Achieving Mucosal Healing in Inflammatory Bowel Diseases: Which Drug Concentrations Need to Be Targeted?

Nathalie Van den Berghe[1], Ann Gils[1] and Debby Thomas[1,*]

Biologicals introduced a major shift in the treatment of patients suffering from inflammatory bowel diseases. Despite providing a tight disease control for many patients, a considerable proportion of patients will fail to respond favorably to treatment or will lose response over time. Therapeutic drug monitoring emerged as a valuable tool to guide clinical decision making as serum drug concentrations have been linked to outcomes. Focusing on mucosal healing as the ultimate treatment goal, different drug concentration thresholds to achieve this outcome have been identified in the literature and are summarized in this review. For therapeutic drug monitoring to be successful in guiding clinical decision making, the used assay, the sampling time point, and the outcome that is aimed for should be taken into account when interpreting drug concentration thresholds. Awareness of these essential aspects among clinicians will improve the implementation of therapeutic drug monitoring and aid in making an evidence-based decision.

Inflammatory bowel diseases (IBDs), the two main subtypes being Crohn's disease (CD) and ulcerative colitis (UC), are chronic inflammatory disorders of the gastrointestinal tract typically characterized by a relapsing-remitting disease course.[1] These diseases usually start early in life, thereby strongly affecting the quality of life and work productivity of young individuals. As IBD remains incurable, patients have to be treated lifelong, which requires cost-effective, tolerable, and safe therapies.

Over the past decades, advances in the understanding of the pathophysiology of these diseases have greatly expanded the treatment options. Next to the classical medications, including 5-aminosalicylic acid, corticosteroids, and immunomodulators, patients can also be treated with biologicals targeting key mediators in this immune-mediated inflammatory disease.[2]

The first biologicals approved for the treatment of IBD were monoclonal antibodies targeting tumor necrosis factor (TNF)-α (infliximab, adalimumab, golimumab, and certolizumab pegol). More recently, biologicals directed against integrins (natalizumab and vedolizumab) and IL-12/23 (ustekinumab) emerged as valuable treatment options (**Table 1**). These drugs introduced a major shift in the treatment of patients suffering from IBD because they have not only shown to reduce symptoms but also avoid the chronic need for steroids, heal mucosal ulcers, and reduce hospitalizations and surgical interventions.[3]

THERAPEUTIC DRUG MONITORING

Biologicals have emerged as an important cornerstone in the treatment of patients with IBD. Nevertheless, the efficacy of these drugs can highly differ between individuals: some patients do not respond to treatment (primary nonresponders), whereas others initially respond to treatment but lose response over time (secondary nonresponders). For anti-TNF drugs, primary and secondary nonresponse rates in patients with IBD have been reported to be 10–30% and 20–40%, respectively.[4,5] Although real-life data for the newer anti-integrin and anti-interleukin drugs is limited and quite variable, similar nonresponse rates as for the anti-TNF drugs have been observed.[6,7]

An inadequate treatment response can be caused by pharmacokinetic or pharmacodynamic issues.[8,9] For instance, treatment failure can be the result of insufficient drug exposure because of an increased clearance due to a high inflammatory disease burden or the presence of neutralizing and/or non-neutralizing antidrug antibodies.[10,11] Alternatively, mechanistic failure can be the reason a patient does not adequately respond to treatment. Mechanistic failure implies that other molecules involved in the inflammatory pathway are the main drivers of the disease rather than the target inhibited by the biological.

Therefore, therapeutic drug monitoring, the measurement of drug concentrations in the blood of the patient, can shed light on what is happening with the drug in the patient and objectively evaluate potential reasons for treatment failure.[12] Currently, a trial-and-error approach is used in which clinicians blindly increase the dose or switch to another drug when a patient is not adequately responding to therapy. On the one hand, this inefficient approach leads to underexposed patients who are unnecessarily switched to another drug instead of optimizing treatment. On the other hand, some patients might be sufficiently exposed and will only benefit from switching to another drug. Therapeutic drug monitoring offers a solution to this problem by distinguishing between lack of response caused by insufficient exposure (subtherapeutic drug

[1]Laboratory for Therapeutic and Diagnostic Antibodies, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Leuven, Belgium.
*Correspondence: Debby Thomas (debby.thomas@kuleuven.be)

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Table 1 Biologicals approved for the treatment of IBDs in the EU and/or the US

Biological	Target	Disease	Route of administration	Dosing schedule
Infliximab	TNF-α	CD/UC	i.v.	5 mg/kg at week 0, week 2, week 6, then every 8 weeks
Adalimumab	TNF-α	CD/UC	s.c.	CD: 80 or 160 mg at week 0, 40, or 80 mg at week 2, then 20 or 40 mg every 2 weeks
				UC: 160 mg at week 0, 80 mg at week 2, then 40 mg every 2 weeks
Golimumab	TNF-α	UC	s.c.	200 mg at week 0, 100 mg at week 2, then 50 mg every 4 weeks if weight < 80 kg (EU) or 100 mg every 4 weeks if weight ≥80 kg (EU and US)
Certolizumab pegol	TNF-α	CD	s.c.	400 mg at week 0, week 2, week 4, then 400 mg every 4 weeks
Natalizumab[a]	α4 integrin	CD	i.v.	300 mg at week 0, then every 4 weeks
Vedolizumab	α4β7 integrin	CD/UC	i.v.	300 mg at week 0, week 2, week 6, then every 8 weeks
Ustekinumab	IL-12/23	CD	First i.v., then s.c.	i.v. infusion of 260 mg if weight ≤ 55 kg, 390 mg if weight 55–85 kg, 520 mg if weight > 85 kg at week 0, then 90 mg s.c. every 8 weeks

CD, Crohn's disease; EU, European Union; IBD, inflammatory bowel disease; i.v., intravenous; s.c., subcutaneous; TNF-α, tumor-necrosis factor-alpha; UC, ulcerative colitis; US, United States.
[a]Only approved in the United States.

concentrations) and lack of response caused by mechanistic failure (therapeutic drug concentrations).[13] Together with a clinical evaluation, measured drug concentrations can help the clinician to define the next treatment step and maximize treatment outcomes.

PHARMACOKINETIC CONSIDERATIONS

In most cases, patients with IBD are treated with a fixed dose of the biological. The concentration of the biological in the blood circulation, however, will differ between patients as it is dependent on absorption, distribution, and elimination of the drug (**Figure 1**). For intravenous administered drugs, the systemic availability is 100% and the peak concentration will be reached within minutes after the end of the infusion.[14] In contrast, the peak concentration of a biological that is administered subcutaneous will only be reached 2–8 days after the injection and will not achieve a peak concentration as high as intravenous administered biologicals.[14]

Even when a biological is administered through the same route, the concentration-time profile can differ remarkably between patients, resulting in a different total exposure to the drug. In some patients, the drug is rapidly absorbed and the peak concentration occurs within 2 days. In other patients, absorption of the drug is slow and the maximum concentration will

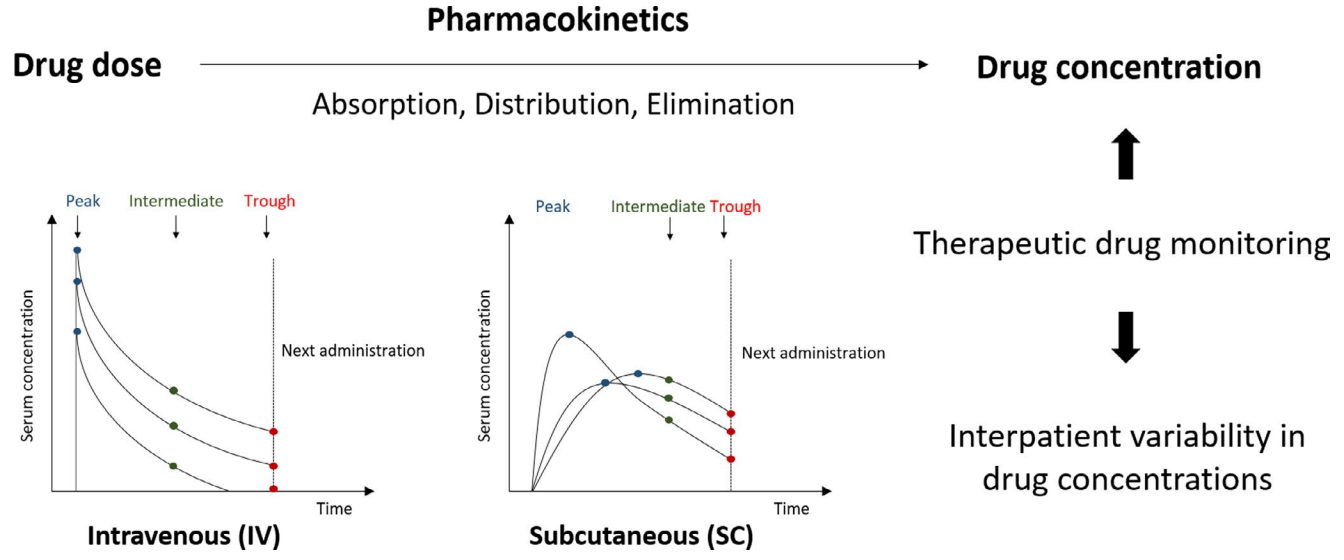

Figure 1 Pharmacokinetic considerations of biologicals. The concentration of the biological in the blood circulation is dependent on absorption, distribution, and elimination of the drug. The concentration-time profile differs between intravenous and subcutaneous administered drugs. Peak concentrations are indicated as blue dots, intermediate concentrations as green dots, and trough concentrations at red dots. Drug concentrations differ between patients (interpatient variability) and within a patient (intrapatient variability).

only be reached several days later. Alternatively, the drug concentration can decline more rapidly in one patient compared with another due to different elimination rates leading to lower drug concentrations at a specific time point. Furthermore, even within one patient the drug concentration at a fixed time point after the drug administration can differ between two dosing intervals. This high interpatient and intrapatient variability in drug concentrations underlines the need for therapeutic drug monitoring.

As the concentration of the drug changes over time, the time of sampling is important. Most frequently, a sample is collected at trough (**Figure 1**, in red), which is the lowest concentration, and in case of intravenous administered drugs, this is exactly before the next administration. For subcutaneous administered drugs, this is not necessarily the case as the concentration of the drug in the blood can further decrease after a new injection, until the new dose is sufficiently absorbed to increase the concentration again. However, for reasons of simplicity, sampling at trough always refers to sampling just before the next administration. Furthermore, trough is stated to be the least variable time point in the dosing interval as it represents the different pharmacokinetic phases, including absorption, distribution, and elimination since its last administration.[15] Alternatively, a sample can be obtained at an intermediate time point (**Figure 1**, in green), in between two administrations, where the drug concentration will be higher than at trough but lower than at peak. Depending on the sampling time point, different pharmacokinetic information can be acquired. A sample collected at trough can give more information on the elimination of drug but less on the absorption or the volume of distribution.[12] Precise estimations of the absorption rate and distribution volume typically require sampling at peak and intermediate time points. For example, a recent study showed that 18 of 26 patients with CD had detectable adalimumab serum concentrations 2 hours after administration of 160 mg adalimumab, which gives an indication on the rate of absorption.[16]

Drug concentrations are most frequently measured in serum as collecting blood is convenient and minimally invasive. However, the target site of the drug is the intestinal mucosa, and drug concentrations measured in serum are consequently considered as surrogate markers. Only one study evaluated drug concentrations in intestinal tissue and observed a positive correlation between anti-TNF in serum and tissue, especially in uninflamed but not in inflamed tissue.[17]

DRUG CONCENTRATION THRESHOLDS TO ACHIEVE ENDOSCOPIC OUTCOMES

With the introduction of biologicals, the therapeutic goal shifted from controlling symptoms and achieving clinical remission to inducing and maintaining mucosal healing.[18] Mucosal healing has shown to greatly improve patient outcomes as it is associated with sustained clinical remission, fewer surgeries and hospitalizations, reduced risk of relapse, and improved ability to work.[19,20] At the present time, current and novel therapeutics are evaluated based on their ability to improve endoscopic outcomes, ultimately aiming at achieving mucosal healing. Therefore, many efforts have been made to determine the minimum drug concentration necessary to achieve mucosal healing. This review will focus on the concentration thresholds of biologicals necessary to attain endoscopic outcomes (**Tables 2** and **3**), starting with the anti-TNF drugs followed by the newer anti-integrin and anti-interleukin biologicals. Because drug concentration thresholds of biologicals in pediatric patients with IBD have not yet been reported in the literature, only studies with adult patients with IBD will be considered.

Infliximab

As infliximab has been approved for the treatment of CD since 1998 by the US Food and Drug Administration and since 1999 by the European Medicines Agency,[21,22] the exposure–response relationship has already extensively been examined. Rather than merely being associated with clinical and biological remission, adequate infliximab concentrations have also been linked to improved endoscopic outcomes.

The first studies showed that detectable trough serum infliximab concentrations were associated with higher rates of endoscopic improvement in both patients with CD and patients with UC.[23,24] Several years later, a *post hoc* analysis of the ACT-1 and ACT-2 trials, which included 728 patients with UC, revealed that higher serum infliximab concentrations were associated with higher rates of mucosal healing at weeks 8, 30, and 54.[25]

More recently, research groups started determining drug concentration thresholds necessary to achieve mucosal healing through receiver-operator characteristics curve analysis. In a retrospective observational study including 78 patients with IBD (53 with CD and 25 with UC), infliximab serum concentrations during maintenance treatment were higher in patients achieving mucosal healing (4.3 μg/mL) vs. patients with endoscopically active disease (1.7 μg/mL).[26] The optimal infliximab threshold concentration associated with mucosal healing was determined to be 5 μg/mL. Incremental gain analysis revealed that the percentage of patients achieving mucosal healing increased with increasing infliximab serum concentrations and reached a near-plateau at 8 μg/mL. Based on these findings, the authors proposed a therapeutic window for infliximab of 6–10 μg/mL aiming for mucosal healing in 80–90% of patients with IBD.

Regarding infliximab induction therapy, several research groups have made an effort to determine drug concentration thresholds associated with endoscopic outcomes, primarily focusing on short-term mucosal healing. In a retrospective study including 54 patients with UC, infliximab concentrations at weeks 2, 6, and 14 were significantly higher in patients achieving mucosal healing at weeks 10–14 compared with those not achieving mucosal healing (22.9 vs. 19.3 μg/mL; 17.6 vs. 10.3 μg/mL; and 7.4 vs. 1.5 μg/mL, respectively).[27] Infliximab concentration thresholds associated with mucosal healing were 28.3, 15, and 2.1 μg/mL, respectively. A similar threshold of 2.1 μg/mL was observed by Van Stappen *et al.*[28] when measuring infliximab concentrations at week 14 in 190 samples from 29 patients with UC using a rapid, lateral flow-based assay.

When considering a less ambitious outcome, Brandse *et al.*[29] observed that median week 6 infliximab concentrations were significantly higher in endoscopic responders (8.1 μg/mL) compared

Table 2 Drug concentration thresholds of anti-TNF biologicals to achieve endoscopic outcomes

Drug	Disease	Time point	Outcome	Drug concentration (μg/mL)		Threshold concentration (μg/mL)	Sensitivity; specificity	PPV; NPV	Drug assay	Ref.
				Responders	Nonresponders					
Infliximab	UC	Week 2	Mucosal healing (weeks 10–14)	22.9	19.3	28.3	–	–	ELISA	27
Infliximab	UC	Week 2	Mayo endoscopic subscore ≤ 1 (week 8)	–	–	18.6	87%; 25%	63%; 56%	ELISA	30
Infliximab	UC	Week 6	Mucosal healing (weeks 10–14)	17.6	10.3	15.0	–	–	ELISA	27
Infliximab	UC	Week 6	Endoscopic response (week 8)	8.1	2.9	6.6	88%; 73%	–	ELISA	29
Infliximab	UC	Week 6	Mayo endoscopic subscore ≤ 1 (week 8)	–	–	10.6	85%; 37%	69%; 60%	ELISA	30
Infliximab	UC	Week 8	Mayo endoscopic subscore ≤ 1 (week 8)	–	–	34.9	72%; 53%	74%; 52%	ELISA	30
Infliximab	UC	Week 14	Mucosal healing (weeks 10–14)	7.4	1.5	2.1	–	–	ELISA	27
Infliximab	UC	Week 14	Mucosal healing (weeks 10–14)	–	–	2.1	100%; 50%	–	LFA	28
Infliximab	UC	Week 14	Mayo endoscopic subscore ≤ 1 (week 30)	–	–	5.1	67%; 63%	72%; 56%	ELISA	30
Infliximab	UC	Week 14	Mayo endoscopic subscore = 0 (week 30)	–	–	6.7	62%; 63%	46%; 77%	ELISA	30
Infliximab	UC	Week 30	Mayo endoscopic subscore ≤ 1 (week 30)	–	–	2.3	74%; 60%	79%; 55%	ELISA	30
Infliximab	UC	Week 30	Mayo endoscopic subscore = 0 (week 30)	–	–	3.8	75%; 60%	53%; 80%	ELISA	30
Infliximab	CD/UC	Maintenance	Mucosal healing (at same time point)	4.3	1.7	5.0	39%; 85%	70%; 62%	ELISA	26
Adalimumab	UC	Week 4	Mucosal healing (weeks 8–14)	10.6	7.4	9.4	67%; 77%	50%; 87%	ELISA	35
Adalimumab	CD/UC	Maintenance	Mucosal healing (at same time point)	6.5	4.2	4.9	66%; 85%	88%; 51%	ELISA	32
Adalimumab	CD/UC	Maintenance	Mucosal healing (at same time point)	6.2	3.1	7.1	32%; 85%	51%; 72%	ELISA	26
Adalimumab	CD	Maintenance	Mucosal healing (at same time point)	14.7	3.4	8.1	91%; 76%	84%; 86%	MSA	33
Adalimumab	CD/UC	Maintenance	Endoscopic healing (at same time point)	13.3	8.5	7.5	62%; 83%	–	MSA	34
Golimumab	UC	Week 6	Mucosal healing (week 6)	3.14	1.70	–	–	–	ECLIA	36
Golimumab	UC	Week 6	Mucosal healing (weeks 30 and 54)	1.22	0.83	–	–	–	ECLIA	36
Golimumab	UC	Week 6	Mucosal healing (week 14)	6.3	3.3	–	–	–	ELISA	37
Golimumab	UC	Week 6	Mucosal healing (week 14)	7.6	4.7	7.4	92%; 57%	80%; 80%	ELISA	39
Certolizumab pegol	CD	Week 8	Endoscopic response (week 10)	19.8	11.5	–	–	–	ELISA	41
Certolizumab pegol	CD	Week 8	Endoscopic remission (week 10)	19.2	12.6	–	–	–	ELISA	41
Certolizumab pegol	CD	Week 8	Endoscopic response (week 54)	28.4	23.4	–	–	–	ELISA	41
Certolizumab pegol	CD	Week 8	Endoscopic remission (week 54)	38.1	19.0	–	–	–	ELISA	41

CD, Crohn's disease; ECLIA, electrochemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; LFA, lateral flow assay; MSA, mobility shift assay; NPV, negative predictive value; PPV, positive; predictive value; TNF, tumor necrosis factor; UC, ulcerative colitis.

Table 3 Drug concentration thresholds of anti-integrin and anti-interleukin biologicals to achieve endoscopic outcomes

Drug	Disease	Time point	Therapeutic outcome	Drug concentration (μg/mL)		Threshold concentration (μg/mL)	Sensitivity; specificity	PPV; NPV	Drug assay	Ref.
				Responders	Nonresponders					
Vedolizumab	UC	Week 2	Mucosal healing (week 14)	31.7	24.3	28.9	62%; 73%	59%; 75%	ELISA	13
Vedolizumab	CD/UC	Week 2	Endoscopic remission (week 52)	24.8	20.0	23.2	–	–	MSA	47
Vedolizumab	CD/UC	Week 6	Mucosal healing (within the first year)	26.8	15.1	18.0	88%; 67%	79%; 80%	ELISA	45
Vedolizumab	CD/UC	Week 6	Mucosal healing (between weeks 14 and 54)	41.7	20.8–26.0	–	–	–	ELISA	46
Vedolizumab	CD/UC	Week 6	Endoscopic remission (week 52)	25.0	17.0	19.8	–	–	MSA	47
Vedolizumab	UC	Week 14	Mucosal healing (week 14)	14.6	9.6	13.9	54%; 85%	48%; 88%	ELISA	13
Vedolizumab	CD	Week 22	Mucosal healing (week 22)	17.4	10.3	13.6	71%; 69%	83%; 52%	ELISA	13
Ustekinumab	CD	Week 4	Endoscopic response (week 24)	23.0	19.6	16.6	88%; 45%	32%; 93%	ELISA	50
Ustekinumab	CD	Week 8	Endoscopic response (week 24)	8.5	6.4	5.0	82%; 45%	30%; 90%	ELISA	50
Ustekinumab	CD	Week 16	Endoscopic response (week 24)	3.4	2.2	2.3	77%; 54%	34%; 88%	ELISA	50
Ustekinumab	CD	Week 24	Endoscopic response (week 24)	2.4	1.8	1.9	67%; 52%	34%; 85%	ELISA	50
Ustekinumab	CD	Week ≥ 26	Endoscopic response (week ≥ 26)	4.7	3.8	4.5	67%; 70%	–	MSA	49

CD, Crohn's disease; ELISA, enzyme-linked immunosorbent assay; MSA, mobility shift assay; NPV, negative predictive value; PPV, positive predictive value; UC, ulcerative colitis.

with endoscopic nonresponders (2.9 μg/mL). At that time point during induction, a serum infliximab concentration higher than 6.6 μg/mL was identified as the threshold for endoscopic response.

Based on data of 484 patients with UC from two randomized controlled trials, Vande Casteele *et al.*[30] identified infliximab concentration thresholds associated with endoscopic outcomes during induction and maintenance therapy. The evaluated endoscopic outcome was either Mayo Clinical endoscopic subscore equal to 0 or Mayo Clinical endoscopic subscore ≤ 1. An infliximab serum concentration of 18.6 μg/mL at week 2 and 10.6 μg/mL at week 6 were associated with a week 8 Mayo Clinic endoscopic subscore ≤ 1. An infliximab serum concentration of 5.1 μg/mL at week 14 and 2.3 μg/mL at week 30 were associated with a week 30 Mayo Clinic endoscopic subscore ≤ 1, whereas higher concentrations of 6.7 μg/mL and 3.8 μg/mL, respectively, were associated with a subscore equal to 0. Additionally, the authors identified an infliximab concentration threshold at an intermediate time point. An infliximab serum concentration of 34.9 μg/mL at week 8 was associated with a week 8 Mayo Clinic endoscopic subscore ≤ 1.

Recently, a population pharmacokinetic–pharmacodynamic model that predicts the proportion of patients with UC with mucosal healing after infliximab induction therapy was developed based on 583 samples from 204 patients with UC.[31] The model estimated an infliximab trough concentration at day 14 of 18.8, 35.1, and 49.8 μg/mL to predict mucosal healing in 55%, 70%, and 75% of patients, respectively.

Adalimumab

Also for adalimumab, multiple studies have evaluated the association between drug serum concentrations and endoscopic outcomes, most of them not specifically looking at a certain time point during treatment but a mixture of time points in maintenance at trough.

In a cohort of 40 patients with IBD (22 with CD and 18 with UC), the median adalimumab trough concentrations were significantly higher in patients with mucosal healing compared with those without (6.5 vs. 4.2 μg/mL).[32] The absence of mucosal healing was associated with a maintenance trough concentration of adalimumab < 4.9 μg/mL. In accordance with these results, Ungar *et al.*[26] observed significantly higher median adalimumab concentrations in patients with mucosal healing than patients with active inflammation based on endoscopy (6.2 vs. 3.1 μg/mL, respectively) in a larger cohort of 67 patients with IBD (58 with CD and 9 with UC). Compared with the previous study,[32] a somewhat higher threshold of 7.1 μg/mL necessary to achieve mucosal healing was identified with a relatively low sensitivity of 32%. Through incremental gain analysis, the authors showed that the percentage of mucosal healers increased with increasing adalimumab serum concentrations, reaching a plateau at 12 μg/mL. Therefore, a therapeutic window of 8–12 μg/mL aiming at 80–90% of patients with IBD achieving mucosal healing was proposed.

In a study that only included patients with CD, a higher adalimumab trough concentration was significantly associated with mucosal healing (14.7 μg/mL in those with mucosal healing vs. 3.4 μg/mL in those without).[33] A cutoff of 8.1 μg/mL discriminated patients with mucosal healing best from those without. Alternatively, Yarur *et al.*[34] evaluated the association between adalimumab drug

concentrations and endoscopic healing when serum sampling was random and not only at trough. This study design reflects a real-life clinical setting as collecting serum just before the drug administration is not always possible. In this cohort of 66 patients with IBD (59 with CD and 7 with UC), mean random adalimumab concentrations were significantly lower in patients with endoscopic inflammation than in those without (8.5 vs. 13.3 μg/mL, respectively) and the adalimumab concentrations threshold best associated with endoscopic healing was 7.5 μg/mL.

In a cohort of 43 patients with UC, Papamichael *et al.*[35] investigated the optimal adalimumab concentration at week 4 to achieve short-term mucosal healing (e.g., within weeks 8–14). Patients with short-term mucosal healing had higher adalimumab concentrations at week 4 compared with patients without (10.6 μg/mL vs. 7.4 μg/mL, respectively). A threshold of 9.4 μg/mL was identified to achieve short-term mucosal healing. Interestingly, when the authors excluded the 5 patients who previously failed infliximab due to primary nonresponse, the threshold lowered to 7.5 μg/mL.

Golimumab

Only limited studies have evaluated the exposure–response relationship of golimumab in patients suffering from UC. *Post hoc* analyses of the PURSUIT study revealed that the proportion of patients achieving mucosal healing generally increased with increasing golimumab concentrations.[36] Moreover, golimumab serum concentrations at week 6 were higher in patients achieving mucosal healing at week 6 (3.14 vs. 1.70 μg/mL) and at both weeks 30 and 54 (1.22 vs. 0.83 μg/mL). No thresholds to achieve mucosal healing were identified in this cohort.

Detrez *et al.*[37] also observed a difference in week 6 golimumab concentrations in patients with mucosal healing (6.3 μg/mL) compared with patients not achieving mucosal healing at week 14 (3.3 μg/mL). However, this difference was not statistically significant, which is most likely due to the small sample size and low mucosal healing rate (4 of 21 patients). In another study of Detrez *et al.*,[38] the decrease in golimumab trough concentration from weeks 2 to 10 was steeper in patients without mucosal healing at 14 weeks of therapy than in patients with mucosal healing, further indicating the association between golimumab exposure and mucosal healing.

Recently, a population pharmacokinetic model was developed based on 631 samples of 56 patients with UC to investigate the relationship between golimumab exposure during induction therapy and mucosal healing.[39] Patients achieving mucosal healing had a higher model-predicted golimumab concentration at week 6 (7.6 μg/mL) compared with patients not achieving mucosal healing (4.7 μg/mL). The authors were the first to identify a golimumab threshold associated with endoscopic outcome and revealed that a golimumab concentration of 7.4 μg/mL at week 6 was the optimal concentration to achieve mucosal healing at week 14.

Certolizumab pegol

In a pooled analysis of nine clinical trials of certolizumab pegol in CD, an association between drug concentrations and clinical response and remission has been observed,[40] but studies concerning the relationship with endoscopic outcomes are fairly limited.

In a study by Colombel *et al.*,[41] higher concentrations of certolizumab pegol at week 8 were observed in patients with endoscopic response (19.8 vs. 11.5 μg/mL in responders and nonresponders, respectively) and remission (19.2 vs. 12.6 μg/mL in remitters and nonremitters, respectively) at week 10. Additionally, quartile analysis revealed that the percentage of patients achieving endoscopic response and remission increased with higher certolizumab pegol concentrations. In addition, when considering maintenance phase, the rates of endoscopic response and remission at week 54 correlated with plasma concentrations of certolizumab pegol at week 8 (28.4 vs. 23.4 μg/mL for response vs. nonresponse and 38.1 vs. 19.0 μg/mL for remission vs. nonremission). Despite the performed receiver-operator characteristics curve analyses, no certolizumab concentration thresholds to achieve endoscopic outcomes were reported in this study.

Natalizumab

To this day, no studies have been performed to investigate the exposure–response relationship of natalizumab in patients with CD. However, natalizumab is only approved for the treatment of CD in the United States and not in the European Union, limiting the interest of therapeutic drug monitoring of natalizumab.

Vedolizumab

More and more evidence regarding the exposure–response relationship of vedolizumab in both patients with CD and patients with UC is emerging. *Post hoc* analyses of the GEMINI1 trial revealed that median vedolizumab trough serum concentrations at week 6 were lower in patients with UC with higher endoscopic subscores.[42] Moreover, there was a trend toward higher rates of deep remission at week 52 with higher vedolizumab trough steady-state serum concentrations.[43] In contrast, a study by Badr Al-Bawardy *et al.*[44] did not observe a correlation between vedolizumab serum concentrations and mucosal healing in either patients with UC or patients with CD. The pooling of different time points in which vedolizumab concentrations were measured could account for the lack of this association.

In a large retrospective cohort including 179 patients with IBD (113 with CD and 66 with UC), vedolizumab trough concentrations at weeks 2 and 14 were significantly higher in patients who achieved mucosal healing at week 14 compared with those not achieving this outcome (31.7 vs. 24.3 μg/mL and 14.6 vs. 9.6 μg/mL, respectively).[13] Moreover, higher vedolizumab trough concentrations during induction predicted better endoscopic outcomes in patients with UC and CD. A trough concentration above 28.9 μg/mL at week 2 and 13.9 μg/mL at week 14 predicted mucosal healing at week 14 in patients with UC. In patients with CD, a vedolizumab trough concentration above 13.6 μg/mL at week 22 was identified as the minimal exposure to achieve endoscopic response at the same time point.

In accordance with these results, Yacoub *et al.*[45] observed higher vedolizumab concentrations at week 6, but not at weeks 2 or 14, in patients achieving mucosal healing compared with patients not achieving mucosal healing within the first year of treatment (26.8 vs. 15.1 μg/mL, respectively). A threshold of 18 μg/mL was identified as the optimal vedolizumab concentration at week 6 to

reach mucosal healing. A similar concentration–response relationship was observed in a study by Liefferinckx *et al.*[46] with overall higher vedolizumab concentrations. At week 6, patients achieving mucosal healing between weeks 14 and 54 had a higher vedolizumab concentration (41.7 μg/mL) compared with patients with mild (26 μg/mL) or severe endoscopic activity (20.8 μg/mL). No thresholds associated with mucosal healing were identified in this study.

Recently, Yarur *et al.*[47] evaluated the association between vedolizumab concentrations during induction and long-term endoscopic remission. In this cohort of 55 patients with IBD (25 with CD and 30 with UC), higher concentrations of vedolizumab at weeks 2 and 6 were noted in patients with endoscopic remission (24.8 vs. 20.0 μg/mL and 25 vs. 17 μg/mL in remitters and nonremitters, respectively) at week 52. A cutoff higher than 23.2 and 19.8 μg/mL at weeks 2 and 6, respectively, discriminated patients with endoscopic remission best from those without.

Ustekinumab

Data concerning ustekinumab, the most recently approved biological for the treatment of CD, are limited but encouraging. In the *post hoc* analysis of the UNITI trials, more patients achieving clinical remission, endoscopic response, and endoscopic remission were noted in the higher ustekinumab concentration quartiles.[48] The ustekinumab targets for clinical remission during maintenance ranged from 0.8–1.4 μg/mL, but no concentration range was identified for endoscopic outcomes.

In an observational study including 62 patients with CD, the ustekinumab trough concentration at week ≥ 26 was significantly higher in patients with an endoscopic response compared with those without (4.7 vs. 3.8 μg/mL).[49] The optimal ustekinumab week 26 threshold concentration associated with endoscopic response was determined to be 4.5 μg/mL. Although no cutoff was identified for mucosal healing, a trend was observed toward higher rates of endoscopic remission in patients with ustekinumab concentrations higher than 4.5 μg/mL compared with patients with concentrations lower than this threshold (28% vs. 11%, respectively).

A similar exposure–response relationship was observed in a prospective study including 86 patients with CD, both during induction and during maintenance therapy.[50] In this study, ustekinumab serum concentrations at weeks 4, 8, 16, and 24 were higher in patients achieving endoscopic response and concentration thresholds of 16.6, 5.0, 2.3, and 1.9 μg/mL, respectively, were identified as the minimal exposure needed to maximize the likelihood of endoscopic response after 6 months. Due to the low mucosal healing rates, the authors could not perform statistically relevant analyses to find the ideal cutoff for mucosal healing. To this day, no study has yet identified ustekinumab concentration thresholds associated with mucosal healing.

PATIENT-RELATED AND TREATMENT-RELATED FACTORS AFFECTING DRUG CONCENTRATIONS

Over the years, several patient-related and treatment-related factors influencing the concentration of a biological have been identified, which can explain the variation observed between patients.

A biological can induce an immune response in a patient leading to the development of neutralizing and/or non-neutralizing antidrug antibodies. Both types of antidrug antibodies increase the clearance of the drug resulting in lower serum drug concentrations. In addition, neutralizing antibodies inhibit the drug's activity by blocking the binding of the drug to its target.[11]

Disease severity of a patient also influences the drug concentration, which is reflected in the observations that lower C-reactive protein, lower fecal calprotectin, higher albumin, and higher hemoglobin have been associated with elevated serum concentrations of biologicals.[13,29,50,51] Likewise, a high induction dose quickly reduces the inflammatory burden leading to a decreased drug clearance and consequently allowing lower maintenance doses.

Other commonly observed patient-related factors that affect the concentration of a drug include sex, weight, and smoking, more specifically, female sex, low body weight, and nonsmoking predict higher drug concentrations.[13,36,50–52] With respect to treatment-related factors, being biological naïve and concomitant use of immunosuppressives have been shown to be associated with higher drug concentrations.[50,53,54]

PRACTICAL RECOMMENDATIONS FOR THE INTERPRETATION OF DRUG CONCENTRATION THRESHOLDS

The large variability in reported drug concentration thresholds needed to achieve mucosal healing makes it difficult for the clinician to know which concentration to target. In order to interpret a drug concentration threshold, several aspects should be considered.

First, the assay that is used to measure the concentration of the biological is of importance. There are different assay formats available to measure drug and antidrug antibody concentrations, including enzyme-linked immunosorbent assay, electrochemiluminescence immunoassay, mobility shift assay, radio-immunoassay, surface plasmon resonance, and lateral flow assay.[55] Each assay format has its strengths and limitations. When one patient sample is measured with different assays, the measured concentration will not be exactly the same. Variability can even exist within a similar assay format developed by two different companies. Numerous efforts have been made to compare different assays and establish the interchangeability.[56–60] Moreover, several groups, including the Monitoring of monoclonal Antibodies Group in Europe and The Biopharmaceutical Pharmacokinetic and Immunogenicity Assessment Group, have been founded with the aim to harmonize testing of drug and antidrug antibodies. Nevertheless, harmonization is challenging, and drug concentrations should always be interpreted relative to the assay used.

Second, the time of sampling is essential. Due to the dosing regimen, the concentration of a biological at a certain time point during induction will not be the same as at a time point during maintenance. This is also reflected in the infliximab concentration thresholds summarized in **Table 1**, where the reported concentration thresholds at weeks 2 and 6 are on average higher than the reported thresholds in maintenance. Furthermore, and even more important, the time of sampling within one dosing interval is crucial to interpret a certain drug concentration threshold. In most cases, sampling is performed at trough, the time point right before

the next administration. However, sampling can also be performed between two administrations where the concentration of a drug will be higher than at trough but lower than at peak. It is consequently crucial to interpret a drug concentration relative to the time of sampling.

Last, a certain drug concentration threshold should always be evaluated with respect to the outcome. Depending on which outcome you want to target, the drug concentration threshold differs. In general, more ambitious outcomes (e.g., mucosal healing) will require higher drug concentrations than less ambitious outcomes (e.g., clinical response). The minimally required drug concentration also depends on how the outcome is defined. In the comprehensive overview above, the definition of mucosal healing sometimes varies between studies. Especially for CD, there is no consensus on the definition of mucosal healing.[61] In some studies, mucosal healing is defined as a Simple Endoscopic Score lower than three, whereas most others use the complete absence of ulcerations as the definition for mucosal healing in CD. Additionally, one should also take into account at which time point the outcome is defined, a drug concentration threshold for short-term mucosal healing will differ from a threshold associated with mucosal healing at 1 or 2 years of treatment.

Despite a standardized assay and a defined sampling time point and outcome, there is still some variability associated with a certain drug concentration threshold that might influence the success of therapeutic drug monitoring in the clinician's own practice. The drug concentration threshold reported in a study is based on the specific patient cohort of that study. The cohort might consist of more patients that have been exposed to other biologicals than biological-naïve patients and, importantly, patients are not always treated according to the standard dosing regimen. For example, if a drug concentration threshold is derived from a group of which most patients are treated with a dosing frequency of 4 weeks instead of the standard 8 weeks, the threshold will consequently be less appropriate to apply in patients treated with the standard 8-week schedule. Of equal importance, the positive and negative predictive values of a drug concentration threshold should be considered to get insight into the expected success rate of the threshold.

CONCLUSIONS

As biologicals constitute a major healthcare expenditure in many countries and the number of approved biologicals for the treatment of IBD is expected to increase, cost-effective use of these drugs is becoming increasingly important. A large body of evidence supports the usefulness of monitoring drug concentrations of biologicals as drug concentrations have been linked to outcomes for anti-TNF as well as anti-integrin and anti-interleukin biologicals. Focusing on mucosal healing as the ultimate treatment goal, different drug concentration thresholds to achieve this outcome have been identified in the literature. However, for therapeutic drug monitoring to be successful in guiding clinical decision making, clinicians should know how to interpret these drug concentration thresholds. In general, the assay used to measure the drug, the time point of sampling, and the outcome that is aimed for are crucial to take into account. Awareness of these essential aspects among clinicians will improve the implementation of therapeutic drug monitoring and aid clinicians in making an informative and evidence-based decision.

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