Dynamics of serum concentrations of antibodies to infliximab: a new approach for predicting secondary loss of response in inflammatory bowel diseases

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Abstract

Background: Antibodies to infliximab (ATI) in serum are associated with secondary loss of response (LOR) to infliximab (IFX) therapy in patients with inflammatory bowel disease (IBD). However, feasible ATI-related predictors of therapy success are lacking and knowledge about individual ATI dynamics is limited. Therefore, this study analyzed whether ATI dynamics are able to predict LOR to IFX therapy and compared their predictive power with known predictors of LOR to IFX.

Methods: This was a retrospective study of patients with Crohn’s disease (CD) or ulcerative colitis (UC) on IFX maintenance therapy and proactive IFX and immunogenicity monitoring in an outpatient clinic in Germany. Slopes of ATI ($S_{ATI}$) and IFX levels (dynamic parameters) and medians of ATI, IFX, C-reactive protein, and fecal calprotectin (static parameters) were calculated over a defined period of time after ATI emergence. Dynamic and static parameters were analyzed for associations with end points infliximab discontinuation due to secondary LOR and total IFX discontinuation.

Results: In all, 500 visits from 38 IBD patients (28 CD, 10 UC) with a median IFX maintenance duration of 68.2 weeks were evaluated. Grouping by $S_{ATI}$ (ATI-N = ATI nondetectable, ATI-$\downarrow$ = negative $S_{ATI}$, ATI-$\uparrow$ = positive $S_{ATI}$) yielded significant differences for outcomes LOR ($p = 0.004$) and total IFX discontinuation ($p = 0.01$). Patients in the ATI-$\downarrow$ group survived significantly longer LOR-free compared with the ATI-$\uparrow$ group ($p = 0.02$). Cox regression confirmed $S_{ATI}$ to be a significant risk factor for LOR ($p = 0.002$). An $S_{ATI}$ cut-off of approximately 2.0 AU mL$^{-1}$ week$^{-1}$ was determined to predict LOR with 83.3% sensitivity and 93.8% specificity.

Conclusion: The ATI slope-based index $S_{ATI}$ is a new feasible diagnostic predictor of LOR in IBD patients. $S_{ATI}$ may facilitate quick therapeutic decisions after ATI emerge.

Keywords: antibodies to infliximab, antibody dynamics, Crohn’s disease, immunogenicity testing, inflammatory bowel disease, infliximab, personalized medicine, secondary loss of response, therapeutic drug monitoring, treatment failure, ulcerative colitis

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The chimeric, monoclonal antibody infliximab (IFX), sold under originator trade name Remicade®, was the pioneering TNF antagonist to receive Food and Drug Administration (FDA) approval for CD therapy in 1998. In the meantime, IFX biosimilars (e.g., Remsima®, Inflectra®) have entered the market. They are equally effective and safe, but cheaper and hence make IFX accessible for more patients in more countries. Although other TNF antagonists and more gut-specific biologics have been introduced since, IFX is still widely prescribed due to the longtime clinical experience and extensive safety profile available.

The efficacy of IFX, however, is impaired by the capability of biologic drugs to elicit immunogenic reactions: anti-drug antibodies may be generated, leading to enhanced drug clearance, direct neutralization of TNF-binding capacities, and adverse reactions (AR) up to anaphylactic reactions. Even if known for decades, antibodies to infliximab (ATI) still constitute a major cause for secondary loss of response (LOR) to IFX therapy, which is observed in about one-third of patients. The resulting inefficient use of the expensive drug places a high economic burden on healthcare systems. Nevertheless, no generally accepted consensus on the details of diagnostics and the optimal therapeutic strategy to manage emerging ATI has been established. Although some large cohort studies, such as the TAXIT (The Trough Concentration Adapted Infliximab Treatment) study, resulted in widely accepted recommendations and therapeutic algorithms, no official consensus on the details of diagnostics and the optimal therapeutic strategy to manage emerging ATI has been established. It is commonly accepted that IFX serum trough levels between 3 and 8µg·mL−1 are associated with favorable clinical outcome and reduced rates of secondary LOR. To maintain these levels, proactive therapeutic drug monitoring (TDM) combined with immunogenicity testing (IT), enabling early intervention, may be more effective than mere reactive monitoring triggered by relapse or LOR. However, a final proof of this hypothesis will require supporting results from further prospective studies. Beneficial therapeutic interventions include higher IFX dosages, shorter infusion intervals, or, to prevent ATI formation, additional therapy with immunomodulators, such as azathioprine, to restore high IFX trough levels. Evidence suggests that high and permanent ATI in serum are correlated with worse outcomes than low and transient ATI levels. However, consensus definitions of ‘high’ versus ‘low’ or ‘transient’ versus ‘permanent’ are lacking and studies on the detailed dynamics of ATI in individual patients are limited. Furthermore, analysis of ATI titers with respect to ‘static’ thresholds is frequently unable to explain or precisely predict clinical outcomes. Individual dynamics of ATI here may offer more valuable information for the clinician.

We therefore analyzed whether ATI dynamics monitored in a defined period of time are able to predict (secondary) LOR to IFX therapy. We furthermore investigated how the performance of ATI dynamics compares with known predictors of LOR, such as ‘static’ ATI titers, IFX trough levels, and inflammation markers. To do so, a real-world cohort of IBD patients on IFX maintenance therapy from a gastroenterologic outpatient clinic with a proactive TDM/IT policy was evaluated retrospectively. In the context of this study, TDM/IT denotes parallel serum IFX trough level determinations and ATI quantification. We suggest the new diagnostic index \( S_{ATI} \), which is indicative of ATI dynamics and was found to be an early predictor of secondary LOR.

**Patients and methods**

**Patients**

A total of 41 consecutive patients treated between April 2016 and March 2020 in a specialized gastroenterologic outpatient clinic (Gastroenterologische Gemeinschaftspraxis, Prof. Dr. P. Langmann and Dr. M. Weikert, Karlstadt, Germany) were recruited for the study. Of the 41 patients, 38 were finally included in our analysis (see method section ‘Assessment of ATI and IFX dynamics’). Only patients with confirmed diagnosis of CD or UC on maintenance therapy with Remicade® (Janssen Biologics B.V., Leiden, Netherlands), Remsima® (Celltrion, Incheon, South Korea), or Inflectra® (Hospira, Lake Forest, IL, USA) were included, whereby disease severity was indicative of IFX application. Patients below legal age, pregnant patients, and patients with less than four TDM/IT measurements were excluded, whereby ATI-positive patients were required to have at least one more TDM/IT recording after the first detection of ATI.

Remission was defined as the absence of clinical and serological signs of inflammation: normal
defecation frequency, absence of visible blood in stool and of intestinal or extraintestinal IBD-associated manifestations, normal levels of C-reactive protein (CRP), and moderate, stable levels of fecal calprotectin (FC). In patients experiencing a relapse in combination with emerging ATI, either IFX dosages were increased or application intervals shortened (normal IFX dosage in maintenance therapy: 5 mg·kg⁻¹ intravenously every 8 weeks). Reasons for discontinuation of IFX therapy were secondary LOR, serious AR to IFX therapy, or insufficient compliance. In the following, the term ‘LOR’ is used for IFX discontinuation due to secondary LOR. The term ‘total IFX discontinuation’ denotes all cases of IFX discontinuation, due to any of the three aforementioned reasons. Nonresponse to IFX induction (primary LOR) was not considered in this study.

The study was approved by the local Ethics Committee (Ethikkommission der Fakultät für Medizin der Technischen Universität München, approval number 289/19 S). Written informed consent was obtained from all patients. No financial compensation was provided. The reporting of this study conforms to the STROBE (Strengthening The Reporting of OBservational Studies in Epidemiology) statement (see checklist in Supplemental Material).19

**Laboratory analyses**

IFX and ATI serum concentrations were analyzed at MVZ Medizinisches Labor Oldenburg GmbH (Oldenburg, Germany) with the IDKmonitor® Infliximab drug-level enzyme-linked immunosorbent assay (ELISA) and the drug-tolerant IDKmonitor® Infliximab total ADA ELISA (both from Immundiagnostik AG, Bensheim, Germany), respectively. Furthermore, CRP in serum was measured turbidimetrically with the Tina-quant® C-Reactive Protein assay on a cobas® 8000 device with cobas c 701 analytical modules (all from Roche Diagnostics, Mannheim, Germany). FC was quantified via ELISA with the RIDASCREEN® Calprotectin assay on a DSX® system (R-Biopharm AG, Darmstadt, Germany). CRP and FC were both analyzed at the outpatient clinic at Würzburg where the patients were treated and determined at 89% and 45% of TDM/IT visits of all patients, respectively.

**Assessment of ATI and IFX dynamics**

IFX and ATI serum levels were monitored proactively, usually before application of the next IFX dose. The initial entry in a patient’s TDM/IT protocol corresponds to the first monitoring visit after successful IFX induction. $T_0$ is defined as therapy duration week at the first detection of ATI. ATI and IFX dynamics were calculated for the time between $T_0$ and the third consecutive TDM/IT visit after $T_0$, here to be denominated as $T_0–3$. Complete $T_0–3$ datasets were available for 38 of the 41 initially included patients. In all ATI-positive patients, as dynamic variables, individual slopes of the two analytes ATI ($S_{ATI}$) and IFX ($S_{IFX}$) were calculated as average ATI or IFX concentration change between visits $T_0$ and $T_3$ calculated as analyte concentration difference $\Delta c(\text{analyte})$ between $T_0$ and $T_3$ divided by the time period $T_0–3$. For clarification, $S_{ATI}$ and $S_{IFX}$ are reported as AU mL⁻¹ week⁻¹ and µg mL⁻¹ week⁻¹, respectively.

$$S_{\text{analyte}} = \frac{\Delta c(\text{analyte})}{T_{0–3}}$$

with analyte = ATI or IFX

In addition, as static variables, (a) median ATI and IFX levels over $T_{0–3}$ (ATImedian, IFXmedian) and
(b) maximal ATI and minimal IFX levels over the entire observation time (ATImax, IFXmin) were determined. Furthermore, median CRP and median FC levels were calculated as static variables over $T_{0.3}$ (CRPmedian, FCmedian). By definition, $T_0$ does not exist for patients with nondetectable ATI. As such, their $S_{ATI}$ equals 0 AU·mL$^{-1}$·week$^{-1}$, and $S_{IFX}$ and IFXmedian cannot be calculated.

### Statistical analysis

Statistical analyses were performed with GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA), R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria), and RStudio version 1.3.1093 (RStudio PBC, Boston, MA, USA). To enable comprehensive statistical analyses, measurement results below the assays’ limit of quantification (LOQ) were set to 0.3 µg·mL$^{-1}$ for IFX (LOQ 0.6 µg·mL$^{-1}$), to 5 AU·mL$^{-1}$ for ATI (LOQ 10 AU·mL$^{-1}$), to 0.02 mg·dL$^{-1}$ for CRP (LOQ 0.04 mg·dL$^{-1}$), and to 10 or 20 µg·g$^{-1}$ for FC (LOQ 20 or 39 µg·g$^{-1}$, depending on the sample dilution). FC measurements above the measurement range were set to 6.0 weeks). In total, 500 TDM/IT results for both ATI and IFX levels were available. For 444 (89%) and 224 (45%) of the 500 TDM/IT visits, CRP serum concentrations and FC concentrations in stool were documented, respectively. A comprehensive summary of the characteristics of the included patients is given in Table 1.

### Characteristics of the $S_{ATI}$ groups

Based on the individual values of $S_{ATI}$, patients were assigned to three groups: ATI-N (nondetectable ATI; $S_{ATI}=0$), ATI↓ (negative $S_{ATI}$), and ATI↑ (positive $S_{ATI}$). The characteristics and comparisons of the various $S_{ATI}$ groups are depicted in Table 2.

No significant differences between the three groups were found for age, sex, diagnosis, AR, immunosuppressive comedication (CM), therapy duration, IFXmin, SIFX, CRPmedian, FCmedian, $T_0$, and reactive dosing adjustments. ATImedian, ATImax, and $S_{ATI}$ differ significantly between the groups ($p<0.0001$). The median $S_{ATI}$ in the ATI↓ group was calculated as $-1.031$ AU·mL$^{-1}$·week$^{-1}$ (interquartile range [IQR] = −2.469 to −0.0421 AU·mL$^{-1}$·week$^{-1}$) and in the ATI↑ group as 4.335 AU·mL$^{-1}$·week$^{-1}$ (IQR = 0.631–8.617 AU·mL$^{-1}$·week$^{-1}$). Furthermore, the rates of LOR and IFX discontinuation in total were significantly different between the groups ($p=0.004$ and $p=0.01$, respectively). For total IFX discontinuation, differences between the ATI slope groups can be attributed to a significantly lower number of patients discontinuing the therapy in the ATI↓ group compared with the ATI↑ group (8.3% versus 70.0%, respectively; $p=0.0062$). With LOR, however, both the ATI↑ group and the ATI-N group exhibited a significantly higher portion of therapy discontinuation (60% with $p=0.028$ and 18.8% with $p=0.046$, respectively) compared with the ATI↓ group (0%). The median time after $T_3$ until LOR was 22.3 weeks (IQR = 15.0–63.5 weeks). LOR types according to Ma and colleagues$^{80}$ for all patients are provided in the Supplemental Material (Table S1). We observed non-immune-mediated pharmacokinetic failure in all ATI-N LOR patients. LOR in ATI↑ patients could be attributed to immune-mediated pharmacokinetic failure in 66.7% of cases, whereas 33.3% experienced mechanistic failure. IFXmedian was significantly higher in the ATI↓ group (5.4 µg·mL$^{-1}$) than in the ATI↑ group (2.1 µg·mL$^{-1}$, $p=0.0076$).

### Results

#### Patient characteristics

Thirty-eight patients were included in the study. The number of TDM/IT visits per patient ranged from 4 to 30 (median, 12), with monitoring intervals ranging from 2.5 to 11.8 weeks (median,
Exemplary individual ATI and IFX courses of patients in the ATI-N, ATI-↓, and ATI-↑ groups are depicted in the Supplemental Material (Figure S2) to illustrate the calculation of $S_{\text{ATI}}$.

**Total IFX discontinuation-free and LOR-free survival**

Survival of the three ATI groups was assessed by Kaplan–Meier analysis for LOR-free and IFX discontinuation-free survival. Significant differences were only observed for LOR ($p = 0.021$, Figure 2(a)), but not for total IFX discontinuation ($p = 0.088$; Figure 2(b)). Pairwise comparisons of LOR-free survival yielded a significantly lower risk of LOR in the ATI-↓ group compared with the ATI-↑ group ($p = 0.015$). LOR-free survival of ATI-N and ATI-↑ and of ATI-N and ATI↓, however, did not differ significantly ($p = 0.098$ and $p = 0.12$, respectively).

**Cox regression**

To further investigate the association of $S_{\text{ATI}}$ and IFX discontinuation, univariate and bivariate HRs were calculated (Table 3). In a univariate analysis with respect to outcome LOR, not only the ATI-related parameters ATImedian ($p = 0.02$), ATImax ($p = 0.001$), and $S_{\text{ATI}}$ ($p = 0.002$), but also age ($p = 0.03$), diagnosis CD ($p = 0.01$), and CRPmedian ($p = 0.02$) were found to be significantly associated with LOR. Parameters significantly associated with LOR in univariate analysis were included in subsequent bivariate analyses (Table 4). The association of $S_{\text{ATI}}$ and LOR, however, remained significant even after correcting for age, diagnosis CD, CRPmedian and ATImedian ($p = 0.01$, $p = 0.01$, $p = 0.02$, and $p = 0.03$, respectively). $S_{\text{ATI}}$ was not correlated significantly with LOR anymore, when its HR was corrected for ATImax ($p = 0.09$). For outcome IFX discontinuation due to any reason, the only significant associations were found with $S_{\text{ATI}}$ (Table 3; $p = 0.03$). To further investigate the correlation of LOR with diagnosis in univariate analysis, nonparametric Kruskal–Wallis test was additionally performed to compare $S_{\text{ATI}}$, ATImedian, and ATImax between CD and UC patients. However, no statistically significant difference was observed (see Table S3 in Supplemental Material).

**Optimal time interval for $S_{\text{ATI}}$ calculation**

Besides $T_{0-3}$, $S_{\text{ATI}}$ was additionally calculated for the time periods $T_{0-1}$ and $T_{0-2}$ (Figure 3) to investigate the minimum monitoring time period for robust LOR prediction. Univariate Cox-proportional hazards models were then calculated for the end point LOR using the various $S_{\text{ATI}}$ calculation bases. Only $S_{\text{ATI}}$ calculated over $T_{0-3}$ yielded a significant association with LOR ($p = 0.002$; Table 5). $S_{\text{ATI}}$ values calculated for the different time periods are shown in the Supplemental Material (Tables S4 and S5).

**Table 1. Patient characteristics.**

| Characteristics                                      | Summary statistics |
|-------------------------------------------------------|--------------------|
| Total, $n$ (%)                                        | 38 [100.0]         |
| Age, years, median (IQR)                             | 39 [28–57]         |
| Female sex, $n$ (%)                                   | 20 [52.6]          |
| Diagnosis CD, $n$ (%)                                 | 28 [73.7]          |
| IFX discontinuation, $n$ (%)                          | 14 [36.8]          |
| Due to LOR*                                           | 9 [23.7]           |
| Due to AR*                                            | 5 [13.2]           |
| Due to poor compliance                                | 1 [2.6]            |
| AR to IFX, $n$ (%)                                    | 11 [28.9]          |
| Immunosuppressive comedication, $n$ (%)               | 21 [55.3]          |
| Therapy duration, weeks, median (IQR)                 | 68.2 [32.2–108.8]  |

AR, adverse reactions; CD, Crohn’s disease; IFX, infliximab; IQR, interquartile range; LOR, loss of response.

*a*One patient discontinued therapy due to both LOR and AR and was counted for both end points.

*b*Includes cutaneous reactions, anaphylactic reactions, and joint pain.

**Refinement of $S_{\text{ATI}}$ cut-off**

The cut-off of $S_{\text{ATI}}$ for LOR was further optimized via ROC analysis (Figure 4). Including ATI-positive cases only ($n = 22$), $S_{\text{ATI}}$ identifies patients experiencing LOR with an area under the curve (AUC) of 0.948 ($p = 0.002$). The optimal $S_{\text{ATI}}$ cut-off was calculated as 2.008 AU mL$^{-1}$ week$^{-1}$, yielding a sensitivity of 83.3% and a specificity of 93.8%.

**Discussion**

We investigated whether ATI dynamics, quantified via a proactive TDM combined with IT, are able to predict LOR in patients with IBD under IFX medication and how their predictive
Table 2. Patient characteristics by ATI slope (S_ATI) group.

| Characteristics | All   | ATI-N | ATI-↓ | ATI-↑ |
|-----------------|-------|-------|-------|-------|
| **Summary statistics** |       |       |       |       |
| Total, n (%)    | 16 (42.1) | 12 (31.6) | 10 (26.3) |       |
| Age, years, median (IQR) | 39 (30 to 57) | 33 (27 to 50) | 43 (26 to 63) | 0.7 |
| Female sex, n (%) | 8 (50.0) | 7 (58.3) | 5 (58.3) | 0.9 |
| Diagnosis CD, n (%) | 13 (81.3) | 10 (83.3) | 5 (50.0) | 0.1 |
| AR to IFX, n (%) | 3 (18.8) | 6 (50.0) | 2 (20.0) | 0.2 |
| CM, n (%)       | 12 (75.0) | 6 (50.0) | 3 (30.0) | 0.07 |
| Therapy duration, weeks, median (IQR) | 63.8 (28.6 to 105.8) | 75.6 (38.9 to 93.9) | 66.0 (31.8 to 117.1) | 0.9 |
| IFX median, µg·mL–1, median (IQR) | NA | 5.4 (3.9 to 9.7) | 2.1 (1.1 to 4.0) | 0.0076** |
| IFX min, µg·mL–1, median (IQR) | 1.3 (0.4 to 2.1) | 1.1 (0.3 to 2.2) | 0.4 (0.3 to 1.5) | 0.4 |
| S_IFX, µg mL–1 week–1, median (IQR) | NA | –0.247 (–0.595 to –0.011) | –0.021 (–0.147 to 0.115) | 0.1440 |
| ATImedian, AU·mL–1, median (IQR) | 5.0 (5.0 to 5.0) | 5.0 (5.0 to 9.1) | 42.5 (20.3 to 140.6) | 0.0017** |
| ATImax, AU·mL–1, median (IQR) | 5.0 (5.0 to 5.0) | 45.6 (17.7 to 57.7) | 205.7 (50.4 to 506.9) | 0.0017** |
| S_ATI, AU mL–1 week–1, median (IQR) | 0.000 (0.0 to 0.0) | –1.031 (–2.469 to –0.421) | 4.335 (0.631 to 8.617) | 0.0017*** |
| CRPmedian, mg·dL–1, median (IQR) | 0.29 (0.05 to 0.74) | 0.21 (0.06 to 0.31) | 0.35 (0.14 to 1.13) | 0.3 |
| FCmedian, µg·g–1, median (IQR) | 288 (51 to 733) | 156 (88 to 493) | 246 (781 to 489) | 0.8 |
| T0, weeks, median (IQR) | 1.1 (0.0 to 3.8) | 1.1 (0.0 to 2.8) | 0.9 (0.0 to 2.8) | 0.4799 |
| DI shorteninga | 14 (87.5) | 11 (91.7) | 9 (90.0) | 0.9 |
| Frequencya | 1.0 (0.0 to 1.0) | 1.5 (1.0 to 2.8) | 1.0 (0.0 to 1.0) | 0.6 |
| Dose increasea | 0.0 (0.0 to 1.0) | 0.0 (0.0 to 1.0) | 0.0 (0.0 to 1.0) | 0.8 |
| Frequencya | 0.0 (0.0 to 1.0) | 0.0 (0.0 to 1.0) | 0.0 (0.0 to 1.0) | 0.9 |
| IPX discontinuation, n (%) | 6 (37.5) | 6 (50.0) | 4 (40.0) | 0.8 |
| Frequencya | 0.0 (0.0 to 1.0) | 0.0 (0.0 to 1.0) | 0.0 (0.0 to 1.0) | 0.9 |
| Due to LOR | 3 (18.8) | 0 (0.0) | 6 (60.0) | 0.0042** |
| Frequencya | 0.0 (0.0 to 1.0) | 0.0 (0.0 to 1.0) | 0.0 (0.0 to 1.0) | 0.9 |
| Due to AR | 2 (12.5) | 2 (16.7) | 2 (20.0) | 0.7 |

AR, adverse reactions; ATI, antibodies to infliximab; ATI-N, ATI nondetectable; CD, Crohn’s disease; CM, immunosuppressive comedication; DI, dosing interval; IFX, infliximab; IQR, interquartile range; LOR, loss of response; S_ATI, slopes of ATI; S_IFX, slopes of IFX.

**Variables DI shortening and dose increase indicate the number of patients who underwent intervention, while respective frequencies indicate the number of interventions per patient.

*Significance levels are indicated by * for 0.01 < p ≤ 0.05, ** for 0.001 < p ≤ 0.01, and *** for p ≤ 0.001.
performance compares with other measures of therapy success. $S_{\text{ATI}}$ was defined as a novel parameter, representing ATI dynamics during approximately 17 weeks after the first ATI-positive TDM/IT result and was found to be strongly associated with LOR. Increasing ATI concentrations in serum indicated a higher risk of LOR. Notably, $S_{\text{ATI}}$ was the best predictor for LOR among all considered ATI, IFX, and inflammation parameters. The predictive potential of $S_{\text{ATI}}$ for LOR is supported by univariate and multivariate comparisons and ROC analysis. Among ATI-positive patients, an $S_{\text{ATI}}$ cut-off of approximately 2.0 AU mL$^{-1}$ week$^{-1}$ yielded high sensitivity and specificity for LOR. In addition to confirming the predictive potential of $S_{\text{ATI}}$, univariate and multivariate analyses revealed that $S_{\text{ATI}}$ exhibits the best predictive performance among all evaluated laboratory parameters. Included laboratory parameters were usually calculated for $T_{0–3}$, that is, the same period of time as $S_{\text{ATI}}$. Only $\text{ATI}_{\text{max}}$ and $\text{IFX}_{\text{min}}$ refer to the entire observation period of the respective patient. A significant association with the risk of LOR was detected for both $S_{\text{ATI}}$ and $\text{ATI}_{\text{max}}$. The magnitude of the HR for $S_{\text{ATI}}$ is as well comparable to that of $\text{ATI}_{\text{max}}$ (1.209 and 1.004, respectively). However, it is generally unclear when $\text{ATI}_{\text{max}}$ is or will be reached, not to mention if it is reached within $T_{0–3}$. As such, $S_{\text{ATI}}$ constitutes the more feasible parameter for the clinician. Overall, our results suggest that the value of ATI dynamics in current TDM/IT protocols for IBD patients is greatly underestimated.

Our study proposes $S_{\text{ATI}}$ as a simple, new laboratory index to predict LOR. The European Crohn’s and Colitis Organisation (ECCO) and American Gastroenterological Association (AGA) do not recommend proactive TDM/IT due to the lack of sufficient data. However, a positive association of ATI titers and secondary LOR with IFX and, consequently, the benefit of proactive IT in IBD have been described in several studies. The detailed dynamics of ATI courses in single patients, though, are still not fully understood, even for IFX as widely prescribed biologic. Nevertheless, evidence suggests that proactive IT provides the best assessment of anti-drug antibody dynamics, rather than sporadic, reactive ‘snap-shot’ measurements. For IFX and adalimumab, proactive TDM/IT was found to be beneficial with respect to prevention of secondary LOR, less frequent ATI emergence, and better mucosal healing. In our study, the proactive TDM/IT regimen enabled precise and early detection of $T_0$, which occurred at a median of 3.4 weeks after the first TDM/IT visit during maintenance therapy, that is, approximately 16 weeks after the first IFX infusion. This result is in good agreement with other studies with similarly frequent proactive monitoring policy. A time point or interval for therapeutic decision-making was not precisely defined in the aforementioned studies. Our results, on the contrary, enable prediction of LOR approximately 17 weeks after $T_0$. The studies focusing on ATI dynamics are limited in number and primarily investigate the transient versus persistent nature of ATI. In
observational and prospective studies, transient ATI seem to be less frequently associated with LOR and to exhibit lower titers compared with persistent ATI.\textsuperscript{16,18,29} Although not the subject of this work, we observed that in the ATI→ group 83.3\% of the patients had ATI-negative sera at the end of follow-up, compared with only 10\% in the ATI↑ group (data not shown). The respective, dominant LOR type observed in each ATI group (immune-mediated pharmacokinetic failure in ATI↑ and non-immune-mediated pharmacokinetic failure in ATI-N patients; see Table S1 in Supplemental Material) supports these results. Also, the significantly lower median ATI titers in the ATI↓ and the significantly higher LOR rates in the ATI↑ group are in good agreement with the reports cited above. Beyond that, our data suggest that \( S_{\text{ATI}} \) may constitute an early predictor of ATI persistence. Our study suggests that proactive IT can contribute to an improved pharmacologic management and might influence clinical decision-making. It remains to be elucidated whether proactive IT can also influence clinical targets such as the rate of mucosal healing or histologic remission in patients with IBD. If this could be shown in further studies, the current recommendations of ECCO and AGA against proactive IT would be obsolete.

### Table 3. Univariate analysis of IFX discontinuation.

| LOR                          | Total IFX discontinuation |
|------------------------------|--------------------------|
|                              | HR 95% CI     p  | HR 95% CI     p  |
| Age                          | 1.046 1.002–1.093 0.03* | 1.009 0.976–1.042 0.6 |
| Female sex                   | 0.794 0.204–3.087 0.7 | 0.964 0.328–2.832 0.9 |
| Diagnosis CD                 | 0.165 0.039–0.695 0.01* | 0.339 0.113–1.016 0.06 |
| AR                           | 0.280 0.035–2.250 0.2 | 1.201 0.400–3.613 0.7 |
| CM                           | 1.309 0.347–4.944 0.7 | 1.352 0.465–3.933 0.6 |
| CRPmedian                    | 4.529 1.391–14.75 0.02* | 2.458 0.937–6.448 0.09 |
| FCmedian                     | 1.001 0.999–1.002 0.3 | 1.001 0.999–1.002 0.5 |
| T0                           | 0.995 0.961–1.031 0.8 | 0.987 0.952–1.024 0.4 |
| DI shortening                | 3×10\(^7\) 0–inf. 0.4 | 0.649 0.079–5.324 0.7 |
| Frequency                    | 0.841 0.493–1.435 0.5 | 0.797 0.512–1.242 0.3 |
| Dose increase                | 0.369 0.088–1.551 0.2 | 0.321 0.097–1.058 0.05 |
| Frequency                    | 0.795 0.327–1.936 0.6 | 0.692 0.336–1.466 0.3 |
| ATImedian                    | 1.008 1.002–1.013 0.02* | 1.005 1.000–1.010 0.1 |
| ATImax                       | 1.004 1.002–1.007 0.001** | 1.003 1.001–1.005 0.1 |
| S\(_{\text{ATI}}\)          | 1.209 1.082–1.351 0.002** | 1.122 1.029–1.224 0.03* |
| IFXmedian                    | 0.717 0.461–1.115 0.08 | 0.853 0.628–1.158 0.3 |
| IFXmin                       | 0.630 0.288–1.378 0.2 | 0.994 0.635–1.556 1.0 |
| S\(_{\text{IFX}}\)          | 6.708 0.450–100 0.1 | 0.591 0.092–3.780 0.6 |

AR, adverse reactions; C, model concordance; CD, Crohn’s disease; CI, confidence interval; CM, immunosuppressive comedication; DI, dosing interval; HR, hazard ratio; IFX, infliximab; LOR, loss of response; \( S_{\text{ATI}} \), slopes of ATI; \( S_{\text{IFX}} \), slopes of IFX.

Significance levels are indicated by * for 0.01 < \( p \) ≤ 0.05 and ** for \( p \) ≤ 0.01.
Table 4. Bivariate analysis of LOR risk.

| Characteristic | HR (95% CI) | \( p(S_{\text{ATI}}) \) | \( p(x) \) | C |
|----------------|-------------|----------------|-----------|---|
| Age            | 1.172       | 1.034–1.330    | 0.01*     | 0.4 | 0.840 |
| Diagnosis CD   | 1.168       | 1.036–1.317    | 0.01*     | 0.08 | 0.851 |
| CRPMedian      | 1.165       | 1.024–1.325    | 0.02*     | 0.3  | 0.845 |
| ATIMedian      | 1.174       | 1.018–1.355    | 0.03*     | 0.5  | 0.777 |
| ATImax         | 1.136       | 0.982–1.313    | 0.09      | 0.07 | 0.755 |

| Characteristic | \( x \) |
|----------------|--------|
|                | HR     |
|                | 95% CI |
|                |        |
|                | \( \rho(x) \) |
|                | C      |

C, model concordance; CD, Crohn’s disease; CI, confidence interval; HR, hazard ratio. HRs for LOR are presented, adjusted for the enlisted covariates \( x \). Data for total IFX discontinuation are not shown, since \( S_{\text{ATI}} \) was the only significant variable in univariate analysis. Significance levels are indicated by * for 0.01 < \( p \) < 0.05 and ** for \( p \leq 0.01 \).

Figure 3. Principle of data selection in the three different \( S_{\text{ATI}} \) calculation bases \( T_{0-1} \) (left panel), \( T_{0-2} \) (center panel), and \( T_{0-3} \) (right panel). Each panel contains the same hypothetical ATI level course in an individual patient and depicts the magnitude of the corresponding average slope (shaded triangle). The two TDM/IT visits considered for the respective \( S_{\text{ATI}} \) calculations \( (T_0 \text{ and end point}) \) are highlighted on the time \( (t) \) axis.

Table 5. Characteristics for the three groups according to \( S_{\text{ATI}} \) calculation base.

| Characteristics | \( T_{0-1} \) | \( T_{0-2} \) | \( T_{0-3} \) |
|----------------|---------------|---------------|---------------|
| Patients, n (%)| 41 (100.0)    | 39 (100.0)    | 38 (100.0)    |
| LOR, n (%)     | 11 (26.8)     | 10 (25.6)     | 9 (23.7)      |
| Length \( T_{0-x} \), weeks, median [IQR] | 5.3 (4.1–8.6) | 12.0 (8.9–16.0) | 17.2 (12.9–22.6) |
| HR (95% CI)    | 1.018 (0.999–1.037) | 1.078 (0.918–1.267) | 1.209 (1.082–1.51) |
| \( p \) value  | 0.1           | 0.4           | 0.002**       |
| C              | 0.621         | 0.548         | 0.783         |

C, model concordance; CI, confidence interval; HR, hazard ratio; IQR, interquartile range; LOR, loss of response. \( x \) denotes the \( x \)th visit after \( T_0 \). Significance level is indicated by ** for \( p \leq 0.01 \).
Besides ATI, several other predictors of LOR have been described, with IFX trough level being the most important one.\textsuperscript{24,30} Considering the lack of standardized strategies for ATI management, IFX therapies are sometimes merely IFX trough level guided.\textsuperscript{1,10–14} Notably, IFX levels and related indices did not predict LOR in our study. Regarding the \(S_{\text{ATI}}\) group comparisons, this surprising observation may, on the one side, be caused by limiting the analyzed data to the time window \(T_{0-3}\). On the other side, this result may be due to the fact that IFX\textsubscript{median} and \(S_{\text{IFX}}\) could not be calculated for ATI-N patients; hence, these patients were not included in the comparisons. However, univariate Cox regression did not yield significant correlations of IFX-dependent parameters with LOR, either, and no patients were excluded for these calculations. Possibly, the aggressive, proactive dose intensification regime imposed variance on IFX trough levels in this work. Even in absence of ATI, IFX courses can fluctuate (see patient P-12 in Figure S2 in Supplemental Material). The importance of ATI monitoring suggested by our study is supported, for example, by Magro and colleagues,\textsuperscript{31} who found ATI and FC monitoring to be more relevant for therapeutic escalation than IFX trough levels. Apart from IFX trough levels, both FC and CRP are known to be associated with therapeutic outcome.\textsuperscript{3,14,16} In our cohort, however, only \(\text{CRP}_{\text{median}}\) but not \(\text{FC}_{\text{median}}\) was correlated with LOR. This may be attributed to the smaller number of available FC results in our cohort. In our univariate analysis, furthermore, age and diagnosis were associated with LOR. Higher age (>60 years) has been described before as risk factor for anti-TNF therapy discontinuation due to AR or LOR, thereby confirming our findings.\textsuperscript{32,33} The increased LOR risk for UC over CD patients that was surprisingly found in univariate analysis is not supported by further literature. Grinman and colleagues,\textsuperscript{34} however, described higher ATI and FC levels for UC than for CD patients, yet without differences in therapy success. The aforementioned and our study both analyzed a small portion of UC patients (Grinman and colleagues: \(n=10\), accounting for 10.5% of included patients; this study: \(n=10\), 26.3%). Hence, the higher ATI titer for UC patients in the study by Grinman and colleagues and the univariate correlation with LOR for diagnosis UC in our study may be the incidental result of underpowered statistics regarding the variable diagnosis. In contrast, Vande Casteele and colleagues\textsuperscript{18} reported results consistent with ours. There were no differences in ATI and IFX levels between CD and UC in their larger cohort (see Table S3 in Supplemental material). Notably, \(S_{\text{ATI}}\) outperformed other variables in our bivariate analysis. Moreover, \(S_{\text{ATI}}\) predicted LOR despite therapeutic interventions.

A possible explanation for the observed association is that the dynamics of the initial immune response against IFX are characteristic for its severity and responsiveness to treatment intervention, such as dose adjustments. First, a steep rise of ATI, reflected by a positive \(S_{\text{ATI}}\), may be indicative of more aggressive immunogenicity by rapidly enhancing IFX clearance.\textsuperscript{35} Second, the 17-week timeframe of \(T_{0-3}\) comprises first interventions to counteract emerging ATI for some patients. Rising ATI despite dose adjustments in the beginning of maintenance therapy may be indicative of lacking ATI responsiveness to dose adjustments and of ATI persistence, which both are associated with higher LOR rates. We therefore suggest \(S_{\text{ATI}}\) as a valuable parameter for clinical decision-making, in combination with accurate clinical evaluation. In case of \(S_{\text{ATI}} >2.0 \text{AU mL}^{-1}\text{week}^{-1}\), IFX therapy may be maintained if the patient remains in remission.\textsuperscript{12} If \(S_{\text{ATI}}\) above the critical cut-off is accompanied by worsening symptoms, therapeutic intervention may be considered, for example, adjustment of IFX dose or immunosuppressive comedication, or switch to a different biologic. In summary, a high \(S_{\text{ATI}}\) should alert the gastroenterologist to pay close attention to the patient.
Strengths of this study encompass its real-world nature and the consistently proactive, high-frequency TDM/IT. Some other relatively monitoring-intense studies16,18,36 determined ATI serum levels between 1 and 5 times per patient (median of 5 available ATI titers per patient in Roblin and colleagues),16 with average TDM/IT intervals of at least 3 months reported for Roblin and colleagues16 and Pugliese and colleagues.36 Vande Casteele and colleagues18 published a more monitoring-intense retrospective study, in which an average of 14 TDM/IT samples per patient were analyzed over a study duration of 12 years. In this study, a remarkably high number (median, 12) and frequency of visits (median, 6.0 weeks) per patient were analyzed. Of note, we obtained highly significant findings despite our small cohort size. Overall, our pilot analysis builds an intriguing base for further studies of $S_{ATI}$ with larger patient cohorts.

Some limitations of our study, however, have to be mentioned. First, the small cohort size may conceal further significant correlations and did not allow to include more than two variables at a time in the multivariate analysis. The presented bivariate analysis therefore has to be interpreted with caution. Second, the retrospective and monocentric design of this study may have introduced some bias. Third, monitoring intervals longer than $T_{0–3}$ for calculation of $S_{ATI}$ were not assessed due to the lack of sufficient data. Fourth, our collective included both CD and UC patients. This approach has also been chosen in other studies, but may introduce some bias.18,23,34 Finally, all reported ATI-related values are only valid for the specific ELISA employed. Since the majority of IFX IT, like in our study, only targets ATI quantification, but not characterization, the question arises whether more information about ATI would improve patient care. ATI comprise patient-individual polyclonal antibody populations that can exhibit different avidities and epitope coverage. Assessment of these characteristics requires alternative methodology, for example, surface plasmon resonance–based biosensors.37–39 In addition, an adequate consensus methodology is required to establish the overdue gold standard for TDM/IT in IFX therapy. First attempts have already been made.40

Beyond IFX therapy, it will be exciting to track further efforts on the deciphering of patient-individual anti-drug antibody dynamics and characteristics for any biologic. Learning more about their similarities or differences and their role in therapy failure will build the basis for a sustainable improvement in biologic therapy.37,41

Conclusion
To conclude, this study suggests $S_{ATI}$ as new diagnostic index for ATI dynamics, allowing to predict LOR in IBD patients. $S_{ATI}$ was calculated from two TDM/IT visits over a short period of time (17 weeks) and provides, along with clinical symptoms, a decision-making aid for further therapeutic management. Thus, $S_{ATI}$ enables quick and standardized reaction to the first detection of ATI. To ensure early ATI detection, we recommend proactive ATI monitoring at every IFX infusion visit. $S_{ATI}$ will then provide reliable predictive information.

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Author contributions
MKG, AFL, MAT, PL, and PBL contributed to the study design. PL and AFL educated patients, collected consent forms, and applied for study approval by the ethics committee. PL was responsible for patient recruitment. AFL and PL collected data. MKG analyzed the data. MKG, MAT, MT, and PBL drafted the manuscript. All authors were involved in data interpretation, contributed important intellectual content to the manuscript through critical revision, and approved the final article.

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References
1. Vulliemoz M, Brand S, Juillerat P, et al. TNF-alpha blockers in inflammatory bowel diseases: practical recommendations and a user’s guide: an update. *Digestion* 2020; 101: 16–26.

2. Khanna R, Bressler B, Levesque BG, et al. Early combined immunosuppression for the management of Crohn’s disease (REACT): a cluster randomised controlled trial. *Lancet* 2015; 386: 1825–1834.

3. Peyrin-Biroulet L, Fiorino G, Buisson A, et al. First-line therapy in adult Crohn’s disease: who should receive anti-TNF agents. *Nat Rev Gastroenterol Hepatol* 2013; 10: 345–351.

4. Danese S, Fiorino G and Peyrin-Biroulet L. Early intervention in Crohn’s disease: towards disease modification trials. *Gut* 2017; 66: 2179–2187.

5. Ye BD, Pesegova M, Alexeeva O, et al. Efficacy and safety of biosimilar CT-P13 compared with originator infliximab in patients with active Crohn’s disease: an international, randomised, double-blind, phase 3 non-inferiority study. *Lancet* 2019; 393: 1699–1707.

6. Gecse KB, Lovász BD, Farkas K, et al. Efficacy and safety of the biosimilar infliximab CT-P13 treatment in inflammatory bowel diseases: a prospective, multicentre, nationwide cohort. *J Crohn’s Colitis* 2016; 10: 133–140.

7. Atiqi S, Hooijberg F, Loeff FC, et al. Immunogenicity of TNF-inhibitors. *Front Immunol* 2020; 11: 312–312.

8. Qiu Y, Chen B-L, Mao R, et al. Systematic review with meta-analysis: loss of response and requirement of anti-TNFα dose intensification in Crohn’s disease. *J Gastroenterol* 2017; 52: 535–554.

9. Vande Castelee N, Ferrante M, Van Assche G, et al. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. *Gastroenterology* 2015; 148: 1320–1329.

10. Dreesen E, Bossuyt P, Mulleman D, et al. Practical recommendations for the use of therapeutic drug monitoring of biopharmaceuticals in inflammatory diseases. *Clin Pharmacol* 2017; 9: 101–111.

11. Papamichael K, Vajravelu RK, Vaughn BP, et al. Proactive infliximab monitoring following reactive testing is associated with better clinical outcomes than reactive testing alone in patients with inflammatory bowel disease. *J Crohns Colitis* 2018; 12: 804–810.

12. Vande Castelee N, Herfarth H, Katz J, et al. American gastroenterological association institute technical review on the role of therapeutic drug monitoring in the management of inflammatory bowel diseases. *Gastroenterology* 2017; 153: 835–857.e836.

13. Katz L, Gisbert JP, Manoogian B, et al. Doubling the infliximab dose versus halving the infusion intervals in Crohn’s disease patients with loss of response. *Inflamm Bowel Dis* 2012; 18: 2026–2033.

14. Colombel J-F, Adedokun OJ, Gasink C, et al. Combination therapy with infliximab and azathioprine improves infliximab pharmacokinetic features and efficacy: a post hoc analysis. *Clin Gastroenterol Hepatol* 2019; 17: 1525–1532. e1521.

15. Baert F, Noman M, Vermeire S, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn’s disease. *N Engl J Med* 2003; 348: 601–608.

16. Robin 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 Crohn’s Colitis* 2015; 9: 525–531.

17. Ungar B, Chowers Y, Yavzori M, et al. The temporal evolution of antidrug antibodies in patients with inflammatory bowel disease treated with infliximab. *Gut* 2014; 63: 1258–1264.

18. Vande Castelee N, Giils A, Singh S, et al. Antibody response to infliximab and its impact
on pharmacokinetics can be transient. *Am J Gastroenterol* 2013; 108: 962–971.

19. von Elm E, Altman DG, Egger M, et al. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *PLoS Med* 2007; 4: e296.

20. 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: 127–145.

21. Feuerstein JD, Nguyen GC, Kupfer SS, et al. American Gastroenterological Association institute guideline on therapeutic drug monitoring in inflammatory bowel disease. *Gastroenterology* 2017; 153: 827–834.

22. Torres J, Bonovas S, Doherty G, et al. ECGO guidelines on therapeutics in Crohn’s disease: medical treatment. *J Crohn’s Colitis* 2020; 14: 4–22.

23. Mc Gettigan N, Afridi AS, Harkin G, et al. The optimal management of anti-drug antibodies to infliximab and identification of anti-drug antibody values for clinical outcomes in patients with inflammatory bowel disease. *Int J Colorectal Dis* 2021; 36: 1231–1241.

24. Vande Casteele N, Khanna R, Levesque BG, et al. The relationship between infliximab concentrations, antibodies to infliximab and disease activity in Crohn’s disease. *Gut* 2015; 64: 1539–1545.

25. Argollo M, Kotze PG, Kakadadasam P, et al. Optimizing biologic therapy in IBD: how essential is therapeutic drug monitoring? *Nat Rev Gastroenterol Hepatol* 2020; 17: 702–710.

26. Assa A, Matar M, Turner D, et al. Proactive monitoring of adalimumab trough concentration associated with increased clinical remission in children with Crohn’s disease compared with reactive monitoring. *Gastroenterology* 2019; 157: 985–996.e982.

27. Papamichael K, Chachu KA, Vajravelu RK, et al. Improved long-term outcomes of patients with inflammatory bowel disease receiving proactive compared with reactive monitoring of serum concentrations of infliximab. *Clin Gastroenterol Hepatol* 2017; 15: 1580–1588.

28. Fernandes SR, Bernardo S, Simões C, et al. Proactive infliximab drug monitoring is superior to conventional management in inflammatory bowel disease. *Inflamm Bowel Dis* 2020; 26: 263–270.

29. Steenholdt C, Al-khalaf M, Brynskov J, et al. Clinical implications of variations in anti-infliximab antibody levels in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2012; 18: 2209–2217.

30. 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: 341–353.

31. Magro F, Afonso J, Lopes S, et al. Calprotectin and the magnitude of antibodies to infliximab in clinically-stable ulcerative colitis patients are more relevant than infliximab trough levels and pharmacokinetics for therapeutic escalation. *EBioMedicine* 2017; 21: 123–130.

32. Desai A, Zator ZA, de Silva P, et al. Older age is associated with higher rate of discontinuation of anti-TNF therapy in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2012; 19: 309–315.

33. de Jong ME, Smits LJ, van Ruijven B, et al. Increased discontinuation rates of anti-TNF therapy in elderly inflammatory bowel disease patients. *J Crohn’s Colitis* 2020; 14: 888–895.

34. Grinman AB, de Souza MdGG, Bouskela E, et al. Clinical and laboratory markers associated with anti-TNF-alpha trough levels and anti-drug antibodies in patients with inflammatory bowel diseases. *Medicine* 2020; 99: e19359.

35. 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: 946–964.

36. Pugliese D, Guidi L, Privitera G, et al. Switching from IFX originator to biosimilar CT-P13 does not impact effectiveness, safety and immunogenicity in a large cohort of IBD patients. *Expert Opin Biol Ther* 2021; 21: 97–104.

37. 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: 2064–2064.

38. Stubenrauch K, Wessels U, Vogel R, et al. Evaluation of a biosensor immunoassay for simultaneous characterization of isotype and binding region of human anti-tocilizumab antibodies with control by surrogate standards. *Anal Biochem* 2009; 390: 189–196.

39. Real-Fernández F, Cimaz R, Rossi G, et al. Surface plasmon resonance-based methodology
for anti-adalimumab antibody identification and kinetic characterization. *Anal Bioanal Chem* 2015; 407: 7477–7485.

40. Gils A, Van Stappen T, Dreesen E, et al. Harmonization of infliximab and anti-infliximab assays facilitates the comparison between originators and biosimilars in clinical samples. *Inflamm Bowel Dis* 2016; 22: 969–975.

41. Thoren KL, Pasi B, Delgado JC, et al. Quantitation of infliximab and detection of antidrug antibodies in serum by use of surface plasmon resonance. *J Appl Lab Med* 2018; 2: 725–736.