

It is well established that stable concentration of circulating ADAs is associated with lower infliximab trough levels as well as lower duration of response [57,58]. Indeed, the strategies to optimize anti-TNF treatment in IBD patients with loss of response look also to the amount and persistence of ADAs during the induction and the maintenance phase of the treatment and their impact on long-term response. Based on the data collected, a therapeutic algorithm in cases of loss of response to anti-TNF agents was proposed: in particular, patients with active disease and low ADA levels during maintenance should receive an increased drug dose, whereas, in case of high ADA levels, the therapy might need to be switched to an alternative anti-TNF agent [59].

The study published by Liefferinckx and collaborators confirmed that long-term responders presented very low ADA levels, suggesting that ADA presence is associated with the need for optimization [60]. Interestingly, among patients who experienced loss of response, naïve patients presented higher trough levels than previously treated patients; however, the occurrence of ADAs was similar in both groups, which suggests that lower infliximab trough levels at induction or just before optimization during maintenance may not be related only to immunogenicity to infliximab.

An important aspect not to be underestimated is related to the concept of transient ADAs. Vande Casteele and colleagues reported that in 15/53 (28%) patients with CD ADAs disappeared over-time and only 2/15 (13%) patients with transient ADAs needed to discontinue infliximab treatment, indicating that their production may be transient and does not always lead to an unsatisfactory clinical outcome [61].

Combination therapy with infliximab or adalimumab and immunosuppressants such as methotrexate or thiopurines may delay or prevent the formation of ADAs [62,63]. However, according to the consensus guidelines of ECCO and ESPGHAN on the medical management of pediatric CD, there is insufficient evidence to define the risk/benefit ratio for mono- or combination therapy in CD children, while it seems that combination therapy for the first 6 months may be associated with a lower rate of antibodies development and loss of response, this benefit should be weighed against the eventually increased lymphoma risk with thiopurines on an individual basis. The use of concomitant low dose MTX maybe safer but is less evidence-based [64].

3.4. Anti-TNF agent pharmacokinetics in children

mAbs as anti-TNF agents have a high molecular weight, limited membrane permeability and limited stability toward gastrointestinal protease activity so, they are not suitable for oral administration.

In children, according to the consensus guidelines of ECCO and ESPGHAN, on the medical management of pediatric CD, infliximab (5 mg/kg) is administered intravenously at 0, 2, 6 weeks during the induction schedule and then every 8 weeks in maintenance. Higher doses (up to 10 mg/kg) and/or shorter intervals (every 4 weeks) may be considered in patients losing response to the drug or with too low drug levels; lower infliximab doses may also be considered when trough levels are above 8–10 μg/ml and remission is achieved [64].

Adalimumab is supplied as a sterile, single-use, preservative-free solution in a prefilled pen for subcutaneous administration. In CD, the drug is given at a dose of 2.4 mg/kg (maximum 160 mg) at baseline and 1.2 mg/kg (maximum 80 mg) at week 2 during the induction therapy, followed by 0.6 mg/kg (maximum of 40 mg) every other week. For patients under 40 kg, dosing regimens may be reduced to 80–40–20 mg; in patients losing response or with low trough levels, weekly injections should be considered [64]. Recent ECCO/ESPGHAN guidelines for UC affected children confirmed the therapeutic schemes proposed for infliximab and adalimumab also in pediatric patients and provided additional recommendation for the use of the other anti-TNF agent available, golimumab [26].

Golimumab is administered subcutaneously as adalimumab: recommended doses for induction are 200 mg at week 0 followed by 100 mg at week 2 for those weighing ≥45 kg. Children with lower weight should be dosed based on body surface area (115 and 60 mg/m^2 at weeks 0 and 2). Maintenance doses every four weeks are 60 mg/m^2 if weight <45 kg and 100 mg if weight ≥45 kg [26]. For the fourth anti-TNF agent, certolizumab-pegol, approved for IBD treatment in adults, data in the pediatric population are very limited, and thus this agent will not be considered in this review.

Intravenous administration of infliximab guarantees 100% drug bioavailability and an immediate distribution. The maximum concentration is achieved within 1 h from the beginning of the infusion; rapid infusion (over 1 h) seems as safe and effective as traditional slower infusions of 120 min [65,66], if the induction doses of infliximab are well tolerated and dose is stable [18,67]. The plasma concentration profile over time is characterized by peak concentrations and trough levels; serum trough levels are higher during the induction phase and tend to stabilize at later time points (after 14 weeks) [67].

Adalimumab subcutaneous administration is advantageous because of patient self-injection and because of more uniform concentration profiles over time at steady state: due to the lymphatic drainage from the injection site, the drug reaches the systemic circulation and plasma concentration increases slowly. In healthy subjects, maximal concentration is achieved in 131 ± 56 h after a single 40 mg dose [68]. Disadvantage of this injection route is the higher interindividual pharmacokinetic variability between patients; a recent report on 28 adult IBD patients has investigated the variability of adalimumab

subcutaneous administration, finding that only 69% of patients showed detectable serum levels of the anti-TNF agent 2 h after the first dose (median (interquartile range, IQR) concentration: 0.6 (0.2–1.2) μg/mL). A fourfold difference in the range of adalimumab concentrations was seen seven days after the first dose (median (IQR): 13.5 (10.5–19.2) μg/mL) [69].

Golimumab subcutaneous monthly administration represents an advantage in patient compliance: maximal concentration is also achieved slowly (3–8 days after injection) and is affected by interindividual variability due to differences in the pharmacokinetics of the drug [70].

Being anti-TNF agents large hydrophilic molecules, their distribution is restricted to the bloodstream and extracellular spaces with very limited penetration into cells and through the blood-brain barrier. The extent of distribution relies upon the rates of extravasation in tissues and distribution in the interstitial space, antibody binding to the tissue components such as cell surfaces, and clearance from tissues, including intracellular uptake and degradation [71]. The apparent volume of distribution (Vd) for anti-TNF agents at steady state is therefore low (4.5–6 L) corresponding to the intravascular space [72]. Fasanmade and collaborators compared pharmacokinetic data of infliximab in 112 pediatric and 580 adult patients with moderately to severely active CD, using two-compartment models with zero-order infusion and first-order elimination. Comparable values for infliximab clearance (CL) and distribution volume in the central (V$_1$) and peripheral compartment (V$_2$) were found. Indeed, CL (SE) was 5.43 (0.15) mL/kg/day, V$_1$ was 54.2 (1.15) mL/kg and V$_2$ 29.2 (2.03) mL/kg in a typical child (older than 6 years, median weight: 42 kg). Corresponding values in a typical adult (median weigh: 68 kg) were 5.39 (0.13) mL/kg/day, 52.7 (0.49) mL/kg and 19.0 (1.53) mL/kg [73]. A similar comparative study between pediatric and adult populations was performed by Xu and collaborators on golimumab treated UC patients. Pharmacokinetic parameters were estimated in 35 children and 966 adults using a one-compartment model with first-order absorption and elimination with similar results in the two groups (apparent CL (5th–95th percentile): 0.692 (0.416–1.57) *versus* 1.05 (0.569–1.91) L/day; apparent Vd: 12.0 (8.29–19.3) *versus* 14.0 (9.14–21.0)L) [74].

Infliximab elimination half-life (t½) is 8–10 days, adalimumab t½ is 10–20 days and golimumab t½ is 5–15 days [67,74]. Renal excretion of anti-TNF mAbs is almost not existent since these agents' molecular weight is too high to be filtered by the kidneys and eliminated in the urine. IgG elimination, both of endogenous ones and of exogenous therapeutic mAbs, occurs mostly through catabolism by lysosomal degradation after intracellular uptake by either pinocytosis or by a receptor-mediated endocytosis process through the Fc-gamma-receptors (FcγRs) expressed on many human immune cells [71,75]. To prevent early clearance, endogenous IgGs bind to the neonatal Fc- or Brambell-receptor (FcRn), which is also useful for salvage and membrane recycling of exogenous therapeutic mAbs. Indeed, both FcγRs and FcRn receptors mediate cellular uptake of antibodies and target IgGs inside endosomes where the acidic environment degrades the IgG–FcγR complexes while reinforcing the binding between the IgG and the FcRn. IgG–FcRn complexes are then recycled

back to the cell surface. As pH returns to neutral values, the binding strength weakens, and IgGs are released; this mechanism is responsible for the long $t\frac{1}{2}$ of mAbs [76].

Increased levels of C reactive protein (CRP) and decreased concentrations of serum albumin have shown to be related to an increased anti-TNF agents clearance, as well as body mass index [73,77]. A colonic loss of infliximab through the inflamed mucosal barrier has been also demonstrated, and high infliximab concentrations in the feces of patients with UC were observed during the first days of therapy and associated to primary nonresponse [78].

4. Predictors of anti-TNF response

A number of factors have been investigated so far to evaluate their predictive effects on anti-TNF efficacy in IBD. As reviewed elsewhere, candidate predictors were either patient-related features (e.g. age, gender, and body mass index) or disease-related features (e.g. disease duration, severity, extent and phenotype, serological markers such as CRP and pretreatment albumin levels and immunological markers) or treatment-related features (e.g. treatment adherence, mucosal healing, concomitant immunosuppressive drugs, drug trough levels, ADAs) [79,80]. Despite all efforts, predicting response to biological treatment is still not an easy task, likely because controversial and/or not sufficiently powered results are

reported in the literature. Indeed, there are a number of published studies, particularly in the pediatric population, limited by the population size analyzed and more prospective large studies are needed to examine the value of these predictive markers [81].

4.1. Therapeutic drug monitoring of anti-TNF agents

Therapeutic drug monitoring (TDM) is a clinical laboratory practice that helps clinicians in drug prescribing procedures. Traditionally, TDM involves measuring drug concentrations in biological fluids and interpreting these concentrations for adjusting further drug dosages in order to maintain plasma or blood drug concentrations within an optimal targeted therapeutic window. In this context, TDM measures mainly the contribution of drug pharmacokinetic variability and is useful only if there is a good relationship between the plasma drug concentration and the therapeutic and/or toxic effect, if there is a low therapeutic index and if an appropriate timing of sampling is established [82]. If these criteria are not met, other strategies to monitor therapeutic response should be attempted.

In IBD, TDM has been used to improve therapy with thiopurines. Indeed, the therapeutic window of active thionucleotides metabolites (TGN) is between 235–450 pmol/8×10^8 erythrocytes and this range correlates with the clinical response. Moreover, methyl mercaptopurine derivatives above 5700

Table 1. Clinical trials on biomarkers and monitoring of anti-TNF in IBD, including pediatric patients (https://clinicaltrials.gov) A: adults; ADM: adalimumab; CD: Crohn's Disease; IFX: infliximab; IBD: Inflammatory Bowel Disease; MINOTOR: Genetic an Functional Studies of Patient With Inflammatory Bowel Disease; MRI: magnetic resonance imaging; P: paediatrics (<18yrs), PAILOT: Pediatric Crohn's Disease Adalimumab Level-based Optimization Treatment Trial; PANTS: Personalising Anti-TNF Therapy in Crohns Disease; PEDICAD: The Role of PET/MRI in the Diagnosis and Treatment of Children and Adolescents With Inflammatory Bowel Diseases; PET: Positron Emission Tomography; TOPIT: Trough Level Optimized Pediatric Inflammatory Bowel Disease Therapy; YA: young adults (18–25 yrs); * on March 2019.

CT Identifiers (CT status*)	Title or acronyms	Study type, Observational model, time perspective	Study AIM description	Biologicals
NCT01971970 (completed)	Biomarkers of anti-TNF treatment in IBD	Observational Cohort (P) Prospective	To identify pretreatment serum level of endogenous anti-TNF and RNA expression profiles in relation to primary clinical response or nonresponse	IFX ADM
NCT01605188 (completed)	Predictors of nonresponse and loss of response in IBD patients treated with anti-TNF	Observational Case-crossover (P, A) Prospective	To identify of demographic, clinical, molecular, and immunological baseline predictors for anti-TNF nonresponse and anti-TNF loss of response	IFX ADM
NCT01605175 (completed)	Predictors of mucosal healing IBD patients treated with anti-TNF	Observational Case-crossover (P, A) Prospective	To identify of demographic, clinical, molecular, and immunological baseline predictors for anti-TNF mucosal healing	IFX ADM
NCT03751722 (completed)	MINOTOR	Observational Cohort (P, A) Prospective	To identify genetic and functional macrophage activation marker of anti-TNF treatment	Anti-TNF
NCT03088449 (Not yet recruiting)	PANTS	Observational Cohort (P, A) Prospective	To identify clinical, serological, and genetic factors that determine primary nonresponse, loss of response and adverse drug reactions to anti-TNF drugs in patients with active luminal CD	IFX ADM
NCT02488005 (completed)	The use of small bowel ultrasound to predict response to Remicade induction	Interventional (P, YA)	To evaluate the changes in bowel wall thickness in response to infliximab treatment	IFX
NCT02256462 (completed)	PAILOT	Interventional (P)	To evaluate the effect of routine ADA drug monitoring-based treatment on trough levels and antibodies to adalimumab (ATAs) during therapy in comparison to clinically-based monitoring on disease activity	ADM
NCT02624037 (recruiting)	Precision IFX: using a dashboard to individualize infliximab dosage	Inteventional (P, A)	To evaluate the selection of IFX dose and dosing frequency in patients according to a pharmacokinetic dashboard software-guided dosing system aimed at maintaining a target trough IFX concentration	IFX
NCT03640637 (Not yet recruiting)	PEDICAD	Observational Cohort (P,YA) Prospective	To evaluate whether PET/MRI scan is an accurate method to diagnose relapses and to monitor the effect of anti-TNF biological treatment	IFX ADM
NCT02522169 (unknown)	TOPIT	Inteventional (P)	To evaluate the IFX-trough-level guided therapy optimization	IFX

Table 2. Predictive infliximab trough levels on clinical outcome in pediatric IBD patients. [1] defined by scoring <10 points on Pediatric Ulcerative Colitis Activity Index (PUCAI) or Pediatric Crohn's Disease Activity Index (PCDAI); [2] defined by scoring <5 points on Harvey Bradshaw Index (HBI); [3] defined by fecal calprotectin cut off of 1000 μg/g; [4] defined as C-reactive protein (CRP) ≤5 mg/L in combination with an erythrocyte sedimentation rate (ESR) ≤20 mm/h; [5] defined as absence of ulcerations; Pts:patients.

INFLIXIMAB MEASUREMENTS		PREDICTION		Pts	
SAMPLING TIME	TROUGH LEVELS median (range)	TIME	ASSOCIATION	N	REF
Week 2	>9.2 μg/mL	Week 14	Clinical remission[1,2]	63	[90]
Week 6	9.8 (8.4–12.6) μg/mL versus 7.1 (4.7–9.8) μg/mL	Week 6	↑ trough levels, clinical remission[1]	49	[87]
Week 6	>2.2 μg/mL	> Week 52	Clinical remission[1,2]	63	[90]
Week 14	20 (0–48) μg/mL versus 4.0 (0.47–25) μg/mL	Week 14	↑ trough levels, ↓inflammation[3]	37	[86].
Week 14	5.0 (3.6–9.1) μg/mL versus 1.0 (0.18–2.7) μg/mL	Week 14	↑ trough levels, clinical remission[1]	49	[87]
Week 14	≥3.11 μg/mL	Week 54	Clinical remission[1]	49	[87]
Week 14	≥3 μg/mL		Clinical remission (64%)[1]	58	[88]
Week 14	4.6 (2.7–11.8) μg/mL versus 1.5 (0.9–3.0) μg/mL	Week 52	↑ trough levels, clinical remission[1)]	35	[99]
Week 14	4.6 (2.5–10.3) μg/mL versus 2.6 (0.3–3.2) μg/mL	Week 52	↑ trough levels, biological remission[4]	35	[99]
	6.0 (3.2–12.0) μg/mL versus 2.6(1.1–3.2) μg/mL	Week 52	↑ trough levels, clinical and biological remission[1,4]	35	[99]
	≥5 μg/mL		Clinical remission (76%)[1]	58	[88]
	≥7 μg/mL		Clinical remission (100%)[1]	58	[88]
Maintenance	5.4 (3.8–8.0) μg/mL versus 4.2 (2.6–6.7) μg/mL	Maintenance	↑ trough levels, clinical remission[1]	52	[99]
Maintenance	5.2 (3.7–7) μg/mL versus 4.2 (2.6–6.5) μg/mL	Maintenance	↑ trough levels, biological remission[4]	52	[99]
Maintenance	7 (4.0–8.2) μg/mL versus 4.4 (2.7–6.8) μg/mL	Maintenance	↑ trough levels, clinical and biological remission[1,4]	52	[99]
Maintenance	6.5 (4.2–9.5) μg/mL versus 3.2 (2.3–5.6) μg/mL	Maintenance	↑ trough levels, endoscopical remission[5]	52	[99]
Maintenance	4.0 (2.0–6.4) μg/mL versus 2.25 (0.5–4.7) μg/mL	Maintenance	↑ trough levels, clinical remission[1,2]	63	[90]
Maintenance > Week 52	≥5 μg/mL	> Week 52	Mucosal healing in 80–90% of subjects[5]	105	[82]

pmol/8 × 10⁸ erythrocytes identify patients at higher risk of hepatoxicity [83]. The utility of TDM for biological treatments is now under investigation [84]. Studies have been conducted mainly in adults with results that are not directly applicable to pediatric patients: indeed, due to their continuously changing anatomical and physiological features during the different developmental stages, children display a large inter-individual variability in pharmacokinetic profiles compared to adult subjects [85]. Several efforts are currently ongoing to optimize the anti-TNF therapy in pediatric IBD patients. On March 2019, a web research conducted on the clinical trials database (https://clinicaltrials.gov) using 'IBD' and 'anti-TNF' and 'child' as keywords returned 43 results: among these, five clinical trials are aimed at identifying predictive biomarkers of treatment response and five are focused on strategies to monitor the effect of biologicals treatment (Table 1).

Numerous studies have demonstrated that anti-TNF drug trough levels and ADA levels are useful for monitoring and assessing response to treatment (Table 2). Trough levels play the best role as predictors of clinical outcome, whereas ADAs are generally investigated in case of loss of therapeutic response and are eventually used to guide patients to alternative anti-TNF agents.

Adequate infliximab exposure during induction therapy was associated with better clinical and/or biological remission. When infliximab was measured in 37 IBD children, the median serum trough levels during induction were 17.6 μg/ml (range 0–48 μg/ml, 48 blood samples analysed at weeks 2 and 6), with significantly lower infliximab levels observed in patients with higher inflammation (median (range) 4.0 (0.47–25) μg/ml, in children with fecal calprotectin >1000 μg/g versus 20 (0–48) μg/ml in those with fecal calprotectin <1000 μg/g, p < 0.005) [86]. Naviglio and coworkers analysed 49 IBD pediatric patients and found that infliximab concentrations were significantly higher in those in clinical remission compared to those who were not, at both week 6 (median (IQR) 9.8 (8.4–12.6) μg/mL versus 7.1

(4.7–9.8) μg/mL, p = 0.044) and week 14 (5.0 (3.6–9.1) μg/mL versus 1.0 (0.18–2.7) μg/mL, p = 0.00039). These authors also reported that an infliximab concentration below the cut-off value of 3.11 μg/mL at the end of induction (week 14) identified therapeutic loss of response during maintenance (week 54) [87]. A previous study also established a cut-off level >3 μg/mL at week 14 for predicting 64% of persistent remission at week 54, and cut-off levels >4, and >7 μg/mL for predicting 76% and 100% of persistent remission, respectively [88]. Van Hoeve and collaborators reported that median infliximab trough levels measured just before the first maintenance infusion were significantly higher in children achieving clinical (4.6 (2.7–11.8) μg/mL versus 1.5 (0.9–3.0) μg/mL), biological (4.6 (2.5–10.3) μg/mL versus 2.6 (0.3–3.2) μg/mL) and combined clinical/biological remission (6.0 (3.2–12.0) μg/mL versus 2.6 (1.1–3.2) μg/mL, all p < 0.002) at week 52 compared to children not meeting these criteria (35 CD and UC patients analyzed) [89]. In a population of 63 pediatric patients, an infliximab trough level >2.2 μg/mL at week 6 predicted infliximab effectiveness beyond 1 year of treatment (p < 0.0001). Moreover, clinical remission at week 14 was predictable by infliximab trough levels >9.2 μg/mL at week 2 (p = 0.02) [90]. In the absence of concomitant immunosuppression, proactive TDM at week 10 may maintain higher infliximab concentrations during maintenance as established in a retrospective cohort of 83 IBD children and adolescents [91]. Very recently, a large multicentric, prospective observational study conducted in UK on CD patients with active luminal disease (clinical trial NCT03088449, PANTS) enrolled 955 patients naïve to infliximab and 655 naïve to adalimumab, including 219 pediatric patients. Primary non-response at week 14 occurred in 24% of patients and was in line with the previously published 10–40%, whereas non-remission at week 54 occurred in 63% of patients, with a higher rate than previously reported (i.e. 30–40%) [92]. In multivariate analysis, the only factor independently associated with primary non-response to both drugs was the low drug concentration at week 14 (p < 0.0001). The optimal drug concentrations at week

14, associated with remission at both week 14 and week 54 were 7 μg/ml for infliximab and 12 μg/ml for adalimumab. This study suggested that a higher target drug concentration might be required during induction than those reported previously: such difference could be ascribable to the more stringent definition of remission used. Obesity, smoking, low albumin concentrations, higher baseline markers of disease activity, and development of immunogenicity were associated with low drug concentrations, which mediated non-remission [92]. The contribution of these factors on anti-TNF concentration and clearance had been repeatedly found in adults [93–95].

The concentration-response relationship was observed also for golimumab in adults, with patients in the highest serum concentration quartiles (>1.72 μg/mL) having greater rates of clinical response and clinical remission when compared with those in the lower quartile values at week 6 [70]. Partial clinical responders (at week 14) showed significantly higher serum golimumab concentration than non-responders, both at week 2 (median (IQR): 10.0 (7.8–10.5) μg/mL versus 7.4 (4.8–8.3) μg/ml, p = 0.032) and at week 6 (5.1 (4.0–7.9) μg/ml versus 2.1 (1.8–4.2) μg/ml, p = 0.037) [96].

During maintenance therapy, median infliximab trough levels in children ranged from 3.5–4.5 μg/mL [97] and differed between patients who achieved clinical remission and those who did not. Also in maintenance, lower trough levels had been consistently related to a poor clinical response in IBD: Choi and coworkers analysed infliximab trough levels of 39 IBD patients in maintenance within 1 year, finding a median (IQR) of 3.99 μg/mL (0.30–21.96) in good responders versus 0.88 μg/mL (0.00–6.80) in patients with poor response to treatment (p = 0.002) [98]. In 49 pediatric IBD patients, serum infliximab concentrations at weeks 14, 22 and 52 were significantly higher in those who responded to anti-TNF at week 54 (week 14: 6.1 (3.8–9.6) μg/mL versus 1.4 (0.35–2.8) μg/mL (p = 0.00038); week 22: 5.2 (2.9–9.0) μg/mL versus 1.0 (0.34–1.9) μg/mL (p = 0.0022); week 52: 3.8 (2.7–6.0) μg/mL versus 1.2 (0.67–1.9) μg/mL (p = 0.025)) [87]. Ungar and collaborators reported a median infliximab trough level during maintenance of 4.0 μg/mL (IQR 2.0–6.4) in responders and of 2.25 (0.5–4.7) μg/ml in nonresponders, when of 63 pediatric patients (66.6% CD) were analysed [90]. Similarly, Van Hoeve and coworkers reported that children in clinical remission (5.4 (3.8–8.0) μg/mL versus 4.2 (2.6–6.7) μg/mL), biological remission (5.2 (3.7–7.7) μg/mL versus 4.2 (2.6–6.5) μg/mL), combined clinical and biological remission (5.7 (4.0–8.2) μg/mL versus 4.4 (2.7–6.8) μg/mL) and endoscopic remission (6.5 (4.2–9.5) μg/mL versus 3.2 (2.3–5.6) μg/mL) had significantly higher levels compared to others (all p ≤ 0.001), as measured in a cohort of 52 paediatric patients (33 CD and 19 UC) [99].

Evidence also support a relation between TNF-α inhibitor drug levels and mucosal healing. Kang and coworkers evaluated 105 pediatric patients with luminal CD who had been receiving anti-TNF therapy for at least 1 year, after which they underwent ileocolonoscopy. After a multivariate analysis, they established the need of a concentration of ≥5 μg/mL for achieving mucosal healing in 80–90% of subjects [100]. In adults, cut-offs for achieving mucosal healing ranging from 6–10 μg/mL to ≥9.7 μg/mL were reported (80% specificity) [101,102]. The authors hypothesized that such discrepancy between children and adults is not due to anti-

TNF pharmacokinetic profiles, but is more ascribable to differences in other factors, such as disease behavior, previous anti-TNF use, and disease duration. Moreover, the time from diagnosis to infliximab treatment was also an independent factor significantly associated with mucosal healing, with patients initiating treatment ≥1 year after diagnosis less likely to experience mucosal healing than those starting treatment within 1 year [100].

4.2. TDM and anti-TNF dose adjustment

Patients who had poor responses and subtherapeutic infliximab trough levels can improve response after dose intensification. In approximately 30% of IBD patients, loss of response occurs because of pharmacokinetic alterations. Such pharmacokinetic-related causes of failure may be overcome with dose elevation and/or interval shortening to target drug levels into the therapeutic window. Most studies had been conducted in adults. Indeed, a significantly higher proportion of CD adult patients (88% versus 65%) with trough levels <3 μg/mL reached remission and a decrease in the median concentration of CRP after dose escalation [103]. A recent observational study investigated retrospectively the outcome in 48 adult IBD patients who underwent a clinical non-immunogenic loss of response (with infliximab plasma concentration trough level >3 μg/ml and without detectable ADAs) and therapy intensification (either dose elevation to 10 mg/kg every 8 weeks or interval shortening to 5 mg/kg every 4/6 weeks). Twenty-three (49%) and 29 (60%) patients reached clinical remission by 6 and 12 months postinfliximab escalation. At both time points, clinical remission was significantly more prevalent for patients with infliximab levels between 3 and 4.54 μg/ml at the time of loss or response compared to others with higher values (p < 0.02). Patients with baseline trough levels above 9 μg/ml were very unlikely to reach clinical remission upon doubling the dose of infliximab [104]; therefore, dose escalation is particularly useful in patients with lower drug concentrations.

Therapy intensification according to physician choice has been performed also in children. Among a small group of 39 pediatric patients, 23 children who had lower median infliximab trough levels (median (IQR) 0.88 (0.00–6.80) μg/mL versus 3.99 (0.30–21.96) μg/mL, p = 0.002) and poor response to treatment, underwent therapy intensification. Almost 81% of them regained the response and increased serum levels [median (IQR): 7.76 (1.96–20.00) μg/mL]. All the remaining patients had no detectable infliximab serum levels and presented ADAs, so that they were switched to another anti-TNF agent, such as adalimumab [98].

The clinical trials TOPIT (NCT02522169) and PAILOT (NCT02256462) are interventional studies on IBD pediatric patients focused on infliximab and adalimumab trough levels, respectively. In TOPIT, infliximab trough levels will be assessed in all children enrolled. In the conventional treatment arm, therapy intensification at physician discretion will be allowed in the presence of clinical signs of disease exacerbation. In the intervention group, the infliximab therapeutic window will be maintained by pharmacokinetic guided decisions based first on trough levels and then on ADA testing. PAILOT proposed a similar study design focused on adalimumab. In the

conventional treatment arm, trough adalimumab levels are measured following physician decision when patients loss of response occurs and therapy is adjusted according to results. In the intervention group, adalimumab monitoring is performed routinely every two months: injection interval is decreased to every week when trough levels are below 5 μg/ml and if levels are undetectable (below 0.3 μg/ml), ADAs are tested and patients will discontinue the study when ADAs are persistently above 8 μg/ml. So far, no results are available for both clinical trials. These studies will help clarifying the role of reactive *versus* proactive TDM in children. Indeed, it is not clear whether measuring TDM in patients with symptomatic remission improves long-term outcomes and the thresholds above which therapy intensification has no sense [105]. Results of similar interventional study on adult IBD patients had already been published [103]. In the TAXIT clinical trial, patients were first dose optimized to have an infliximab trough concentration within the interval of 3–7 μg/mL (optimization phase) and then randomized to receive either a clinically based or a concentration- and ADA-based dosing of infliximab. Clinical and biochemical remission at 1 year after optimization was considered. The major benefit was related to the initial dose optimization, resulting in a higher proportion of CD patients in remission, in safe reduction of the dose and in substantial drug cost savings. No additional benefit was observed for prospective concentration-based over clinically based dosing of infliximab, although a lower proportion of flares was observed in the former arm [103].

Therapy personalization could be achieved also by drug reduction when elevated trough levels (>7 μg/mL) are detected. Such approach could be useful for saving treatment costs (up to 28% reduction) and for reducing the incidence of side effects [103].

4.3. Analytical assays in anti-TNF TDM

Different assays have been set up to measure infliximab, adalimumab, golimumab and ADA concentrations. Among these, there are: the drug-sensitive solid-phase immunoassays, the radioimmunoassay (RIA), the homogeneous mobility shift assays (HMSA) and the cell reporter gene assays (RGA). Recently the fiber-optic surface plasmon resonance (FO-SPR) and lateral flow (LF) assays have been introduced, allowing a rapid readout of the results [106] (Figure 1).

The enzyme-linked immunosorbent assay (ELISA) is probably the most common solid-phase immunoassay used in clinical practice. ELISA can be performed according to various protocols: the plate could be either coated with antigen (direct and indirect ELISA) or with antibody (sandwich ELISA) and the detection method can be either direct (a labeled primary antibody that reacts with the antigen) or indirect (a labeled secondary antibody, usually a horseradish peroxidase (HRP)-conjugated antibody). The final color due to enzyme substrate addition is directly proportional to the analyte concentration and is measured spectrophotometrically. A special case of sandwich ELISA is the bridging ELISA. In this assay, the antigen is used both for capturing the antibody and for detection. This test is currently the most used method as a screening test to determine the presence of ADAs [107,108]. Recently, a new ELISA test (Immundiagnostik, Bensheim, Germany) has been introduced in the market that allows a reliable determination of ADAs even in the presence of anti-TNF agents; therefore, it is ideal for therapy monitoring when a measurable drug concentration is expected. So, this test is an opportunity for the clinicians to monitor and optimize the therapy early on. The advantages of ELISA test are: the possibility to automate tests, the lower costs and the possibility of high throughput analysis. However, excluding Immunodiagnostik assay, ELISA test can be used to measure anti-TNF agents or ADAs, but not both simultaneously. Another disadvantage is that the detection of ADAs is affected by several washing steps. In particular, ELISA tests may only be able to detect high-affinity antibodies because those with low affinity (IgG4) may be neutralized after the several washing steps. Moreover, since the ELISA tests have a response time of about 4–8 h, their use is limited in proactive strategies [108].

RIA is similar to ELISA in terms of sensitivity and specificity, and cannot detect ADAs in presence of the drugs; unlike ELISA, RIA is able to detect low affinities antibodies, such as IgG4 which represent a significant proportion of ADAs [107]. However, the use of a radioactive agent, iodine 125 (^{125}I), the higher costs, and the prolonged incubation time needed (overnight incubation) limit its practical use.

To overcome some of these limitations, newer techniques have been designed: HMSA and RGA. HMSA overcomes many limitations of the solid-phase ELISA method because the antibody and antigen-binding reactions take place in a homogeneous liquid-phase condition, enhancing detection of all immunoglobulin isotypes and all subclasses of IgG. The test permits to dissociate drug-ADA complexes, and hence can quantify them independently, thanks to high-pressure liquid chromatography and size exclusion chromatography [109]. RGA is a relatively inexpensive test that permits to obtain quantitative measurements instantaneously. Lallemand et al. have set up an assay able to quantify TNF agent and ADAs using human erythroleukemic K562 cells transfected with a *Nuclear Factor Kappa B (NFK*B) regulated luciferase reporter gene construct. TNF-α will activate *NFκB* signaling pathway, leading to luciferase production. If anti-TNF neutralizes TNF-α, the luciferase activity decreases whereas if ADAs are present, this will block anti-TNF activity, resulting in higher levels of TNF-α and higher luciferase activity [110]. Based on the same scheme, Gils and colleagues have used *NFκB* induced-IL-6 fibrosarcoma cells for anti-TNF antagonism [111]. The advantages of these cell-based assays are that both drugs and ADA levels are evaluated with a high sensitivity within 2 h [111]. Steenholdt and colleagues reported that infliximab detection was comparable between the four different assays (ELISA, RIA, HMSA and RGA). In particular, despite variable analytical properties, the assays have shown that IFX detection correlated significantly (p < 0.0001) [112].

An important innovation for TDM has been the introduction of FO-SPR and LF assays that allow a rapid readout of the results. FO-SPR permits to study the interaction between two molecules in real time, one immobilized on a chip and the other flowing through a microfluidic system over the chip surface. The major advantage is that it can determine anti-TNF and ADA concentrations simultaneously and rapidly (approximately 20 min), measuring the refractive index changes caused by interactions between the drug or ADAs
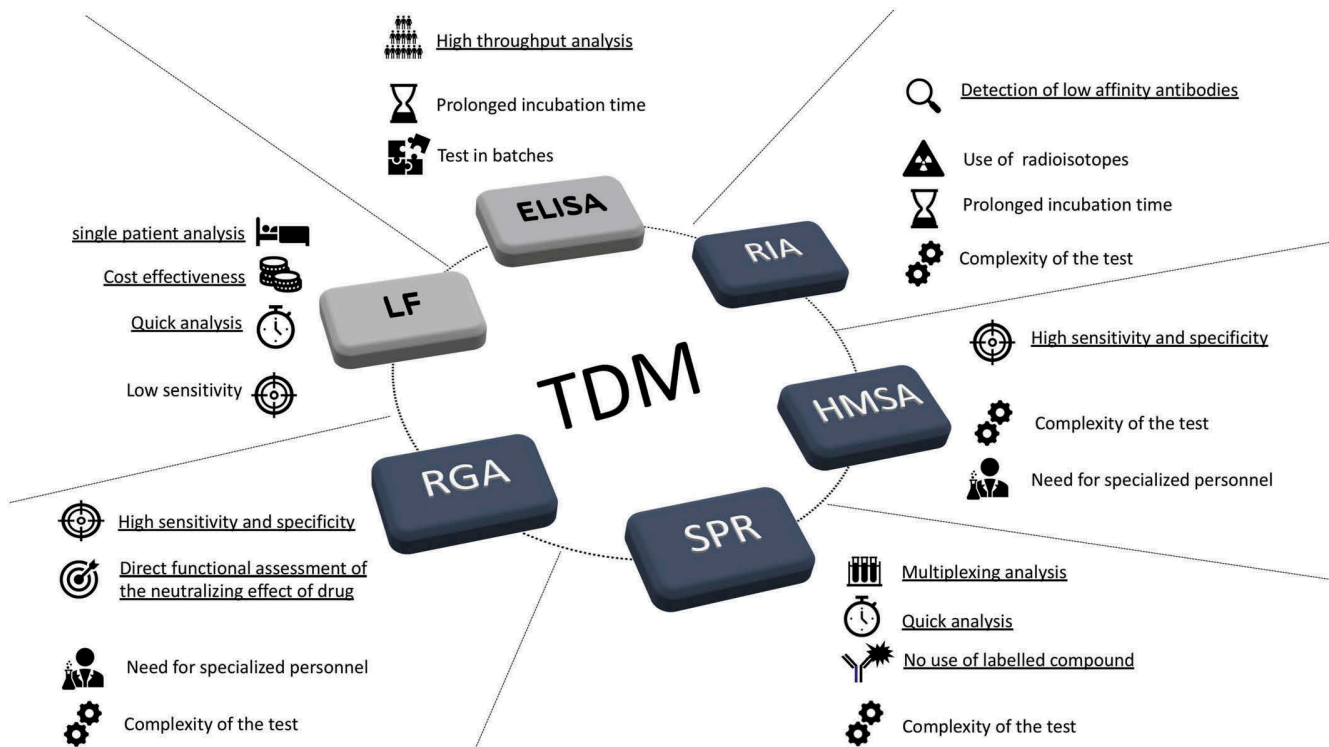
Figure 1. Schematic representation of the different methods used for therapeutic drug monitoring (TDM). The advantages are underlined. In light grey the solid-phase immunoassays, in particular the enzyme-linked immunosorbent assay (ELISA) and Lateral Flow (LF), in dark grey the other ones: radioimmunoassay (RIA), the homogeneous mobility shift assays (HMSA), the cell reporter gene assays (RGA) and the surface plasmon resonance (SPR) assay.

present in solution and a specific functionalized capture antibody immobilized on the optical fiber [113]. Another advantage of this technique is that it does not require labeled compounds and avoids long incubation steps, reducing the complexity of the test [114].

Another technique implemented in TDM is the LF assay. The Quantum Blue (Bühlmann Laboratories, Schönenbuch, Switzerland) has been the first rapid LF test for TDM introduced in the market, and consists in a small device (i.e.: Quantum Blue® Reader) that analyzes the test membrane spotted with the patient serum, returning a quantitative value of signal intensity for a Test and a Control line. The assay permits to quantify drug (infliximab and adalimumab) trough levels within 15 min. In this assay, recombinant TNF-α is conjugated to gold colloids that are released into the reaction system as the sample is applied. The drug present in the sample will bind to the gold conjugate. The monoclonal antibody, highly specific for the analyte bound on the test membrane, binds the complex of gold conjugate and the drug, resulting in coloring of the Test Line. The remaining free TNF/gold conjugate will bind to the Control Line. Other companies have introduced later similar LF systems on the market: the R-Biopharm product (RIDA Quick) is based on principles similar to Quantum Blue and permits to quantify infliximab or adalimumab in 20 min; instead, Grifols LF (Promonitor Quick) can quantify ADAs directly from the blood or serum of patient in 30 min. LF assays showed good agreement with traditional ELISA assays and for this reason may represent a viable option for real-time therapeutic drug monitoring [115]. According to the needs

of the clinicians, the use of LF assays could be useful for proactive strategies permitting to have an immediate result in order to change clinical decision.

In conclusion, ELISA assays are commonly used for TDM; however, their results are not immediately available to clinicians because they require several hours of work performed by appropriately trained personnel, and are cost-effective only if several patients' samples are evaluated simultaneously. RIA is similar to ELISA in terms of sensitivity and specificity, but the use of radioisotopes makes this technique more complex to set up and expensive, limiting its use in clinical practice. HMSA overcomes many limitations of the solid-phase ELISA method permitting to analyze the drug and ADAs separately thanks to high-pressure liquid chromatography and size exclusion chromatography. Unlike these methods, RGA cell-based assay provides a direct functional assessment of the neutralizing effect of drug and permits to evaluate both drugs and ADA levels with a high sensitivity and in a short time. HMSA and RGA are both sensitive and specific techniques, but the complexity of the analysis limits their widespread use. For this reason, the use of newer techniques is becoming increasingly important. LF assays are cost effective for the analysis, mostly in the context of a limited number of samples. Other advantages of these devices include the relative ease of operation and the quick turnaround time for results (15–30 min). However, FO-SPR is also a promising test that should be better exploited in clinical practice because of the ability of quantifying the drug and ADAs simultaneously and rapidly.

5. Conclusion

In conclusion, there is a consensus across above-mentioned studies that higher anti-TNF trough levels are associated with a better clinical outcome both in induction and maintenance therapy in IBD children, and that early anti-TNF serum measurements could be predictive also of long-term response. However, it is still not clear what the best predictive time of sampling is: most frequently, trough levels are measured at the end of induction (week 14) although important correlation with remission had been established also at earlier time points (Table 2). Moreover, the ideal target drug plasma concentration to achieve is still an open question: a therapeutic window of 3–5 µg/mL is generally accepted, but evidences support that this therapeutic window differs among infliximab and adalimumab and that, likely, there is the need of higher values for a sustained clinical and endoscopical remission. Therefore, it can be supposed that the ideal value is probably different for the specific target; i.e. it may be even higher if the target is resolution of perianal disease. The introduction of rapid tests for the quantification of drugs and ADAs levels should further improve the TDM of biologics also permitting proactive strategies.

6. Expert opinion

Concentrations of anti-TNF agents and ADAs are strikingly associated with therapeutic response of patients with IBD, also in children. However, most studies performed so far consider trough concentrations and propose a reactive paradigm for therapeutic adjustment, with increase of anti-TNF dose or change to an alternative agent performed when the patient has been already clearly underdosed for a critical therapeutic period, such as induction therapy; moreover, adjustment is done waiting for the following administration of the drug, that for most agents may imply more than two weeks of additional inadequate exposure. To improve clinical utility, monitoring of anti-TNF should be shifted from a reactive to a proactive paradigm. This should involve assessment of infliximab concentrations at an earlier time point than trough levels, in order to adjust therapy ideally during or immediately after anti-TNF administration. Results from preliminary studies on proactive adjustment of anti-TNF therapy support the feasibility and utility of this approach [84]. Moreover, evaluation of more articulated and informative pharmacokinetic models than single time-point concentrations, such as calculation of individual drug clearance, adjusted also for relevant pharmacodynamic, clinical and demographic covariates, describing patient development and growth, may provide further benefit in discriminating patients at risk for inadequate exposure, as early as possible, and in adjusting the therapeutic strategy accordingly [116]. More clinical studies to prove utility of more extensive pharmacokinetic evaluations to improve therapy with anti-TNF in pediatric IBD patients are needed [117].

ADA production in patients is an important determinant of low anti-TNF concentration and increased susceptibility to hypersensitivity. More efficient determination of ADAs should be evaluated, with studies overcoming the limitation of detecting ADAs just when anti-TNF concentration is low or absent: the possibility to detect ADA production as early as possible, even before a clinically relevant decrease in anti-TNF concentration is important. Further molecular characterization of ADAs may also be important, in order to identify those with a higher probability of persistent *versus* transient inhibition of anti-TNF. Molecular tools that allow multitasking molecular assays on patients' serum are needed, allowing measurement at the same time of the drug and ADA concentration, distinguishing among different ADA subtypes, e.g. those directed against the antigen binding region of the anti-TNF agents *versus* other parts of the therapeutic antibody [118].

For clinical implementation of monitoring of anti-TNF agents and ADAs, molecular assays that provide drug concentration efficiently, with an accurate and rapid quantification are of great importance. Recent development of point-of-care assays, such as LF assays, has been done: these assays have been demonstrated to provide reliable quantification of anti-TNF agents, more efficiently than standard plate-based ELISA assays, that require to process many samples at the same time to be economically efficient [115]. Further improvements of anti-TNF quantification approaches could, however, be pursued: in particular, diagnostic tools that allow real-time tracking of circulating therapeutic agents can be envisioned [119]. These innovative approaches have been already developed for small endogenous molecules, such as glucose. Innovative biosensor could be developed also for biological agents such as anti-TNF agents.

Further studies for the identification of additional patient-specific molecular biomarkers of anti-TNF efficacy, such as germline pharmacogenomic variants or epigenetic features (e.g. long noncoding RNA expression, methylation of candidate genes in DNA from relevant tissues) could be performed, as these markers could allow stratification of patients with increased probability of inadequate clinical response before starting expensive and potentially ineffective treatments [120], such as those based on anti-TNF agents.

In the future, therefore, for patients with pediatric IBD, monitoring of anti-TNF agents will be done at the point-of-care, in real time, using pharmacokinetic/pharmacodynamic models adjusted for patient pharmacogenomic, development and clinical state, allowing to identify patients at risk for lack of clinical efficacy or adverse effect at an early stage of therapy, proactively adjusting the therapeutic strategy.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

References

1. D'Haens GR, Sartor RB, Silverberg MS, et al. Future directions in inflammatory bowel disease management. J Crohns Colitis. 2014;8 (8):726–734.
2. Duijvestein M, Battat R, Vande Casteele N, et al. Novel therapies and treatment strategies for patients with inflammatory bowel disease. Curr Treat Options Gastroenterol. 2018;16(1):129–146.
   ● **Comprehensive review about IBD treatment.**
3. Peyrin-Biroulet L, Deltenre P, de Suray N, et al. Efficacy and safety of tumor necrosis factor antagonists in Crohn's disease: meta-analysis of placebo-controlled trials. Clin Gastroenterol Hepatol. 2008;6 (6):644–653.
4. Ns D, Hart A, De CP. Systematic review: predicting and optimising response to anti-TNF therapy in Crohn's disease - algorithm for practical management. Aliment Pharmacol Ther. 2016;43(1):30–51.
   ● **Interesting review with the proposal of an algorithm for clinical adjustment of anti-TNF drugs.**
5. Ordas I, Eckmann L, Talamini M, et al. Ulcerative colitis. Lancet. 2012;380(9853):1606–1619.
6. Torres J, Mehandru S, Colombel JF, et al. Crohn's disease. Lancet. 2017;389(10080):1741–1755.
7. Mahid SS, Minor KS, Soto RE, et al. Smoking and inflammatory bowel disease: a meta-analysis. Mayo Clin Proc. 2006;81 (11):1462–1471.
8. Sakamoto N, Kono S, Wakai K, et al. Dietary risk factors for inflammatory bowel disease: a multicenter case-control study in Japan. Inflamm Bowel Dis. 2005;11(2):154–163.
9. Abraham C, Cho JH. Inflammatory bowel disease. N Engl J Med. 2009;361(21):2066–2078.
10. Sartor RB. Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. Nat Clin Pract Gastroenterol Hepatol. 2006;3 (7):390–407.
11. Jostins L, Ripke S, Weersma RK, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature. 2012;491(7422):119–124.
12. Liu JZ, van Sommeren S, Huang H, et al. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. Nat Genet. 2015;47(9):979–986.
13. Ng SC, Shi HY, Hamidi N, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet. 2018;390 (10114):2769–2778.
14. Kappelman MD, Moore KR, Allen JK, et al. Recent trends in the prevalence of Crohn's disease and ulcerative colitis in a commercially insured US population. Dig Dis Sci. 2013;58 (2):519–525.
15. Johnston RD, Logan RF. What is the peak age for onset of IBD? Inflamm Bowel Dis. 2008;14(Suppl 2):S4–5.
16. Barton JR, Gillon S, Ferguson A. Incidence of inflammatory bowel disease in Scottish children between 1968 and 1983; marginal fall in ulcerative colitis, three-fold rise in Crohn's disease. Gut. 1989;30 (5):618–622.
17. Schildkraut V, Alex G, Cameron DJ, et al. Sixty-year study of incidence of childhood ulcerative colitis finds eleven-fold increase beginning in 1990s. Inflamm Bowel Dis. 2013;19(1):1–6.
18. Turunen P, Kolho KL, Auvinen A, et al. Incidence of inflammatory bowel disease in Finnish children, 1987–2003. Inflamm Bowel Dis. 2006;12(8):677–683.
19. Benchimol EI, Manuel DG, Guttmann A, et al. Changing age demographics of inflammatory bowel disease in Ontario, Canada: a population-based cohort study of epidemiology trends. Inflamm Bowel Dis. 2014;20(10):1761–1769.
20. Pigneur B, Seksik P, Viola S, et al. Natural history of Crohn's disease: comparison between childhood- and adult-onset disease. Inflamm Bowel Dis. 2010;16(6):953–961.
21. Van Limbergen J, Russell RK, Drummond HE, et al. Definition of phenotypic characteristics of childhood-onset inflammatory bowel disease. Gastroenterology. 2008;135(4):1114–1122.
22. Lichtenstein GR. New ACG Guideline for the Management of Crohn's Disease. Gastroenterol Hepatol. 2018;14(8):451.
23. Lichtenstein GR, Loftus EV, Isaacs KL, et al. ACG clinical guideline: management of crohn's disease in adults. Am J Gastroenterol. 2018;113(4):481–517.
   ● **Updated clinical guidelines for the treatment of Crohn's disease.**
24. Jovani M, Danese S. Vedolizumab for the treatment of IBD: a selective therapeutic approach targeting pathogenic a4b7 cells. Curr Drug Targets. 2013;14(12):1433–1443.
25. Teng MW, Bowman EP, McElwee JJ, et al. IL-12 and IL-23 cytokines: from discovery to targeted therapies for immune-mediated inflammatory diseases. Nat Med. 2015;21(7):719–729.
26. Turner D, Ruemmele FM, Orlanski-Meyer E, et al. Management of paediatric ulcerative colitis, part 1: ambulatory care-an evidence-based guideline from European Crohn's and Colitis organization and European society of paediatric gastroenterology, hepatology and nutrition. J Pediatr Gastroenterol Nutr. 2018;67(2):257–291.
   ● **Updated clinical guidelines for the treatment of ulcerative colitis in children.**
27. Hanauer SB, Feagan BG, Lichtenstein GR, et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. Lancet. 2002;359(9317):1541–1549.
28. Colombel JF, Sandborn WJ, Reinisch W, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. N Engl J Med. 2010;362(15):1383–1395.
29. Hebuterne X, Lemann M, Bouhnik Y, et al. Endoscopic improvement of mucosal lesions in patients with moderate to severe ileocolonic Crohn's disease following treatment with certolizumab pegol. Gut. 2013;62(2):201–208.
30. Colombel JF, Rutgeerts P, Reinisch W, et al. Early mucosal healing with infliximab is associated with improved long-term clinical outcomes in ulcerative colitis. Gastroenterology. 2011;141 (4):1194–1201.
31. Beutler B, Cerami A. The biology of cachectin/TNF–a primary mediator of the host response. Annu Rev Immunol. 1989;7:625–655.
32. Feldmann M, Maini RN. Anti-TNF alpha therapy of rheumatoid arthritis: what have we learned?. Annu Rev Immunol. 2001;19:163–196.
33. Quetglas EG, Mujagic Z, Wigge S, et al. Update on pathogenesis and predictors of response of therapeutic strategies used in inflammatory bowel disease. World J Gastroenterol. 2015;21 (44):12519–12543.
34. Billmeier U, Dieterich W, Neurath MF, et al. Molecular mechanism of action of anti-tumor necrosis factor antibodies in inflammatory bowel diseases. World J Gastroenterol. 2016;22(42):9300–9313.
35. Guo Y, Lu N, Bai A. Clinical use and mechanisms of infliximab treatment on inflammatory bowel disease: a recent update. Biomed Res Int. 2013;2013:581631.
36. Hanauer SB, Sandborn WJ, Rutgeerts P, et al. Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial. Gastroenterology. 2006;130(2):323–333. quiz 591.
37. Sandborn WJ, Hanauer SB, Rutgeerts P, et al. Adalimumab for maintenance treatment of Crohn's disease: results of the CLASSIC II trial. Gut. 2007;56(9):1232–1239.
38. Ben-Horin S, Kopylov U, Chowers Y. Optimizing anti-TNF treatments in inflammatory bowel disease. Autoimmun Rev. 2014;13 (1):24–30.
39. McLean LP, Cross RK. Adverse events in IBD: to stop or continue immune suppressant and biologic treatment. Expert Rev Gastroenterol Hepatol. 2014;8(3):223–240.

40. Wheat CL, Ko CW, Clark-Snustad K, et al. Inflammatory Bowel Disease (IBD) pharmacotherapy and the risk of serious infection: a systematic review and network meta-analysis. BMC Gastroenterol. 2017;17(1):52.

41. Cheifetz A, Smedley M, Martin S, et al. The incidence and management of infusion reactions to infliximab: a large center experience. Am J Gastroenterol. 2003;98(6):1315–1324.

42. Vultaggio A, Matucci A, Nencini F, et al. Anti-infliximab IgE and non-IgE antibodies and induction of infusion-related severe anaphylactic reactions. Allergy. 2010;65(5):657–661.

43. Cheifetz A, Mayer L. Monoclonal antibodies, immunogenicity, and associated infusion reactions. Mt Sinai J Med. 2005;72(4):250–256.

44. Crandall WV, Mackner LM. Infusion reactions to infliximab in children and adolescents: frequency, outcome and a predictive model. Aliment Pharmacol Ther. 2003;17(1):75–84.

45. Mocci G, Marzo M, Papa A, et al. Dermatological adverse reactions during anti-TNF treatments: focus on inflammatory bowel disease. J Crohns Colitis. 2013;7(10):769–779.

46. Weinblatt ME, Fleischmann R, Huizinga TW, et al. Efficacy and safety of certolizumab pegol in a broad population of patients with active rheumatoid arthritis: results from the REALISTIC phase IIIb study. Rheumatology (Oxford). 2012;51(12):2204–2214.

47. Weinblatt ME, Schiff M, Valente R, et al. Head-to-head comparison of subcutaneous abatacept versus adalimumab for rheumatoid arthritis: findings of a phase IIIb, multinational, prospective, randomized study. Arthritis Rheum. 2013;65(1):28–38.

48. Keystone EC, Genovese MC, Klareskog L, et al. Golimumab, a human antibody to tumour necrosis factor {alpha} given by monthly subcutaneous injections, in active rheumatoid arthritis despite methotrexate therapy: the GO-FORWARD Study. Ann Rheum Dis. 2009;68(6):789–796.

49. Urquhart L. Market watch: top drugs and companies by sales in 2017. Nat Rev Drug Discov. 2018;17(4):232.

50. Pentek M, Lakatos PL, Oorsprong T, et al. Access to biologicals in Crohn's disease in ten European countries. World J Gastroenterol. 2017;23(34):6294–6305.

51. Church PC, Hyams J, Ruemmele F, et al. The continental divide: anti-TNF use in pediatric IBD is different in North America compared to other parts of the world. Can J Gastroenterol Hepatol. 2018;2018:3190548.

52. Gonczi L, Vegh Z, Golovics PA, et al. Prediction of short- and medium-term efficacy of biosimilar infliximab therapy. Do trough levels and antidrug antibody levels or clinical and biochemical markers play the more important role? J Crohns Colitis. 2017;11(6):697–705.

53. Yanai H, Lichtenstein L, Assa A, et al. Levels of drug and antidrug antibodies are associated with outcome of interventions after loss of response to infliximab or adalimumab. Clin Gastroenterol Hepatol. 2015;13(3):522–530 e2.

54. Vermeire S, Gils A, Accossato P, et al. Immunogenicity of biologics in inflammatory bowel disease. Therap Adv Gastroenterol. 2018;11:1756283X17750355.

55. van Schie KA, Hart MH, de Groot ER, et al. The antibody response against human and chimeric anti-TNF therapeutic antibodies primarily targets the TNF binding region. Ann Rheum Dis. 2015;74(1):311–314.

56. Chirmule N, Jawa V, Meibohm B. Immunogenicity to therapeutic proteins: impact on PK/PD and efficacy. Aaps J. 2012;14(2):296–302.

57. Nanda KS, Cheifetz AS, Moss AC. Impact of antibodies to infliximab on clinical outcomes and serum infliximab levels in patients with inflammatory bowel disease (IBD): a meta-analysis. Am J Gastroenterol. 2013;108(1):40–47. quiz 48.

58. Roblin X, Marotte H, Leclerc M, et al. Combination of C-reactive protein, infliximab trough levels, and stable but not transient antibodies to infliximab are associated with loss of response to infliximab in inflammatory bowel disease. J Crohns Colitis. 2015;9(7):525–531.

59. Ordas I, Feagan BG, Sandborn WJ. Therapeutic drug monitoring of tumor necrosis factor antagonists in inflammatory bowel disease. Clin Gastroenterol Hepatol. 2012;10(10):1079–1087. quiz e85–6.

60. Liefferinckx C, Minsart C, Toubeau JF, et al. Infliximab trough levels at induction to predict treatment failure during maintenance. Inflamm Bowel Dis. 2017;23(8):1371–1381.

61. Vande Casteele N, Gils A, Singh S, et al. Antibody response to infliximab and its impact on pharmacokinetics can be transient. Am J Gastroenterol. 2013;108(6):962–971.

62. Bond A, Dodd S, Fisher G, et al. Concurrent immunomodulator therapy is associated with higher adalimumab trough levels during scheduled maintenance therapy. Scand J Gastroenterol. 2017;52(2):204–208.

63. Ben-Horin S, Waterman M, Kopylov U, et al. Addition of an immunomodulator to infliximab therapy eliminates antidrug antibodies in serum and restores clinical response of patients with inflammatory bowel disease. Clin Gastroenterol Hepatol. 2013;11(4):444–447.

64. Ruemmele FM, Veres G, Kolho KL, et al. Consensus guidelines of ECCO/ESPGHAN on the medical management of pediatric Crohn's disease. J Crohns Colitis. 2014;8(10):1179–1207.

65. McConnell J, Parvulescu-Codrea S, Behm B, et al. Accelerated infliximab infusions for inflammatory bowel disease improve effectiveness. World J Gastrointest Pharmacol Ther. 2012;3(5):74–82.

66. Clare DF, Alexander FC, Mike S, et al. Accelerated infliximab infusions are safe and well tolerated in patients with inflammatory bowel disease. Eur J Gastroenterol Hepatol. 2009;21(1):71–75.

67. Ordas I, Mould DR, Feagan BG, et al. Anti-TNF monoclonal antibodies in inflammatory bowel disease: pharmacokinetics-based dosing paradigms. Clin Pharmacol Ther. 2012;91(4):635–646.

68. Lichtenstein GR, Panaccione R, Mallarkey G. Efficacy and safety of adalimumab in Crohn's disease. Therap Adv Gastroenterol. 2008;1(1):43–50.

69. Vande Casteele N, Baert F, Bian S, et al. subcutaneous absorption contributes to observed interindividual variability in adalimumab serum concentrations in Crohn's disease: a prospective multicentre study. J Crohns Colitis. 2019.

70. Sandborn WJ, Feagan BG, Marano C, et al. Subcutaneous golimumab induces clinical response and remission in patients with moderate-to-severe ulcerative colitis. Gastroenterology. 2014;146(1):85–95. quiz e14–5.

71. Ryman JT, Meibohm B. Pharmacokinetics of monoclonal antibodies. CPT Pharmacometrics Syst Pharmacol. 2017;6(9):576–588.

72. Klotz U, Teml A, Schwab M. Clinical pharmacokinetics and use of infliximab. Clin Pharmacokinet. 2007;46(8):645–660.

73. Fasanmade AA, Adedokun OJ, Blank M, et al. Pharmacokinetic properties of infliximab in children and adults with Crohn's disease: a retrospective analysis of data from 2 phase III clinical trials. Clin Ther. 2011;33(7):946–964.

74. Xu Y, Adedokun OJ, Chan D, et al. Population pharmacokinetics and exposure-response modeling analyses of golimumab in children with moderately to severely active ulcerative colitis. J Clin Pharmacol. 2019;59(4):590–604.

75. Patel KR, Roberts JT, Barb AW. Multiple variables at the leukocyte cell surface impact Fc gamma receptor-dependent mechanisms. Front Immunol. 2019;10:223.

76. Mould DR, Sweeney KR. The pharmacokinetics and pharmacodynamics of monoclonal antibodies–mechanistic modeling applied to drug development. Curr Opin Drug Discovery Dev. 2007;10(1):84–96.

77. Ternant D, Ducourau E, Perdriger A, et al. Relationship between inflammation and infliximab pharmacokinetics in rheumatoid arthritis. Br J Clin Pharmacol. 2014;78(1):118–128.

78. Brandse JF, van Den Brink GR, Wildenberg ME, et al. Loss of infliximab into feces is associated with lack of response to therapy in patients with severe ulcerative colitis. Gastroenterology. 2015;149(2):350–5 e2.

79. Lopetuso LR, Gerardi V, Papa V, et al. Can we predict the efficacy of Anti-TNF-alpha agents? Int J Mol Sci. 2017;18:9.

80. Naviglio S, Giuffrida P, Stocco G, et al. How to predict response to anti-tumour necrosis factor agents in inflammatory bowel disease. Expert Rev Gastroenterol Hepatol. 2018;12(8):797–810.

● Comprehensive review on therapy personalization and anti-TNF agents use in IBD.

81. Carman N, Mack DR, Benchimol EI. Therapeutic drug monitoring in pediatric inflammatory bowel disease. Curr Gastroenterol Rep. 2018;20(5):18.

82. Kang JS, Lee MH. Overview of therapeutic drug monitoring. Korean J Intern Med. 2009;24(1):1–10.

83. Dubinsky MC, Lamothe S, Yang HY, et al. Pharmacogenomics and metabolite measurement for 6-mercaptopurine therapy in inflammatory bowel disease. Gastroenterology. 2000;118(4):705–713.

84. Lega S, Bramuzzo M, Dubinsky MC. Therapeutic drug monitoring in pediatric IBD: current application and future perspectives. Curr Med Chem. 2018;25(24):2840–2854.

85. Batchelor HK, Marriott JF. Paediatric pharmacokinetics: key considerations. Br J Clin Pharmacol. 2015;79(3):395–404.

86. Hamalainen A, Sipponen T, Kolho KL. Serum infliximab concentrations in pediatric inflammatory bowel disease. Scand J Gastroenterol. 2013;48(1):35–41.

87. Naviglio S, Lacorte D, Lucafo M, et al. Causes of treatment failure in children with inflammatory bowel disease treated with infliximab: a pharmacokinetic study. J Pediatr Gastroenterol Nutr. 2019;68(1):37–44.

88. Singh N, Rosenthal CJ, Melmed GY, et al. Early infliximab trough levels are associated with persistent remission in pediatric patients with inflammatory bowel disease. Inflamm Bowel Dis. 2014;20(10):1708–1713.

89. van Hoeve K, Dreesen E, Hoffman I, et al. Adequate infliximab exposure during induction predicts remission in paediatric patients with inflammatory bowel disease. J Pediatr Gastroenterol Nutr. 2019; 68(6):847-853.

90. Ungar B, Glidai Y, Yavzori M, et al. Association between infliximab drug and antibody levels and therapy outcome in pediatric inflammatory bowel diseases. J Pediatr Gastroenterol Nutr. 2018;67(4):507–512.

91. Lega S, Phan BL, Rosenthal CJ, et al. Proactively optimized infliximab monotherapy is as effective as combination therapy in IBD. Inflamm Bowel Dis. 2019;25(1):134–141.

92. Kennedy NA, Heap GA, Green HD, et al. Predictors of anti-TNF treatment failure in anti-TNF-naive patients with active luminal Crohn's disease: a prospective, multicentre, cohort study. Lancet Gastroenterol Hepatol. 2019;4(5):341-353.
● Clinical prospective study evaluating the predictors of treatment failure for anti-TNF drugs in Crohn's disease.

93. Brandse JF, Mould D, Smeekes O, et al. A real-life population pharmacokinetic study reveals factors associated with clearance and immunogenicity of infliximab in inflammatory bowel disease. Inflamm Bowel Dis. 2017;23(4):650–660.

94. Dotan I, Ron Y, Yanai H, et al. Patient factors that increase infliximab clearance and shorten half-life in inflammatory bowel disease: a population pharmacokinetic study. Inflamm Bowel Dis. 2014;20(12):2247–2259.

95. Fasanmade AA, Adedokun OJ, Olson A, et al. Serum albumin concentration: a predictive factor of infliximab pharmacokinetics and clinical response in patients with ulcerative colitis. Int J Clin Pharmacol Ther. 2010;48(5):297–308.

96. Detrez I, Dreesen E, Van Stappen T, et al. Variability in golimumab exposure: a 'Real-Life' observational study in active ulcerative colitis. J Crohns Colitis. 2016;10(5):575–581.

97. Hoekman DR, Brandse JF, de Meij TG, et al. The association of infliximab trough levels with disease activity in pediatric inflammatory bowel disease. Scand J Gastroenterol. 2015;50(9):1110–1117.

98. Choi SY, Kang B, Lee JH, et al. Clinical use of measuring trough levels and antibodies against infliximab in patients with pediatric inflammatory bowel disease. Gut Liver. 2017;11(1):55–61.

99. van Hoeve K, Dreesen E, Hoffman I, et al. Higher infliximab trough levels are associated with better outcome in paediatric patients with inflammatory bowel disease. J Crohns Colitis. 2018;12(11):1316–1325.

100. Kang B, Choi SY, Choi YO, et al. Infliximab trough levels are associated with mucosal healing during maintenance treatment with infliximab in paediatric Crohn's disease. J Crohns Colitis. 2019;13(2):189–197.

101. Ungar B, Levy I, Yavne Y, et al. Optimizing anti-TNF-alpha therapy: serum levels of infliximab and adalimumab are associated with mucosal healing in patients with inflammatory bowel diseases. Clin Gastroenterol Hepatol. 2016;14(4):550–557 e2.

102. Papamichael K, Rakowsky S, Rivera C, et al. Infliximab trough concentrations during maintenance therapy are associated with endoscopic and histologic healing in ulcerative colitis. Aliment Pharmacol Ther. 2018;47(4):478–484.

103. Vande Casteele N, Ferrante M, Van Assche G, et al. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. Gastroenterology. 2015;148(7):1320–9 e3.

104. Ungar B, Ben-Shatach Z, Ben-Haim G, et al. Infliximab therapy intensification upon loss of response: is there an optimal trough level? Dig Liver Dis. 2019;S1590-8658(19)30099-4.

105. Ma C, Battat R, Jairath V, et al. Advances in therapeutic drug monitoring for small-molecule and biologic therapies in inflammatory bowel disease. Curr Treat Options Gastroenterol. 2019;17(1):127–145.

106. Vande Casteele N. Assays for measurement of TNF antagonists in practice. Frontline Gastroenterol. 2017;8(4):236–242.

107. Jani M, Isaacs JD, Morgan AW, et al. Detection of anti-drug antibodies using a bridging ELISA compared with radioimmunoassay in adalimumab-treated rheumatoid arthritis patients with random drug levels. Rheumatology (Oxford). 2016;55(11):2050–2055.

108. Wadhwa M, Knezevic I, Kang HN, et al. Immunogenicity assessment of biotherapeutic products: an overview of assays and their utility. Biologicals. 2015;43(5):298–306.

109. Wang SL, Ohrmund L, Hauenstein S, et al. Development and validation of a homogeneous mobility shift assay for the measurement of infliximab and antibodies-to-infliximab levels in patient serum. J Immunol Methods. 2012;382(1–2):177–188.

110. Lallemand C, Kavrochorianou N, Steenholdt C, et al. Reporter gene assay for the quantification of the activity and neutralizing antibody response to TNFalpha antagonists. J Immunol Methods. 2011;373(1–2):229–239.

111. Gils A, Vande Casteele N, Poppe R, et al. Development of a universal anti-adalimumab antibody standard for interlaboratory harmonization. Ther Drug Monit. 2014;36(5):669–673.

112. Steenholdt C, Bendtzen K, Brynskov J, et al. Clinical implications of measuring drug and anti-drug antibodies by different assays when optimizing infliximab treatment failure in Crohn's disease: post hoc analysis of a randomized controlled trial. Am J Gastroenterol. 2014;109(7):1055–1064.

113. Lu J, Van Stappen T, Spasic D, et al. Fiber optic-SPR platform for fast and sensitive infliximab detection in serum of inflammatory bowel disease patients. Biosens Bioelectron. 2016;79:173–179.

114. Beeg M, Nobili A, Orsini B, et al. A surface plasmon resonance-based assay to measure serum concentrations of therapeutic antibodies and anti-drug antibodies. Sci Rep. 2019;9(1):2064.

115. Curci D, Lucafo M, Cifu A, et al. Determination of serum infliximab concentration by point-of-care devices in children with inflammatory bowel disease. J Pediatr Gastroenterol Nutr. 2019; May 28, epub ahead of print.

116. Danhof M, de Lange EC, Della Pasqua OE, et al. Mechanism-based pharmacokinetic-pharmacodynamic (PK-PD) modeling in translational drug research. Trends Pharmacol Sci. 2008;29(4):186–191.

117. Ramsey LB, Mizuno T, Vinks AA, et al. Learning health systems as facilitators of precision medicine. Clin Pharmacol Ther. 2017;101(3):359–367.

118. Krishna M, Nadler SG. Immunogenicity to biotherapeutics - the role of anti-drug immune complexes. Front Immunol. 2016;7:21.

119. Ferguson BS, Hoggarth DA, Maliniak D, et al. Real-time, aptamer-based tracking of circulating therapeutic agents in living animals. Sci Transl Med. 2013;5(213):213ra165.

120. Lucafo M, Franca R, Selvestrel D, et al. Pharmacogenetics of treatments for inflammatory bowel disease. Expert Opin Drug Metab Toxicol. 2018;14(12):1209–1223.