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Clinical utility of anti-TNFα trough levels and anti-drug antibodies in the management of inflammatory bowel disease

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Abstract

1. Introduction

The inflammatory bowel diseases (IBD) are chronic inflammatory diseases of the gastrointestinal (GI) tract that are associated with significant morbidity and disability. Over the past few decades, biological therapy with anti-Tumor Necrosis Factor agents (anti TNF-α) has emerged as a corner stone of treatment for IBD. Despite significant response and remission rates, clinicians are often confronted to secondary loss of responses or side effects during long term maintenance. There is emerging data from the literature suggesting that infliximab trough levels might help clinicians tailoring therapy and overcome on-treatment IBD flares.

2. Study aims

To evaluate the indications for trough anti-TNF-α and ADA serum testing, the prevalence of ADA and the therapeutic range of anti-TNF trough level measures and to assess the clinical impact of trough anti-TNF-α and ADA serum levels on routine management of IBD patients

3. Study Design

This is a retrospective study of anti-TNF trough level and ADA serum determinations in IBD patients receiving anti-TNF agents. We included all therapeutic drug monitoring measurements performed from 05.03.2013 to 23.04.2014. A total of 70 patients where included between two tertiary referral centers.

3.1 Inclusion criteria

1. Adult patients affected with CD or UC. 2. Patients for whom an anti-TNF-α trough level and / or anti-drug antibodies were performed at CHUV. 3. Current treatment by anti-TNF- α agents.

3.2. Exclusion criteria

1.No IBD diagnosis. 2. Adults patients suffering from another chronic inflammatory disease requiring treatment with anti-TNF-α such as rheumatoid arthritis. 3. Treatment with anti-TNF-α interrupted before the assays.

Results

In our patients (n=70) appear no male or female predominance and the average age was 40 years. Most tests (n=117) were performed for Crohn's disease (73%). A large subset of our patients had been previously managed with immunomodulators (54.%). However, few cases were on combination therapy (10%). The great majority (79%) for the indication of the determinations were performed as "medical follow up". The percentage of patients with an IFX trough level within the desired range (3-8 µg/ml) is at first determination rather low, but tends to increase during the subsequent determinations. Of 117 total tests assessed the results impacted treatment decisions in approximately 40% of the cases.

Conclusion

Therapetic drug monitoring will guide the induction, the dose titration to prevent disease flares and finally it will guide interventions for cases with loss of response to biologicals. However, some knowledge gaps still need to be addressed to confirm this strategy. We feel that after testing drug and ADA levels, clinicians may gain a sense of saliency in relation to decision-making not only in cases with poor response to biologicals but even in stable case

Keywords:
Inflammatory bowel disease/ trough levels/ anti-TNF-α/l adapted strategy /swiss

List of symbols and abbreviations

ADA	Anti-drug-antibody
CD	Crohn's disease
EIMs	Extraintestinal manifestations
ESR	Erythrocyte sedimentation rate
FBM	Faculté Biologie et Médecine
GWAS	Genome-wide association studies
G6PD	Glucose 6-phosphate dehydrogenase
IBD	Inflammatory bowel disease
UC	Ulcerative colitis
TNF-α	Tumor necrosis factor α
ADA	Anti-drugs-antibody
ELISA	Enzyme-linked immunosorbent assay
HMSA	Homogenous mobility shift assay
IFX	Infliximab
Y	Years
NSAIDs	No-steroidal Anti-inflammatory Drugs
NS	Non significant
RIA	Radioimmunoassay
WF	Weighting factor
TDM	Therapeutic drug monitoring

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Index

1. Introduction

The inflammatory bowel diseases (IBD), which are comprised of Crohn's disease (CD), ulcerative colitis (UC) and indeterminate colitis, are chronic inflammatory diseases of the gastrointestinal (GI) tract that are associated with significant morbidity and disability[1-6]. Apart from GI manifestations, IBD are associated with extra-intestinal manifestations (EIM)[7].

Over the past few decades, biological therapy with anti-Tumor Necrosis Factor agents (anti TNF-α) has emerged as a corner stone of treatment for IBD and its EIMs. Therapy with these agents can achieve mucosal healing, reduce the number of hospitalizations and reduce surgery and ameliorate quality of life [8]. Although a large number of patients benefit from those treatments, a limiting factor for the use of these agents is primary non-response, loss of response or intolerance [9]. A precise rate of primary non-response is not easy to find in the literature especially because of different use of definitions, but it is estimated that primary non-responders rates correspond to around 40% of anti-TNF treated patients[10]. In addition, in patients initially responding to treatment, secondary loss of response can be noted in 21% to 46% of the cases[10]. To tackle those limiting factors and try to improve disease control, several clinicians make use of therapeutic drug monitoring strategies to guide management of IBD when TNF failure occurs.

1.1 Epidemiology

The geographic distribution of IBD is highly variable [11]. For instance, the prevalence of IBD is higher in northern Europe and North America than in Asia and the southern hemisphere [12]. These diseases are observed predominantly in developed nations [13]. However the incidence, especially in countries previously thought to have a low incidence, is rising [14].

It is estimated that 1.4 million people in the United States of America (USA) are affected with IBD, whereas in Europe approximately 2.2 million suffer from this condition [14]. In Switzerland, between 12 000 and 16 000 patients suffer from IBD [15, 16]. In general UC is more prevalent than CD [17].
The male/female ratio is 0.8 for CD, and 1.5 for UC [18]. IBD can occur at any age with a peak incidence between 25-45 year [1, 3, 5, 19]. In adults UC is the most common form of IBD, whereas for children it is CD [12].

1.2 Etiology, Pathogenesis and risk factors

The ultimate cause of IBD is not well established [9, 20, 21], but significant progresses have been made [2]. Overall, IBD can be considered as a dysregulation of the mucosal immune responses to commensal gut flora in a genetically predisposed host [1, 5, 11, 17, 20, 22].
Under normal conditions, equilibrium between the luminal content and the mucosal immune system exists. IBD can be the consequence of a breakdown balance in the gut mucosa, which results in an aberrant immune response against the commensal flora [1, 5, 7, 11, 17].
This abnormal interaction leads to activation of adaptive immune responses that result in the production of inflammatory cytokines such as IL-1, IL-6, IL-8 and tumor necrosis factor alpha (TNF-α) [7, 20, 22]. These cytokines are capable of maintaining a strong inflammatory state in susceptible hosts [20].

Figure 1: Immunological mechanisms of IBD pathogenesis [1].

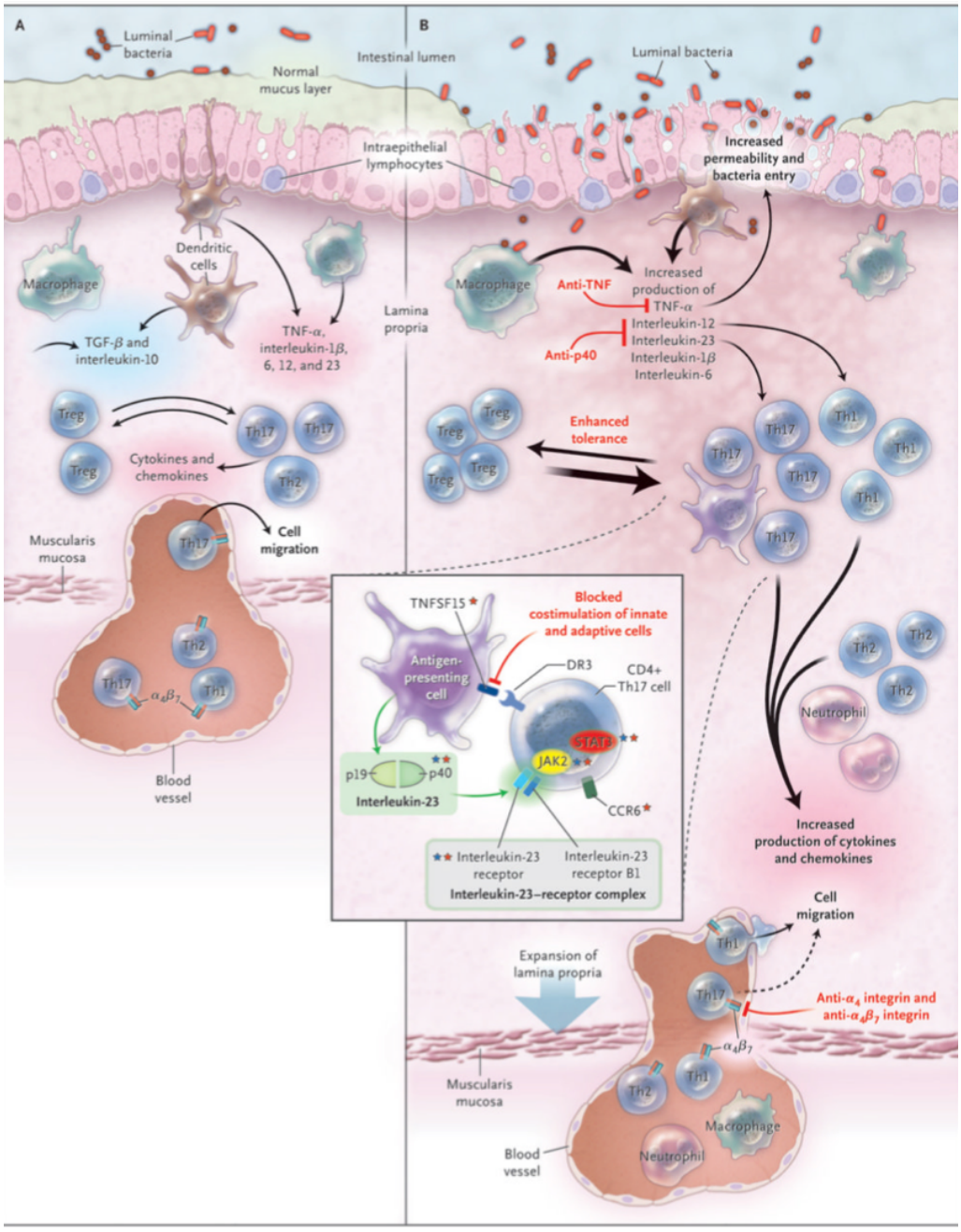


Figure 1. Immunological mechanisms of IBD pathogenesis [1] . Section A shows, in a healthy person, a normal lamina propria and some immune cells which secrete cytokines. These are pro-inflammatory and anti-inflammatory. An homeostatic equilibrium is maintained. On the contrary, section B shows a person with intestinal inflammation. The mucosal layer is interrupted and epithelial tight junctions, which normally maintain intestinal permeability, are disrupted. In IBD, immune cells react and produce pro-inflammatory cytokines, which recruit additional leukocytes by preserving a cycle of inflammation. Red labels denote some therapeutic approaches [1].

1.2.1 Risk factors

Two main categories of risk factors have been described for IBD:

Genetic predisposition [23],[24] : Evidence for a genetic factor associated with IBD comes from several observations. First, a positive family history increases disease risk in a significant manner [23, 25]. For example, for CD, the concordance rate for monozygotic twins is approximately 50%[11]. Moreover there appears to be concordance with disease distribution in family members [11]. In comparison, concerning UC, the concordance of monozygotic twins is only 16% [11]. IBD are more common in whites and Jews, and indeed Jewish descent is an independent risk factor for the disorder [21]. Finally, and most importantly, several genome-wide association studies (GWAS) have identified specific genetic anomalies associated with IBD. Some of the major genetic mutations include genes implicated in innate immune responses such as: NOD2, ATG16L1 and on the adaptive immunity such as IL-23R.

Environmental: in addition to ethnic and genetic predisposition, several environmental factors have been described as disease modifiers including in a non-exhaustive way [23]: antibiotic use, smoking, appendicectomy, breast feeding, sleep and stress. All environmental factors that have putative roles in disease onset and flares are generically grouped under the label "exposome" [26].

According to the so-called "hygiene hypothesis", the low frequency of infections in developed countries causes an increasing incidence of various conditions. The role of poor hygiene as a prevention of IBD has been reported [25] Furthermore, developing countries, which recently changed their lifestyle, have seen their incidence of IBD increased [17, 18].

A critical component of the "exposome" is the microbiome, defined as the entire intestinal commensal bacterial community [21]. The literature shows that a reduced diversity and an alteration of the composition and the organization of the commensal microbiota can drive intestinal inflammation in genetically susceptible individuals [21]. To date, it is currently unclear whether microbial changes associated with IBD are a cause or consequence of disease. It is also unclear whether therapeutic strategies aimed at modulating microbial composition are promising as most studies on probiotics failed to show any benefits and fecal microbiota transplantation shows marginal benefits, if any.

Smoking is a relevant behavioral factor that modulates IBD development and intensity.. It is indeed associated with an increased risk of CD onset and disease exacerbation. In contrast, it is partially protective from UC [14]. Non-smokers and former smokers both have an increased risk of UC, whereas smoking exacerbates the course of CD. [27]. Nationally, using the data from SIBDCS (n=1770) where patients were defined as smokers or non-smokers based on self-declaration we noted that, around 30% of all IBD patients are smokers at present day [28]. In this study, Smoking quantities were greater in Crohn's disease patients compared to UC. This peculiarity is observed in Europe as well [28]. Importantly, women with CD had the highest prevalence of smoking.

As mentioned earlier, **appendicectomy** protects from the development of UC but can increase the risk of CD [14]. One possible explanation for increased incidence of CD is the fortuitous discovery of CD in appendectomy piece.

Finally, some drugs have been associated with IBD development including non steroidal anti-inflammatory drugs [12] and oral contraceptives [14]. Both of these drugs may indeed trigger disease flares and worsening of the underlying IBD.

It is logic to think if what we eat every day has an impact the onset or the course of IBD, but right now clinicians are not yet able to fully answer to the question. Some studies, particularly on animal models, suggested that certain nutrients can reduce intestinal inflammation and on the contrary other can be deleterious. In the literature we find some progress of the understanding of the impact of a diet on IBD, but clear data are limited and controversial. For example the efficacy of omega-3 fatty acids for the maintenance of a medically induced remission in CD is not yet confirmed. [13]

In conclusion, those data suggest a clear role for environmental factors in IBD factors that is currently under intense investigation in Switzerland and worldwide.

1.3 Clinical manifestations of IBD

1.3.1. Comparison between UC and CD

CD and UC are two well-distinguished diseases. However, there are sometimes overlaps in their clinical presentation. The diagnosis and characterization of these diseases rely on clinical, endoscopic, radiological and pathologic criteria. [3, 13, 25, 29] A comparison between these two IBD is depicted in table 1.

Table 1: A comparison between these two inflammatory bowel diseases.

IBD	**Ulcerative colitis** (UC)	**Crohn's disease** (CD)
Localization	Rectum : 50% Left colon : 20-30% Pancolitis : 20% Rare: Backwash ileitis, ceacal patch	**Any segment** of the intestinal tract[4, 20] Small intestine-colon: (50-60%) Rectum: 30% Small intestine: 25% Rare: esophagus, and stomach.
Age	Two peaks of prevalence: 40-49 and 60-69 have been observed [16].	The main prevalence of the diagnoses: 17 and 40 years [21]. Peak of prevalence: 30-39 years [16].
Symptoms	Rectal bleeding, diarrhea, urgency, tenesmus, abdominal pain, fever, extra intestinal manifestations [17].	Chronic diarrhea, abdominal pain, weight loss, intestinal obstructive symptoms, extra intestinal manifestations [13].
Pathology	**Non-transmural** = confined to the mucosa and submucosa[1, 11]	**Transmural** = inflammation affects the entire gastrointestinal wall [1, 5, 11, 13].
Evolution of the disease	Chronic inflammatory condition relapsing and remitting course[30] • 25% of patients are in remission approximately 3-7 years after diagnosis [25]. • 18% of patients have a relapse every year [25]. • 57% of patients experience periodic relapses [25]. • The colectomy rate is estimated at 24% after 10 years after diagnosis [25].	CD can evolve in different phenotypes. Non-stricturing non- penetrating: 80% Other phenotypes: structuring, penetrating and perianally penetrating [21]. • 10-30% of patients undergo exacerbation one year after diagnosis[25] • 15-25% of patients show a slight disease activity • 55-65% of patients are in remission, of which only 10-13% achieved a long remission over several years [25].

		• 80% of patients with CD will require surgery during their lifetime [31].
Diagnosis	No gold standard Endoscopic, histological, radiological, biochemical investigations, negative stool examination for infectious cause are needed [32].	No gold standard Distribution, behavior, clinical, endoscopic, histologic and radiologic features is required. Exclusion of intestinal inflammation such as infection, ischemia.
Main disease severity scores	**Truelove-Witts** severity score [33] : **mild:** <4 stools / day, pulse <90bpm, no fever, no anemia normal erythrocyte sedimentation rate (ESR) or normal CRP [30, 33]. Erythematous mucosa [20] **moderate:** 4-6 stools / day, minimal signs of systemic toxicity [30, 33] Mucosal bleeding and ulcerated [20] **severe:** > 6 stools / day containing blood, systemic toxicity, (fever, tachycardia, anemia, ESR and CRP increased) [30, 33]. Mucosal bleeding and ulcerated [20]	Crohn's disease activity index (**CDAI**) [34] **mild:** CDAI of 150-220 The patient is eating well. No dehydration, fever, abdominal mass and no abdominal sensitivity to pressure. No weight loss> 10%, no obstruction. CRP below the standard[13] **moderate:** CDAI of 220-450 the treatment of mild ineffective: fever, weight loss, abdominal pain, nausea, occasional vomiting without obstruction. CRP above the norm [13] **severe:** CDAI >450 (BMI <18), signs of obstruction, pain or signs of abscess. CRP increased[13]

1.3.2. Extra-intestinal manifestations

Figure 2: The major extraintestinal manifestations (EIMs) and associate autoimmune disorders in IBD form[21]

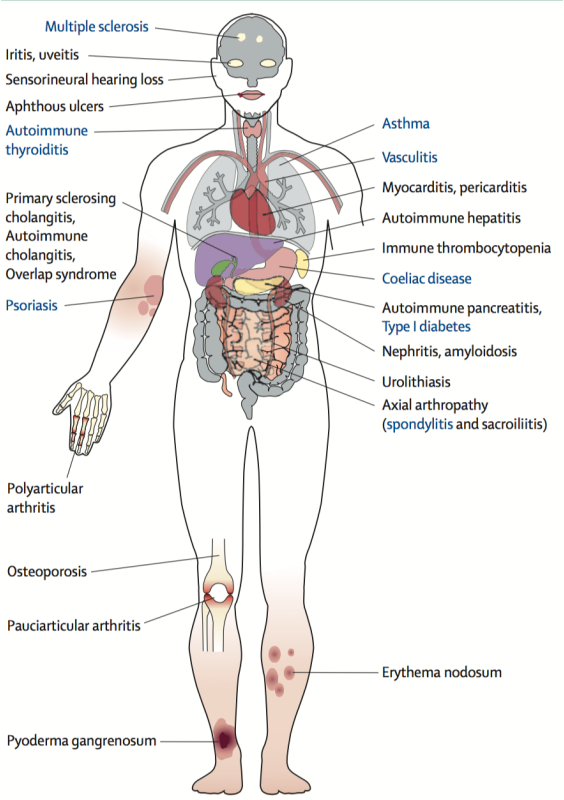


Figure 2. EIMs are a common problem in IBD, many patients with CD or UC can develop one of this EIMs during their life. Figure 2 shows the most important one. EIMs can be categorized in three different groups [35]: 1. reactive manifestations associated with intestinal disease activity (polyarticular arthritis and erythema nodosum) or not (pyoderma gangrenosum, uveitis). 2. non-IBD-specific describing a major susceptibility to autoimmune disease (thyroid disease, insulin-dependent diabetes). 3. IBD-related aggravations due to metabolic complications (thromboembolic events, osteoporosis).

1.3.3. Disease complications

Surgery is not considered a final curing option in CD and should be used only in specific situations such as abscesses, internal fistulas that are not responding to medical treatment, fibrostenotic strictures, bowel obstruction or cancer [21]. Regarding UC about 20-30% of patients need surgery during their lifetime. Urgent surgery is indicated when a life-threatening complication appear for example fulminant colitis. An elective procedure is considered when the disease is refractory or a colorectal cancer is discovered [17]. Screening for disease complications such as colorectal cancer, abscess, malnutrition, osteoporosis, anemia, opportunistic infections and monitoring for medication adverse effects is required to optimizing the medications.

1.4 Drug management

Since the exact mechanisms underlying IBD are not identified, a curative therapy does not exist [13]. Various drugs exist to induce and maintain disease remission. The mainstay of

pharmacological therapy is described in table 2. Apart from the pharmacological control of disease, management of IBD patients also includes general measures. These include: improving patient adherence to medication, smoking cessation, avoidance of exacerbating medications such as non steroidal anti-inflammatory medication, oral contraceptives and antibiotic.

Table 2: Pharmacological therapy for IBD.

Drug	Commercial name	Characteristics
Mesalazine = 5-aminosalicylic acid (5-ASA)	**Asacol® Asazine® Pentasa® Salofalk®**	The derivatives of aminosalicylate drugs are widely used. Especially in cases of mild or moderate severity of UC [19, 32] Side effects: Rare. Headache, nausea, rash, skin, thrombocytopenia [36], interstitial nephritis or nephrotic syndrome [32],pancreatitis, hepatitis,[32] bloody diarrhea (not to be confused with disease worsening) [36]
Azathioprine and 6-mercaptopurine (Immununomodulateurs)	**Imurek®, Purinéthol®**	Side effects: hematological, hypersensitivity reactions, infections, pancreatitis, gastrointestinal-intolerance. Mild increased risk of lymphoma.
Methotrexate (antimetabolite and antifolate drug)	**Methoject®**	Side effects: hepatotoxicity, pulmonary fibrosis, and myelosuppression, contraindicated during pregnancy and a reliable method of birth control should be used.
Corticosteroids		Corticosteroids induce remission, but do not prevent relapse [19]. Not used for maintenance. Side effects: Cushing Syndrome , psychiatric disturbance, infections, cataract and glaucoma, gastrodudenal mucosal injury, metabolic syndrome , sodium and water retention , osteoporosis [32] Recommendations: Calcium and Vit D supplementation, bisphosphonate if more than 3-month therapy [32], wean slowly to avoid adrenal insufficiency.
Anti-TNF-α	**Remicade®=** infliximab **Humira®=**adalimumab **Cimzia®**= certolizumab **Simponi®**= golimumab	Used for induction and maintenance [19] . Side effects: see body of the manuscript Contraindications: -Patients suffering form an active or untreated -Tuberculosis (TB). -Current or recent neoplasia. -Sepsis. -Optic neuritis. -Infusion reaction[19] - Severe congestive heart failure [7]

1.4.1 Anti TNF-α treatment

Anti-TNF-α therapies are central in current treatment strategies for IBD. However loss of response, intolerance and immunogenicity are limiting factors.

Over the past 15 years, with the introduction of anti-TNF, treatment strategies for CD and UC have changed dramatically [37]. Additionally treatment goals have also shifted to include mucosal healing, as well as clinical remission, reduction in risk of neoplasia, reduction in hospitalization and surgery and improvement in quality of life [38]. In Western Europe about 60% of patients with CD are treated with immunomodulators and 30% with biological therapies [39].

Immunomodulators such as azathioprine, mercaptopurine or methotrexate act in a generic and non-specific way. On the contrary, biologics are highly engineered proteins, which target specific inflammatory cytokines that are involved in the pathogenesis of the disease [19].

The main indication for TNF inhibitors is active moderate to severe CD or UC, which did not respond to first line therapy and are refractory or cortico-dependent. In case of moderately active disease and corticosteroid/immunosupressor refractory, anti-TNF-α is an appropriate option [40]. Also in severe colitis they show an important role. The efficacy for induction for IFX is around 60-70% and a remission rate of about 30-40% [37, 41, 42].

Indications for TNF inhibitors in CD include moderate to severe active ileal and/or colonic disease, when the patient is steroid-refractory, -dependent, or -intolerant.. TNF inhibitors are also indicated in case of fistulizing disease [41].

Adverse effect of anti-TNF-α therapy exists, such as anaphylactic reactions and infections. Rare but serious cases of, melanoma skin cancer, drug induced lupus and psoriasis have been noted[43]. However hazard ratio for serious infection for infliximab is 1.77, but prednisone is associated with double of risk of serious infection[19].

Following drugs are available

Infliximab is a chimeric monoclonal IgG1 antibody (75% human, 25% murine), which binds soluble and cell-surface TNF-α [41, 44] . It is administered intravenously: 5mg/kg at weeks (0, 2, 6) for the induction and every 8 weeks for the maintaining dose [7].

Adalimumab is a fully humanized monoclonal IgG1 antibody [41]. It is administrated subcutaneously; 160 mg at week 0 and then 80 at week 2 and finally 40mg every second week.

Certolizumab pegol is an antibody Fab' fragment conjugated with a polyethylene glycol molecule. It is administrated subcutaneously; the induction dose is 400 mg at weeks (0,2,4) then every 4 weeks [7]. Certolizumab pegol is registered in Switzerland [41]. We were not able to find any recommended blood level for this agent.

Infliximab and adalimumab are effective for induction and maintenance of remission in both CD and UC while certolizumab pegol has been approved for use in CD patients [41, 45].

Figure 3: Mode of action of anti-TNFα agents [7].

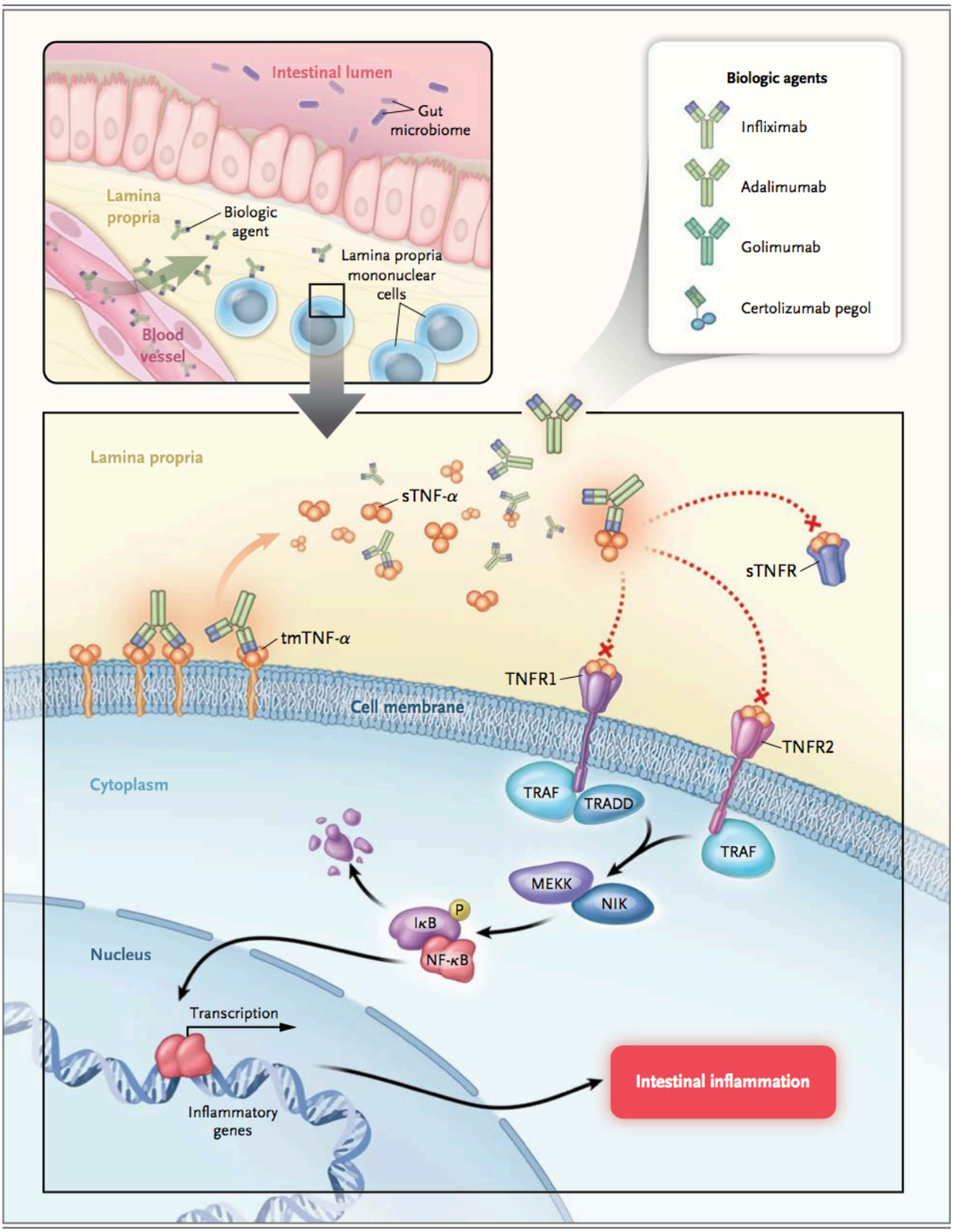


Figure 3. Anti-TNF-α agents bind the precursor transmembrane TNF- α and the soluble TNF-α, neutralizing the pro-inflammatory signaling

1.4.2 Loss of response to anti TNF-α agents and appropriate strategies

Despite significant response and remission rates with anti-TNF-α, clinicians are often confronted with primary and secondary loss of response or side effect during long-term therapy. Therefore, my master project is focused on therapeutic drug monitoring of anti TNF-α agents to optimize their use in IBD patients.

Primary non-response is observed when no clinically relevant response after the induction can be observed. Secondary non-response is observed when the clinically response is not sustained for more than 12 months. Therapeutic drug monitoring (TDM) might offer a rational approach in case of secondary non-response [37].
Monoclonal antibodies like biological drugs act like foreign antigens for the humoral immune system, which generate high affinity antibodies against biological drugs. These specific antibodies may reduce the efficacy of TNF-α antagonists [37].

Lose of response occurs approximately in 40% of the patients, who initially respond to TNF-α antagonists [46].

In case of a primary non-response anti-TNF-therapy is considered non-effective in about a third of patients for this specific case. A non-TNF pathological mechanism can be considered, so switching to another anti-TNF is not advisable [10].

There are emerging data from the literature suggesting that infliximab trough levels might help clinicians tailoring therapy and overcome on-treatment IBD flares. In fact measurement of serum drug and anti-drug-antibody (ADA) concentrations has the potential to guide health professional's decision, optimize treatment and reduce healthcare costs [47]. For IFX a reasonable target of trough level for a positive predictive value and sustained remission is 3.0-7.0 microgram/ml [47]. There are more limited data for Adalimumab, but they indicate that a concentration less than 5 micrograms/mL is seen in patients with active disease [47]

The clinical presentation of ADAs related side effects of anti-TNF includes either erythemas at the injection site or anaphylactoid reactions with hypotension, dyspnea and flushing. Serum sickness with fever, rash or arthralgia may also occur [48].

ADAs increase drug clearance, negatively influence the pharmacokinetics (PK) of anti-TNF and therefore the clinical outcome [37]. It is true, however, that ADAs are not the only element that increase the clearance of biologics. In fact low albumin levels and male sex increase the clearance too [44].

Higher Anti-TNF-alpha levels, more specifically higher IFX levels are associated with higher rate of clinical remission. Conversely, the presence of ADA has been linked to poorer disease outcome [49]: an 8 microgram/mL concentration of ADA or less is associated with few relapses than higher concentration [47].

ELISA (enzyme-linked immunosorbent assay), HMSA (homogenous mobility shift assay), RIA (radioimmunoassay) are the most commonly used methods to measure trough level drug and ADA [47]. In this study anti-TNF-a and ADA are measured using a commercially available ELISA. Trough level refers to the lowest point to which levels of a drug fall in the blood between doses. In order to have an accurate estimation, trough level should be measured just before the administration of the next dose.

Following preliminary consensus has been recommended for cases with secondary loss of response [47]:

Table 3: Adapted proposed strategy for tailored therapy with anti TNF-α agents [47].

	ADA-negative	ADA-positive
Anti-TNF < threshold	Increase dosage	Switch to another anti TNF-α
Anti- TNF ≥ threshold	Change of class of drugs Example ustekinumab **Stelara®**	Monitor the activity of the disease

The most appropriate strategy varies from patient to patient, but also from availability and cost.

If the situation permits, increasing the frequency or increasing the dose of biological drugs is an appropriate strategy before switching to another anti-TNF-α. In addition we have to remember that switching is an effective strategy, but can reduce therapeutics options the use of the former drug in the future [41].

Using IFX as an example: if the infliximab concentration is subtherapeutic and ADA level are low, doctors have the interest of increasing the dosage of infliximab. On the contrary if the patients have low concentration and detectable ADA switching to another TNF-α antagonist result with better outcomes. When the situation is a little be more complicated; trough level is high, but ADA also high, three options are available change of TNF inhibitor, addiction of immunosuppressant or change of class [44]. In patients with supratherapeutic drug concentration, who are feeling well, the possibility to reduce de dosage it is also possible [47].

During the use of biological drugs injection-site reaction and anaphylactic reactions have been reported. In this cases switching to another biological drugs is needed [41].

1.4.3 New treatment approaches

As mentioned earlier, although efficient, anti-TNF agents fail to offer any benefit in a significant proportion of patients. Over the past years, several novel pathophysiologic mechanisms of disease have been targeted for drug interventions. Overall, current treatment strategies target lymphocyte trafficking through integrin blockade (vedolizumab) or alternate cytokines such as IL12 or IL-23 (ustekinumab). [21].

2. Study aims

1. To evaluate the indications for trough anti-TNF-α and ADA serum testing
2. To determine the prevalence of ADA and the therapeutic range of anti-TNF trough level measures
3. To assess the clinical impact of trough anti-TNF-α and ADA serum levels on routine management of IBD patients

3. Study Design

This is a retrospective study of anti-TNF trough level and ADA serum determinations in IBD patients receiving anti-TNF agents. We included all therapeutic drug monitoring measurements performed from 05.03.2013 to 23.04.2014. A total of 70 patients where included between two tertiary referral centers: clinic La Source and the service of Gastroenterology of the CHUV.

Patients have been identified from blood samples (marked as "IBD") sent to the Service d'immunologie et d'allergologie, CHUV, for measurement of anti-TNF-α and ADA concentrations. A chart review to evaluate the indications for trough levels and clinical management has been performed. The whole process of data collection and analyzing data has been approved by the scientific committee of the Swiss IBD Cohort Study (SIBDCS) and also approved by our local ethics committee (VD).

Disease phenotype has been classified according to the Montreal classification as mentioned in Table 4.

Table 4: Montreal classification[50]

IBD	**Ulcerative colitis** (UC)	**Crohn's disease** (CD)
Localization	Montréal Classification Inflammation limited to the rectum (E1), Inflammation limited to the splenic flexure (E2), Inflammation extends to the proximal splenic flexure (E3)	Montréal Classification Terminal ileum (L1), Colon (L2), Ileocolon (L3), Upper GI tract (L4), Upper GI tract + distal disease (L4+L3)
Age	Montréal Classification <16 years (A1), 16-40 y (A2), >40y (A3)	Montréal Classification <16 years (A1), 17-40 y (A2), >40y (A3)
Behavior and severity	S0 Clinical remission S1 Mild UC S2 Moderate UC S3 Severe UC	Montréal phenotype classification: non-stricturing non-penetrating (B1) or stricturing (B2) and penetrating (B3) Perianal fistulae and abscesses (indicated with p) are no longer included in penetrating phenotype.

3.1 Inclusion criteria

1. Adult patients affected with CD or UC.

2. Patients for whom an anti-TNF-α trough level and / or anti- drug antibodies were performed at CHUV.

3. Current treatment by anti-TNF- α agents.

3.2 Exclusion criteria

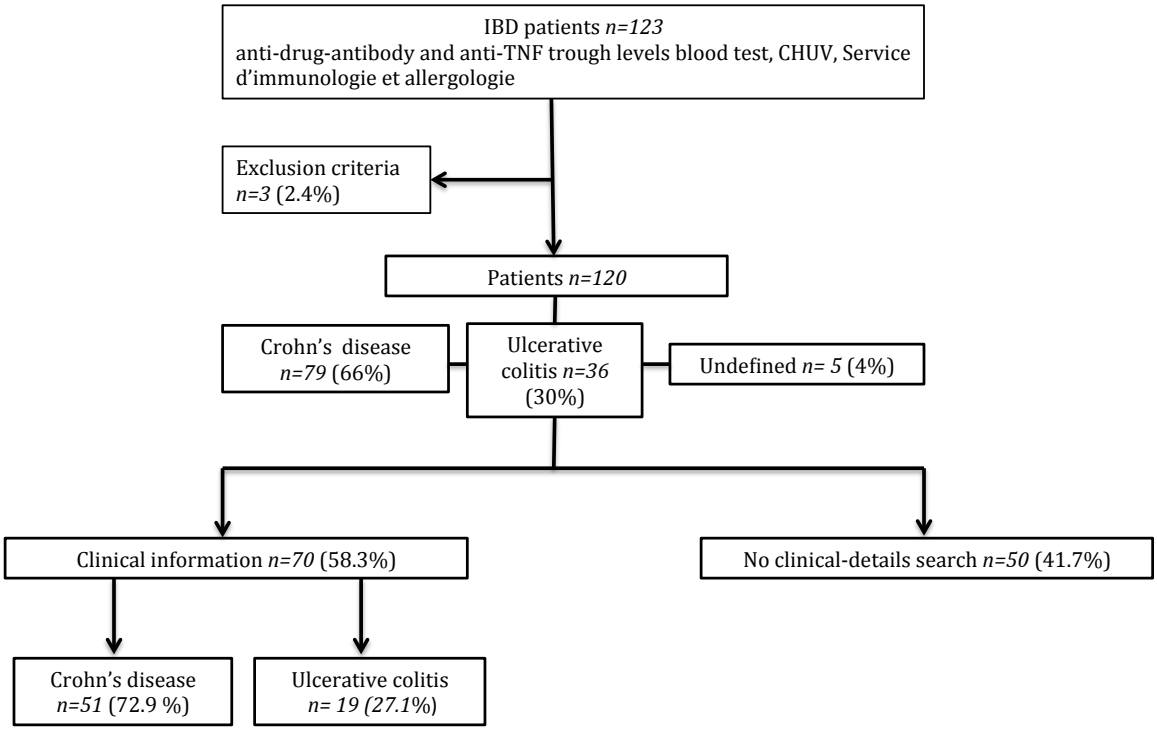
1.Not IBD diagnosed.
2. Adults patients suffering from another chronic inflammatory disease requiring treatment with anti-TNF-α such as rheumatoid arthritis.
3. Treatment with anti-TNF-α interrupted before the assays.

4. Results

4.1 Selection criteria

Between August and September 2014, I was given the opportunity to access a spreadsheet provided by the service of immunology of CHUV based on the informations given by GI doctors, containing samples from123 patients affected by IBD treated with anti-TNF-α, who underwent ADA and trough concentration testing between 1 January 2013 and 31 December 2013. Three patients were excluded because of an age of 18 or less (N=2) or because patient was not affected by an IBD (N=1). The remaining 120 patients suffered from CD (N=79), UC (N=36) or from an undefined IBD (N=5). Due to time limitation and reduced access to the clinical records, exclusively 70 cases were successfully analyzed.

Figure 4: Selection criteria.



4.2 Clinical demographic

The characteristics of the 70 patients appear in table 5. There were no male or female predominance and average age was 40 years. Most tests were performed for Crohn's disease (73%). Disease distribution was pretty homogenous with a majority of CD patients having an L3 (ileo-colonic) phenotype. A large subset of our patients had been previously managed with immunomodulators (54.29%). However, few cases were on combination therapy (10%). The mean time to initial testing and start of the biological was 19 months. The mean time between the diagnosis and testing was 72 months.

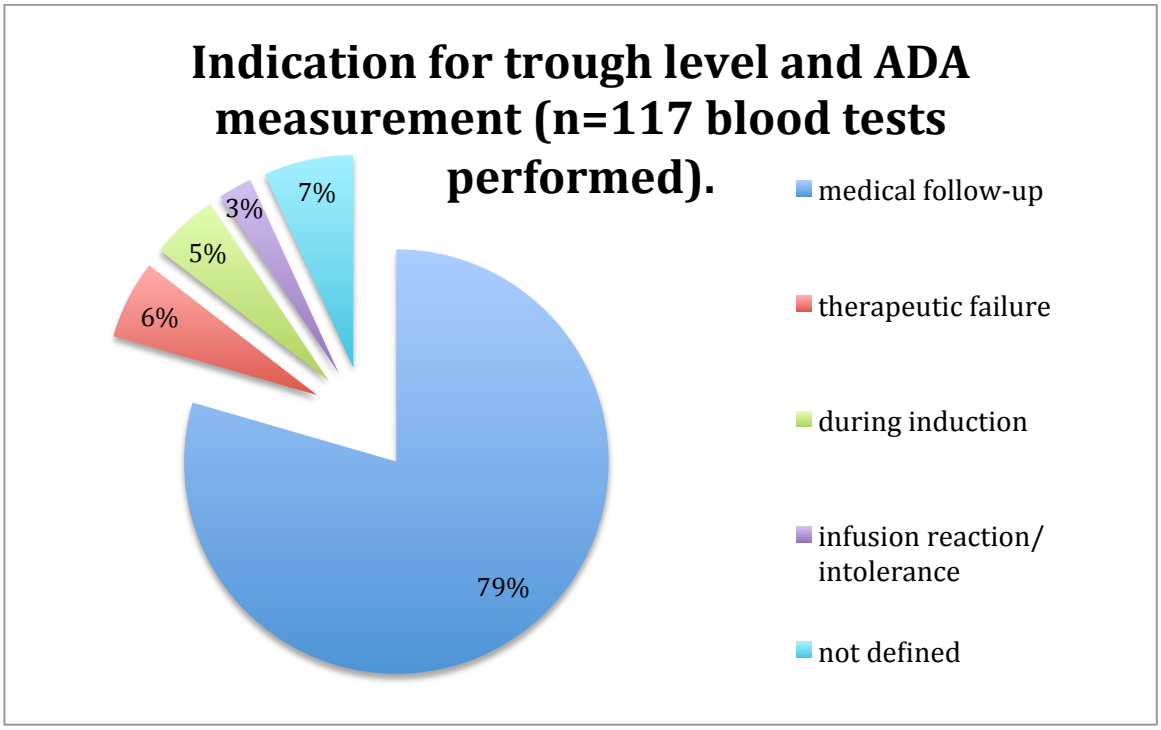
Table 5: Patients characteristics.

Patients characteristics n=70	
Female/male (% female)	33/37(47%)
Age average (range, SD)	40 (20-77; 14)
UC/CD (%CD)	19/51 (73%)
Montréal distribution	
UC	
E1	0 (0%)
E2	5 (26.32%)
E3	5 (26.32%)
Not defined	9 (47.36%)
CD	
L1	13(25.49%)
L2	9 (17.65%)
L3	20 (39.22%)
L4	1 (1.96%)
Not defined	8 (15.69%)
Smoking status	
Current smoker	19 (27.14%)
Non smoker	28
Undefined	23
Timing of assay determinations	
Time elapsed between start of biological drugs and first assay (months, range)	19 (1-104)
Time elapsed between date of diagnosis of IBD and first assay (months)	72 (5-404)
Concomitant immunomodulators	
Never use	2 (2.86%)
Current AZA	5 (7.14%)
Current 6-MP	2 (2.86%)
Current methotrexate	0
Prior use of immunomodulator	38 (54.29%)
Not defined	23 (32.86%)

3.3 Indication

The great majority (79%) of the determinations were performed as “medical follow up”. The indication pre-treatment evaluation was actively searched, but no clinicians performed the determination as “pre-treatment evaluation”. The main indication for testing was “medical follow up”. Therapeutic failure (7%) and possible autoimmune / delayed hypersensitivity reaction (5%) were further relevant indications (Figure 5).

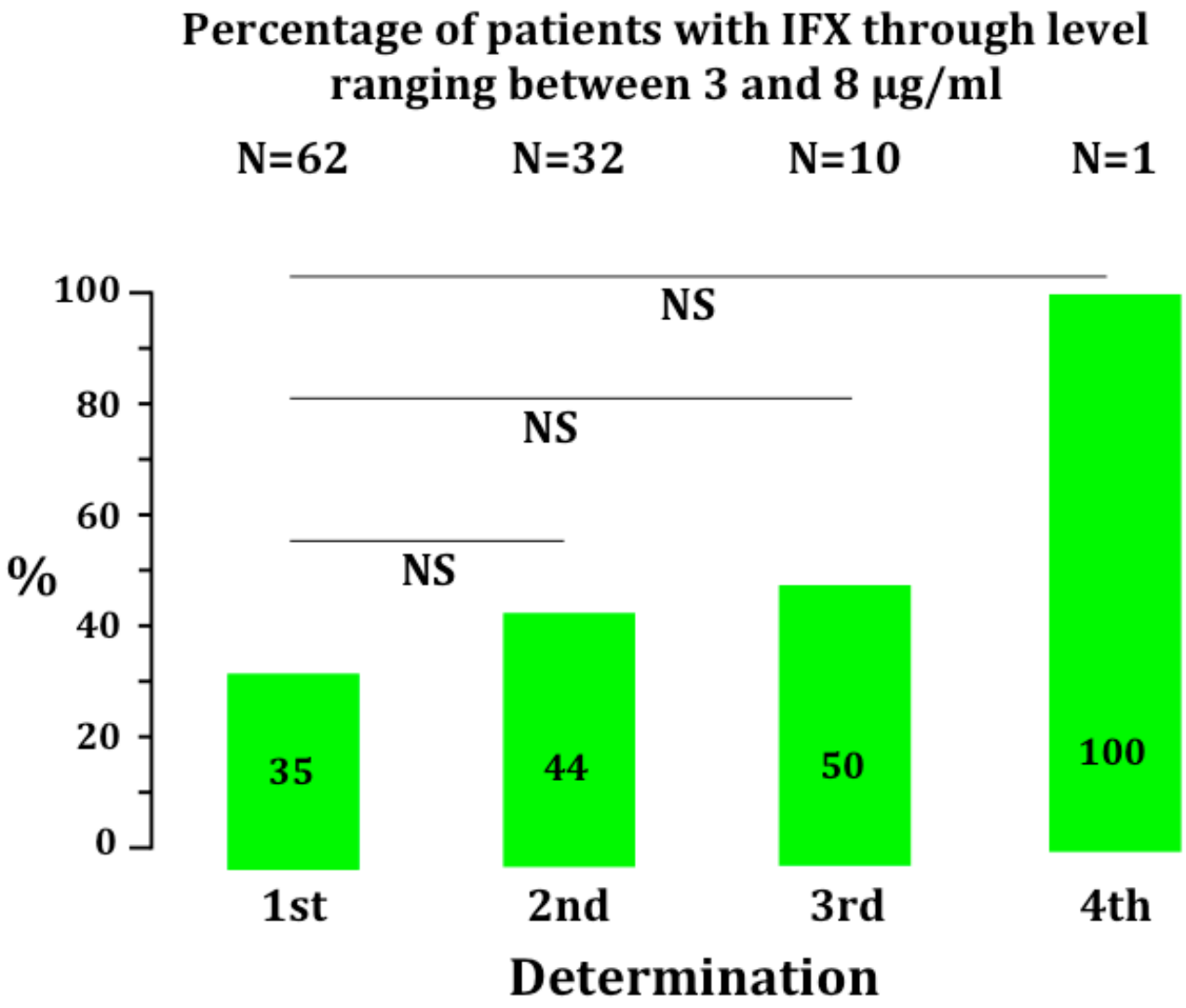
Figure 5: Indication for trough level and ADA measurement (n=117 blood tests performed).



4.4 Assays results

The percentage of patients with an IFX trough level within the desired range (between 3 and 8 µg/ml) is at first determination rather low but tends to increase during the subsequent determinations (Fig. 6). This tendency was not found to be significant (Fisher exact test). At least 100 patients should be analyzed to reach statistical significance assuming proportions of trough anti-TNF-α similar to that noted in the table.

Figure 6: Percentage of patients with IFX trough level ranging between 3 and 8 µg/ml in four determinations.



4.5 Clinical approach

Figure 7 shows the clinical management after all assays. Of 117 total tests assessed the results impacted treatment decisions in approximately 40% of the cases.

Figure 7: Clinical management after all assays. Number of assays performed (n=117).

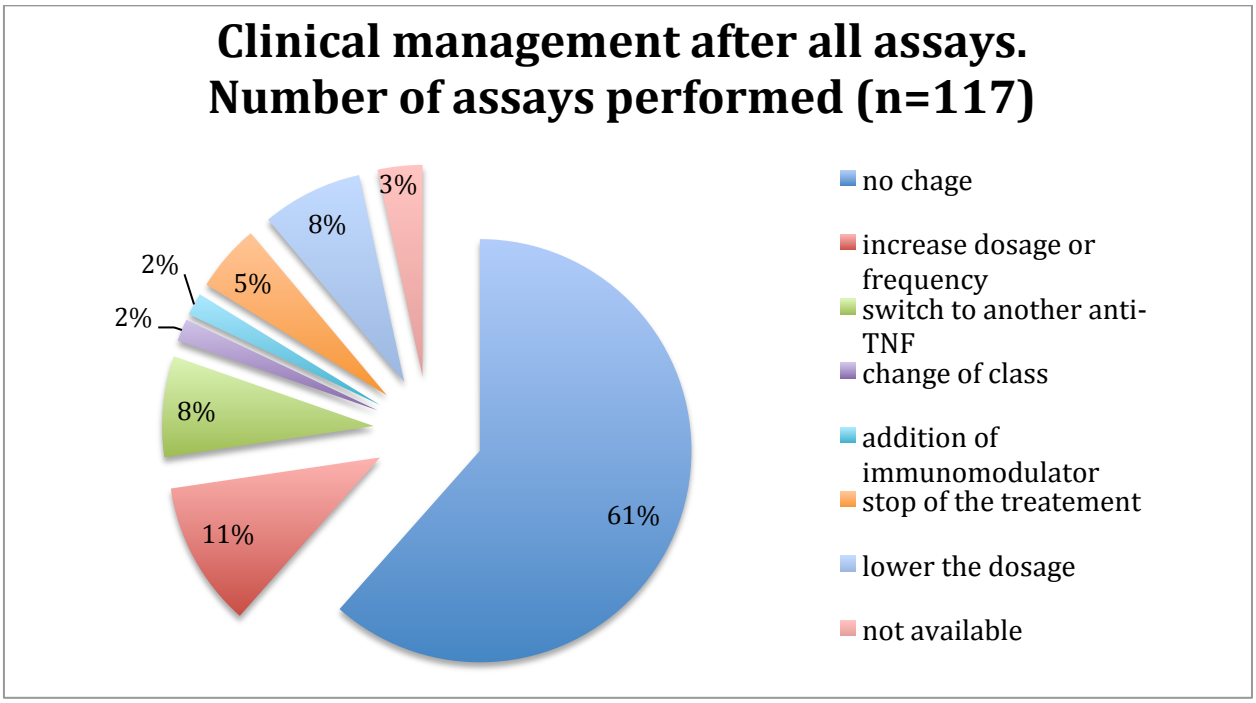


Figure 8: Clinical management

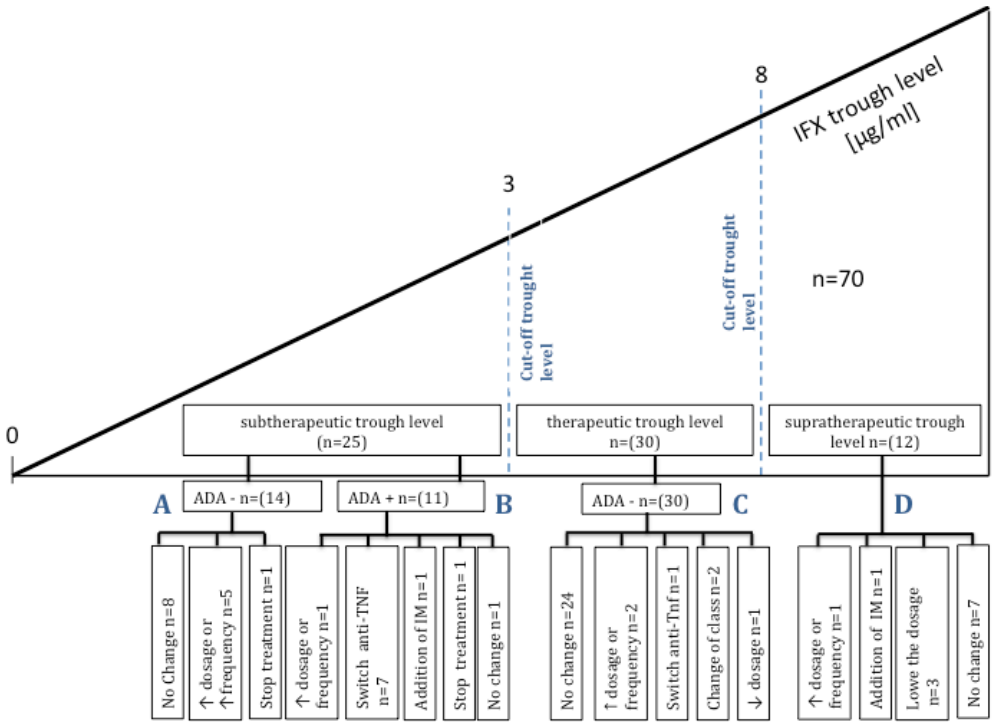


Figure 8 shows the management following the first determination trough level of anti-TNFα and ADA

This figure depicts that anti-TNFα trough level and ADA determination was followed by a change in management in approximately one third of the cases.

In 14 patients (section A) without ADA, the trough level was subtherapeutic. In no more than 5 of these patients the anti-TNFα dose or frequency were increased. In the majority of the patients the management was not adjusted (likely because that disease was not active).

7 out of 11 patients (section B) were found to have ADA and subtherapeutic trough levels the management anti-TNFα was discontinued and replaced by another one.

In 30 cases (section C) without ADA the through level was within therapeutic ranges. In 24 (80%) of these patients the management was not modified.

In 12 (Section D) patients the anti-TNFα trough level was supratherapeutic. In no more than 3 of these patients the dosage was reduced.

5.Discussion

Inflammatory bowel diseases have an important impact on the quality of life of affected patients and the increasing number of cases worldwide is a growing concern. The management of inflammatory bowel disease patients who are refractory both to treatment with first-line agents such as glucocorticoids, 5-aminosalicylates or antibiotics as well to second-line agents such as azathioprine and methotrexate is well recognized and challenging.

Well-controlled trials support the use of anti-TNF-α in the treatment of these conditions. These agents are typically highly effective for induction and maintenance of clinical remission. However, not all patients respond and a proportion of them lose response over time. Low trough circulating levels and the development of antibodies to anti-TNFα drugs are the main mechanisms that have been proposed to explain why some patients do not respond to these drugs.

Available studies indicate that measuring drug and ADA levels can guide the appropriate intervention and results in more efficient use of the drugs [29, 51-53].

a) The data collected by the group of Rochester suggest that measuring drug and ADA levels impacts treatment decision in approximately three quarters of 155 patients with loss of response or partial response after initiation of infliximab [52].

b) The report by a French group included 82 IBD patients having a disease flare while on treatment with adalimumab. The results indicate that assessing drug and ADA levels has an impact on management in approximately two thirds of the cases [53].

c) A retrospective analysis including a total of 247 pediatric and adult patients with IBD while on treatment with infliximab or adalimumab was performed in Israel. The authors of the report noted that measurement of anti-TNF drug level and ADA is useful for guiding the management of more than two thirds of patients with a loss of response to the mentioned biological [29].

d) A Belgian study including 263 IBD-patients demonstrated that targeting infliximab trough level to 3–7 mg/mL results in a more efficient use of the drug [51].

Mean age was very similar in the four [29, 51-53] aforementioned studies and in our cases (approximately 40 years). The female to male ratio was approximately 1.3 in two studies [52], [53], approximately 1.0 in the other two [29],[51] and 1.1 in our experience. Our study, performed in two tertiary referral centers, represents the first Swiss analysis addressing the clinical

utility of the determination of anti TNF-α trough level and ADA in patients with IBD. The majority of our patients were managed exclusively with biological, but without immunomodulators. The patients were tested, on average, approximately one year and a half after initiation of biological treatment. The great majority of tests (approximately 80%) were performed, similar to the Belgian study [51], for medical follow up. This fact likely indicates that many patients were on maintenance therapy. Although our study was not designed to measure disease activity at the time of trough level determination, we assume that most patients in this category were probably asymptomatic in the maintenance phase. The results indicate that clinicians consider these determinations useful and trustful, as indicated by the fact that anti TNF-α dosing was often adapted according to available recommendations (more frequently in cases with subtherapeutic trough level and ADA-positive cases than in cases with supratherapeutic trough level) [47]. The apparent discrepancies with recommendations noted in a large minority of cases likely result from the fact that clinicians' decisions are mainly based on IBD disease activity, and possibly is also influenced by co-morbidities, and socio-economical factors. We feel that after testing drug and ADA levels, clinicians may gain a sense of saliency in relation to decision-making not only in cases with poor response to biologicals but even in stable cases.

Two IBD-cases included in this survey deserve particular attention and may be used to further address the issue of monitoring anti-TNF-α through level and ADA.

A non-smoking 24-year old woman was found to suffer from CD L3. The disease failed to remit on IFX-treatment with satisfactory trough levels (>8 μg/ml) and absent ADA and later on adalimumab-treatment again with satisfactory trough levels (>8 μg/ml) and absent ADA. We feel that management with compounds with different mode of action such as natalizumab, alicaforsen or vedolizumab might be prescribed in this intriguing case.

A non-smoking 46-year old man with UC E2 on long-term treatment with IFX suddenly developed severe edema and arthritis immediately after administering this biological agent. In retrospect the patient was found to have trough anti-TNF-α level <0.3 μg/ml and ADA strongly positive. Awareness of ADA level might have prevented the potentially life threatening reaction.

Some limitations in this study deserve mention. The major limitation of the study resides in its retrospective nature. Consequently, no standardized scoring system was used to correlate the disease activity with the circulating anti TNF-α through level. Furthermore, we were sometimes not able to reconstruct the reasons underlying some clinical decisions. For example, in some situations, clinicians do not increase the dosage in patients with subtherapeutic trough level because of a low disease activity, showing that doctors have other influences on the decisions making processes. Moreover, the number of patients included in the analysis was rather low. Finally, our results, which were collected in two tertiary referral centers, might perhaps not be extrapolated for cases concomitantly treated with immunomodulators.

The results of our study, taken together with those of the literature [29, 51-53], might prompt to recommend the determination of anti-TNF-α trough and ADA level in IBD-patients. Based on those observations, we propose the following attitude with respect to on-treatment monitoring:

a) Increasing the dose of anti-TNF-α therapy is advised in patients with anti-TNF α trough levels <3 µg/ml and without ADA (an perhaps also in patients with levels <5 µg/ml)

b) Decreasing the dose of anti-TNF-α might be recommended in patients in remission and with drug level >8 µg/ml.

c) Patients developing high levels of ADA are less likely to benefit from dose intensification.

d) In patients with persisting disease activity despite therapeutic drug level near to 8 µg/ml and no anti-drug antibodies (see case presented below), it is appropriate to switch to a drug with a different mode of action.

e) Patients with very high levels of ADA are at high risk (like the male subject presented below) of severe allergic reactions.

6. Conclusion

The TDM suggested in this survey is expected to avoid repeated bouts of IBD. In particular, it is likely that TDM will soon evolve into a 3-tiered approach: it will first guide the induction; it will subsequently dictate dose titration to prevent disease flares; and finally it will guide interventions for cases with loss of response to biologicals. However, some knowledge gaps still need to be addressed to confirm this strategy. Specifically, future research needs to explore dose optimization protocols, preferably using more sensitive laboratory assays. One hopes that this will lead to refinement of personalized treatment strategies in IBD.**(54)**

Keywords:
Inflammatory bowel disease/ trough levels/ anti-TNF-α/l adapted strategy /swiss

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