Clinical Utility of Antihuman Lambda Chain-based Enzyme-linked Immunosorbent Assay (ELISA) Versus Double Antigen ELISA for the Detection of Anti-infliximab Antibodies

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Background: Anti-infliximab antibodies (ATIs) are associated with lower serum infliximab (IFX) trough levels and diminished clinical response. The current most prevalent method for detection of ATI is a double-antigen (DA) enzyme-linked immunosorbent assay (ELISA) utilizing IFX for ligand and detection antibody. Serum IFX interferes with ATI measurement in this method. An alternative ELISA using antihuman lambda chain (AHLC) antibody for ATI detection may be less amenable to this interference. The aim of our study was to compare the performance of AHLC-ATI versus DA-ATI for prediction of clinical response and evaluate the clinical significance of positive ATI in the presence of detectable IFX levels in IFX-treated inflammatory bowel disease (IBD) patients.

Methods: In all, 63 patients' sera were analyzed for IFX levels and antibody levels by AHLC and DA. The results were compared with the clinical response to IFX. Percentage of patients with IFX+ATI+ status among IFX-treated patients and the clinical outcome of IFX+ATI+ patients were assessed.

Results: ATIs were demonstrated in 22/63 (34.9%) and 18/63 (28.5%) sera of patients by AHLC and DA assay, respectively ($P = 0.6$). Detectable ATI and in IFX was detected in four patients (6.3%) by AHLC but not by DA assay. IFX+ATI+ status was documented in 8.7% of available sera and was associated with a trend for loss of response.

Conclusions: AHLC and DA ELISA are equally effective for ATI detection in patients with undetectable serum IFX. AHLC ELISA detects ATI in some patients with detectable serum IFX. This IFX+ATI+ status may be a harbinger of evolving loss of response to the drug.

(Inflamm Bowel Dis 2011;000:000–000)

Key Words: Crohn's disease, ulcerative colitis, anti-TNF antibodies, infliximab, anti-infliximab antibodies

Infliximab (IFX) is a chimeric mouse-human monoclonal anti-tumor necrosis factor (TNF) antibody effective for induction and maintenance of remission in Crohn's disease (CD) and ulcerative colitis (UC). However, 30%–84% of the patents lose response to the medication along the course of the treatment.[1–5] Trough levels of IFX significantly correlate with clinical response to IFX in CD[6,7] and UC[8] patients.

Anti-infliximab antibodies (ATI) develop in up to 61% of the patients.[6] The development of ATI is correlated with increased clearance and lower serum levels of IFX as well as increased risk for infusion reactions in CD patients.[6] However, data regarding the relationship between development of ATI and clinical response to IFX in inflammatory bowel disease (IBD) patients has been equivocal. One possible reason for this may be ascribed to the technique of conventional double-antigen (DA) enzyme-linked immunosorbent assays (ELISAs) for ATI measurement, which employs IFX as the capture antigen and labeled IFX as the detection antibody. Consequently, this method is susceptible to various technical limitations, including the inability to detect monovalent IgG4 ATI, which could possibly lead to false-negative results on the one hand, and plastic rheumatoid factor interactions yielding false-positive results[7] on the other. Moreover, IFX in serum competes with the detection antibody, thereby precluding the detection of ATI. This situation is commonly reported as inconclusive ATI and is reported in clinical trials and case series. For instance, inconclusive results of ATI levels were reported in 72% of patients included in the SONIC study[8] and in 39% of UC patients in a case series from Toronto.[9]

Additional Supporting Information may be found in the online version of this article.

Received for publication August 27, 2011; Accepted September 15, 2011.

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DOI 10.1002/ibd.21919

Published online in Wiley Online Library (wileyonlinelibrary.com).

These limitations of the DA technique hamper the ability to accurately define the role of ATI levels in predicting loss of response to IFX.

As an alternative to the DA technique, a radioimmunoassay technique for measuring ATI levels was described for both rheumatoid arthritis and CD patients.[10,11] Both of these studies demonstrated a significant correlation between ATI levels and loss of clinical response to IFX. However, this method is more cumbersome and is not widely available yet.

Another alternative to the DA ELISA was developed in our laboratory. It comprises an ELISA employing antihuman lambda chain (AHLC) conjugated antibody in the detection phase, taking advantage of the fact that IFX is composed of kappa chains. Notably, the presence of ATI demonstrated by this method correlates with loss of clinical response in CD patients treated with IFX.[7] Conceptually, the implementation of AHLC-conjugated antibody in the detection phase may allow detection of IgG4 ATIs and also diminish the magnitude of the interference caused by positive IFX levels, but this has not been hitherto tested. In fact, while the method of testing may bear significant implications for the ability to detect ATI and may thus impact clinical decisions derived from these results, so far there are no studies comparing the different ATI assays with respect to their clinical utility. Moreover, the inability of DA ELISA to detect ATI in the presence of IFX renders it impossible to accurately assess the evolution of antidrug antibody response over time and to investigate the clinical significance of coexistence of ATI with IFX.

Therefore, the aim of this study was to compare the accuracy of ATI detection by each of these two ELISA techniques (DA and AHLC), as defined by its correlation with clinical response or loss of response to treatment with IFX. The secondary objective was to evaluate the clinical characteristics and outcome of IFX-treated CD patients who developed ATI in the presence of detectable serum IFX (double positivity [DP]) as compared with patients who have never developed DP.

PATIENTS AND METHODS

Patient Population and Clinical Outcomes

IBD patients attending the gastroenterology departments of Sheba Medical Center and Rambam Health Care Campus and treated with IFX were included in the study. All patients gave written informed consent and the study was approved by the ethics committees of the Sheba Medical Center and the Rambam Health Care Campus. IFX trough levels and antibodies to IFX (by AHLC method) were available for 215 patients and were measured routinely immediately before the next infusion or upon a decision of the treating physician to discontinue IFX therapy.

To delineate the interaction between IFX in serum and the technical ability to detect ATI, sera were also deliberately obtained shortly after the infusion (6–21 days) in several patients with known ATI at trough-time measurements. These early postinfusion sera were subjected to IFX and AHLC-ATI measurements. In addition, in order to compare the performance of the AHLC method with another ATI detection methodology, ATI were also measured by a DA assay in a sample group ($n = 63$) out of the entire 215 patient cohort.

The clinical data were retrieved from the patients' charts and electronic records and reviewed retrospectively for the purpose of this study. The correlation of DA and AHLC ATI results with the clinical response of patients at the time of serum sampling was compared. Clinical response was defined as improvement or remission of CD-related symptoms coupled with a decision of the treating physician to continue IFX therapy at the current dosing and schedule.[14] Loss of response was defined by lack of improvement or aggravation of disease symptoms and by a decision to increase the dose or shorten the dose interval of IFX further, add immunomodulator therapy or corticosteroids, switch to another anti-TNF medication (Adalimumab) or refer for CD-related surgery.[14] Patients were excluded if loss of response was attributed to a non-CD related cause such as CMV or *Clostridium difficile* colitis.

Determination of IFX and ATI Levels

IFX levels were determined as previously described.[7] ATI were measured in sera samples by a commercially available DA ELISA and by the antihuman lambda antibody (AHLC) detection ELISA.

The AHLC ELISA was performed as previously described.[7] For the measurement of ATI by the DA detection method, we used a commercially available qualitative ATI DA assay: TNF-α-blocker-ADA (Immundiagnostik, Bensheim, Germany) and followed the instructions supplied by the manufacturer.

Spiking Experiments with Exogenous IFX

In order to assess the influence of presence of IFX in the serum on DA and AHLC assays more accurately, we performed spiking experiments with exogenously added IFX. For this purpose, several sera with known high levels of ATI and undetectable IFX were selected. These sera were pre-incubated for 60 minutes at room temperature with graded concentrations of extrinsic IFX (25, 5, 7.5, 10 μg/mL). ATI concentrations were then measured by each of the two techniques as described above.

Clinical Outcome of Patients with Positive ATI in the Presence of Detectable IFX (DP)

IFX treated patients who were found to have an IFX+ATI+ trough serum result on at least one occasion were identified from among the entire cohort ($n = 215$) of CD patients treated with IFX. Patients who had complete clinical

TABLE 1. Detection of ATI by AHLC and DA ELISA

	AHLC	DA	*P*-value
Patients in clinical remission ($n = 33$)	5 (15%)[a]	1 (3%)	0.2
Patients with loss of response ($n = 30$)	17 (57%)[b]	17 (57%)	1
Total ($n = 63$)	22 (35%)	18 (29%)	0.56

[a]Three patients with DP.
[b]One patient with DP.DA, double antigen detection ELISA assay; AHLC, antihuman lambda chain antibody-based ELISA; DP, double positivity (IFX+ ATI+).

and laboratory data available were included and were followed up for at least 6 months from first occurrence of double positivity (index infusion) or until onset of loss of response. Clinical response was defined as previously described at the index infusion (immediately following the determination of IFX trough level and ATI status) and after 6, 12 and 24 months from the index infusion.

Statistical Analysis

Continuous variables were analyzed by two-tailed Student t-test and categorical variables were analyzed by Fisher Exact test. All statistics were performed using MedCalc software (Mariakerke, Belgium). $P < 0.05$ was considered significant.

RESULTS

IFX and ATI Levels (Table 1)

Sixty three patients' sera were analyzed by both DA and AHLC. Out of the 63 tested patients, 33 (52%) clinically responded to IFX and 30 (48%) experienced loss of response (LOR) at the time of sampling. Forty-two out of 63 (66.7%) of the patients were being treated with a conventional maintenance dose of IFX (5 mg/kg every 8 weeks) at the time of sampling and 21/63 (33.3%) were receiving escalation regimens of IFX (5 mg/kg every 6 weeks, 5 mg/kg every 4 weeks, or 10 mg/kg every 8 weeks).

Detectable IFX levels were found in 40/63 patients (63.4%). A positive IFX trough level was detected in 31/33 (93.9%) clinically responsive patients and 9/30 (30%) of patients experiencing LOR. DA ELISA has demonstrated ATI in 18 (28.5%) patients. Seventeen of these patients were experiencing LOR to IFX when tested for ATI, and one patient was clinically responsive. AHLC antibody-based ELISA has demonstrated ATI in 22 (34.9%) patients ($P = 0.59$ for AHLC vs. DA, Fisher Exact test). Seventeen of these patients were experiencing LOR to IFX when tested for ATI, and five patients retained response to the drug. The degree of interrater agreement between the two methods (kappa value) for detection of ATI was 0.72 (95% confidence interval [CI] 0.54–0.9).

Four of the patients with positive AHLC-ATI had detectable serum IFX. All these patients tested negative for ATI by DA-ELISA. Thus, when only IFX-negative patients were included ATI were demonstrated in 17/23 and 18/23 patients by the AHLC and DA methods, respectively.

Spiking Experiments with Extrinsic IFX

In order to further test the performance of the different ELISAs in the presence of serum IFX, we conducted a spiking experiment by addition of extrinsic IFX. Nine serum samples with known high ATI levels and undetectable IFX by the AHLC method were tested by addition of graded concentrations of IFX followed by measurement of ATI by AHLC ELISA. As shown in Figure 1, the addition of 2.5 μg/mL of extrinsic IFX resulted in a 76% mean decline in the measured ATI levels by the AHLC method, compared to the measurement in the absence of exogenous IFX. Interestingly, further escalation of the concentration of added IFX to 5, 7.5, and 10 μg/mL did not have an additive influence on measured ATI levels.

Despite the addition of 2.5 μg/mL of extrinsic IFX, in three out of nine (33%) sera tested by AHLC method, the level of ATI remained above the reported positive cutoff value (1.7 μg/mL) and in one additional patient (11.1%) the ATI level was reported as borderline (1.5 μg/mL).

Three of these serum samples, which were either positive or borderline for ATI in the presence of extrinsic IFX by the AHLC technique, were also tested by the DA assay. In all these samples the addition of 2.5 μg/mL of extrinsic IFX completely abolished the optical density reading of ATI and resulted in undetectable ATI levels.

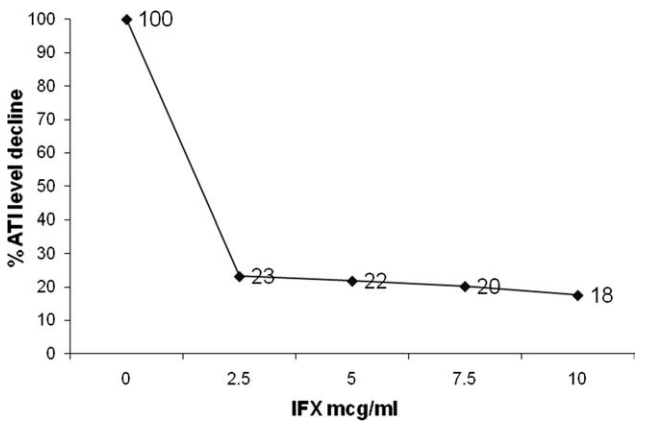

FIGURE 1. ATI levels in the presence of extrinsic infliximab. IFX, infliximab; ATI, anti-infliximab antibodies; DA, double antigen method; AHLC, anti-human lambda chain conjugated antibody method.

TABLE 2. Serum Infliximab and Anti-infliximab Antibody Levels in the Early Postinfusion Period

Sample *n*	Days Postinfusion	IFX,[a] μg/mL	ATI,[b] μg/mL
1	6	63.9	2.3
2	7	55	1.73
3	9	6	2.44
4	10	20.9	1.4
5	10	37	0.7
6	15	0.4	5.9
7	21	0.2	10

[a]IFX, infliximab (positive >1 μg/mL).
[b]ATI, anti-infliximab antibodies (positive >1.7 μg/mL, borderline 1.4-1.7 μg/mL).

Early Postinfusion Levels of IFX and ATI

In seven patients who had undetectable trough levels of IFX and positive ATI, another blood sample was deliberately obtained within several days after the infusion (Table 2) and tested for the presence of ATI by the AHLC methodology. All five samples obtained between days 6–10 of the index infusion were positive for the presence of IFX. In three of these samples ATIs were simultaneously detected.

Two serum samples obtained after 15 and 21 days, respectively, were positive for ATI and had no detectable IFX (Table 2).

The time course of IFX and ATI measurements at the index infusion, the next infusion and the in-between measurement is shown in Figure 2 for two exemplary patients. These findings show that AHLC is able to detect ATI in the presence of IFX in vivo, and that the pharmacokinetics of IFX clearance by ATI may pass through an intermediate stage of IFX+ATI+ before reaching the trough status of IFX−ATI+, at least in some patients.

Prevalence and Clinical Significance of the Double-positive Phenotype (ATI+IFX+)

To assess the prevalence of double-positive IFX+ATI+ phenotype among our IFX-treated patients, a search of all AHLC ATI ELISA results performed at both participating medical centers was performed. A total of 963 samples of IFX-treated patients were available for 215 individual IBD patients. A double-positive phenotype was obtained in 40/215 (18.6%) of IBD patients (32 CD and 8 UC patients). The double-positive phenotype occurred in some patients on several occasions, so the overall prevalence of the double-positive phenotype was 84/963 serum samples (8.7%).

Complete clinical and laboratory data for a follow-up period of at least 6 months from the first occurrence of the double-positive phenotype or until onset of LOR was available for 16/32 patients (Fig. 3). The clinical data of these 16 DP patients are depicted in Supporting Table 1.

As shown, out of the included 16 double-positive patients, 11 (68.8%) were in clinical remission when double positivity was encountered. After 6 and 12 months, 10 (62.5%) and 9 (56.3%) patients were in clinical remission, respectively. Twelve out 16 patients were followed up for at least 24 months. By this timepoint, two (21.4%) patients were still in clinical remission and 10 (78.6%) lost response to IFX. Five of the patients who lost response to IFX were treated with shortening of the therapeutic interval or increasing the dose, three patients were switched to adalimumab, and two patients were referred to surgery.

Evolution of IFX and ATI Status of DP Patients

The evolution of IFX and ATI status of the included patients is depicted in Figure 4. Seven (43.8%) had developed undetectable serum trough IFX levels after 6.3 ± 3.3 months, which was accompanied by loss of clinical response in five (71.4%) of these patients. Positive ATI were persistent in 12 (75%) of the patients and in four patients the ATI concentration dropped below the detection level after 6.7 ± 6.2 months. Seven patients had persistent

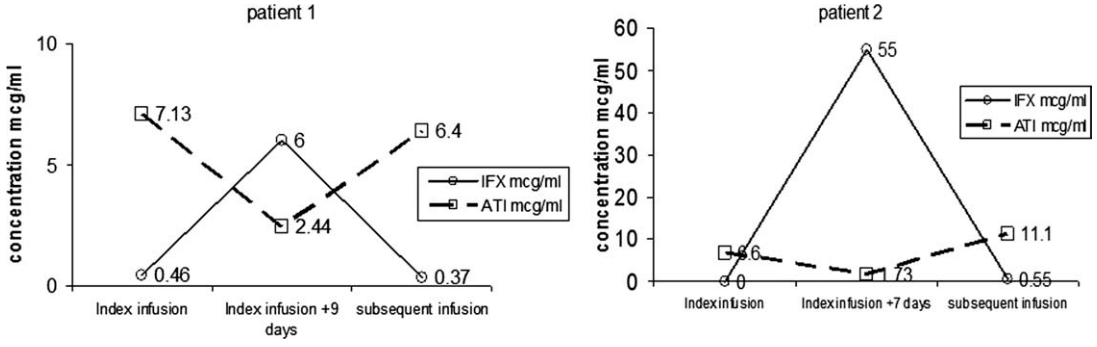

FIGURE 2. Early postinfusion levels of infliximab and ATI. IFX, infliximab; ATI, anti-infliximab antibodies.

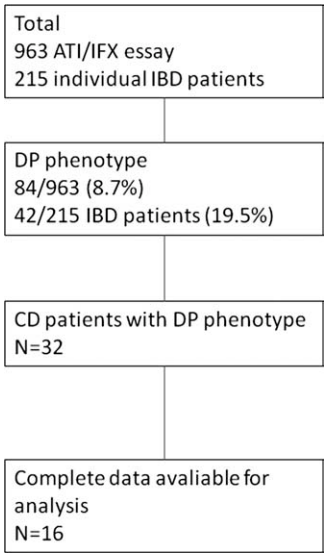

FIGURE 3. Flowchart for selection of double positive patients included in the study. IFX, infliximab; ATI, anti-infliximab antibodies; DP, double positivity (positive ATI in presence of detectable serum infliximab).

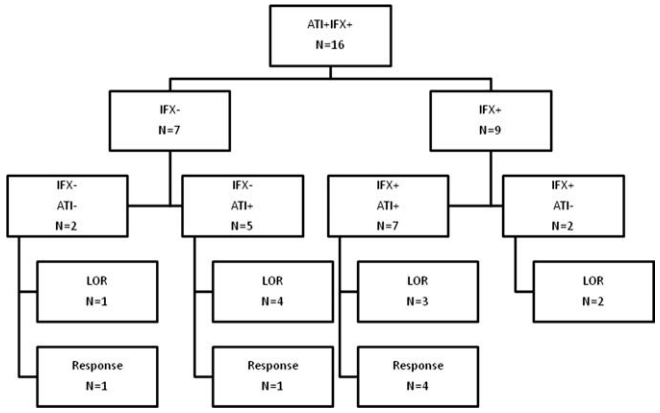

FIGURE 4. Clinical course and evolution of serum infliximab and ATI levels in DP patients. IFX, infliximab (+ − detectable serum infliximab; − − no detectable infliximab present in the serum). ATI, anti-infliximab antibodies (+ − detectable serum ATI; − − no detectable infliximab present in the serum). DP, double positivity (positive ATI in presence of detectable serum infliximab). LOR, loss of response (includes all patients who developed loss of response at any point of follow-up). Sustained response includes all patients who did not develop LOR at any point of follow-up. ATI and IFX status are reported as measured at time of development of LOR for patients who developed LOR, and until the end of follow-up for patients with sustained response.

DP status; four of these patients had sustained their response for 17.7 ± 5.3 months.

DISCUSSION

To the best of our knowledge, the present study is the first to compare two methods of ATI detection and investigate their clinical utility. The results show that the AHLC-based ELISA performs with a similar accuracy as a tested commercial DA-based ELISA for the detection of ATI in patients with undetectable serum IFX. However, AHLC-based ELISA was able to detect ATI in the sera of four patients with detectable serum IFX that were undetectable by the conventional DA-based ELISA. As 3/4 of these IFX+ATI+ were still responding at the time of sampling, the DA technique showed a numeric trend toward better specificity and positive predictive value of ATI detection for clinical LOR.

What are the implications of these findings for predicting clinical outcome and directing medical interventions in IFX-treated patients? There was a significant correlation between detectable ATI (by both methods) and loss of clinical response as reported by the treating physician. This finding is consistent with the previous data published by our group.[7] However, the correlation of ATI status with clinical course is not perfect, since nearly half of the patients losing response may not have detectable ATI in serum by any of the methods (Table 2). Conversely, a proportion of patients may still enjoy a sustained response in the face of detectable ATI. A number of factors may explain these observations. Loss of response may occur due to causes other than immunogenicity, antibodies may be nonneutralizing and neutralizing antibodies may have escaped detection due to the "window phenomena." The latter may be unmasked if patients are tested again at a later timepoint postinfusion. In this respect, the AHLC and DA assays achieved an overall comparable correlation with the clinical outcome in our study, likely because of the relatively small sample size.

In the lack of a gold standard technique for ATI measurement, comparing the technical performance accuracy of ATI assays may be problematic, especially regarding their ability to detect ATI in the presence of IFX. When measured within 10 days from IFX infusion, an IFX+ATI+ pattern was demonstrated in 3/3 patients with known positive ATI and undetectable serum trough IFX level before the index infusion. This observation is consistent with the existing data regarding IFX pharmacokinetics[12] and attests to the in vivo validity of this double-positive status. Moreover, double positivity for ATI and IFX was also reproducible in spiking experiments in vitro. Notably, addition of extrinsic IFX in concentrations that are commonly detected in clinical practice resulted in a moderate reduction in measured ATI levels by the AHLC method, presumably due to displacement of polyclonal/heterologous low-affinity ATI. However, ATI levels remained above the cutoff levels of positivity in 3/9 (33%) of the patients at serum concentrations of IFX commonly occurring in clinical practice. In contrast, addition of extrinsic IFX resulted in undetectable ATI in all tested samples by the DA technique. These experiments corroborated that the AHLC method is less

affected by the presence of IFX in serum compared to the DA-based assays and is still able to detect "true" ATI in this situation, at least in some patients.

From the clinical perspective, ATI+IFX+ pattern can also be demonstrated in samples obtained 6–8 weeks from IFX infusion. When tested by the DA ELISA, these patients' ATI status would have been reported as "inconclusive." This ATI status was associated with higher rates of steroid-free remission[8] and longer median duration of remission.[13] Because all of these patients had detectable serum IFX that is associated with a superior clinical outcome compared to patients with undetectable trough IFX levels,[6,8] this observation is not surprising. However, in the subgroup of patients who develop positive ATI when IFX is still detectable in the serum, this may serve as an indicator of evolving immune response to IFX that would result in future low trough levels and loss of response. This specific patient population has never been previously addressed due to technical inability to detect this double positivity status.

We examined the clinical outcome of these double-positive patients. Thirty-one percent of these patients experienced LOR upon detection of double positivity status, while 10/12 patients (78%) developed LOR within 24 months of an index ATI+IFX+ measurement. Interestingly, development of LOR was associated with abatement of trough serum IFX level in only half of the patients. This observation supports the existence of an alternative pathway for development of LOR, which may be nonimmune or mediated by neutralizing antibodies that do not influence the drug level as measured by available solid-phase ELISA assays. Thus, coexistence of detectable serum IFX and ATI may have a considerable clinical significance. This finding could be an indicator of a present or evolving immune response to IFX and imminent development of LOR. Conceptually, the ability to predict this reaction early may prove useful for introducing preemptive interventions to prevent the occurrence of LOR to IFX. Nonetheless, further studies in a larger cohort of patients are required to clarify the significance of these findings and to elucidate the value of potential preventive strategies once IFX+ATI+ status is detected.

Our study has several limitations. The clinical data for the patients were collected retrospectively, and no objective clinical scoring system was employed. A prospective study employing established clinical and endoscopic scoring system is required for the formal clinical validation of the AHLC-ATI ELISA. However, from the practical point of view, the clinician's decision to discontinue or escalate IFX coupled with subjective patients' clinical deterioration probably reflects the real-life assessment of the severity of the disease and the clinical decision making.[14] Another limitation is that the assays examined are solid-phase assays and a fluid phase test such as a radioimmunoassay (RIA) was not tested in the present study. Nonetheless, RIA is more cumbersome and less available in clinical laboratories compared to standard ELISA techniques. Notwithstanding, it would be of value as a next step to perform similar comparative studies of both solid phase and fluid phase methodologies for ATI detection. Finally, the number of patients with ATI+IFX+ was small and does not enable us to draw any statistically definitive conclusions. However, it does point to the existence and significance of this phenomenon as a possible harbinger of a future abatement of serum drug level and development of LOR.

In conclusion, the AHLC antibody-based ELISA method is at least as accurate as the DA-based ELISA for detection of anti-IFX antibodies. In a subgroup of patients this method identifies ATI in the presence of detectable IFX in the serum. This finding may be clinically significant for being a forerunner of LOR to IFX in some patients, and for possibly allowing us to undertake early preventive interventions.

REFERENCES

1. Hanauer S, Feagan B, Lichtenshtein G, et al. Maintenance infliximab for Crohn's Disease: the ACCENT 1 randomized trial. Lancet. 2002; 359:1541–1549.
2. Sands BE, Anderson FH, Bernstein CN, et al. Infliximab maintenance therapy for fistulizing Crohn's disease. N Engl J Med. 2004;350: 876–885.
3. Schnitzler F, Fidder H, Ferrante M, et al. Long-term outcome of treatment with infliximab in 614 patients with Crohn's disease: results from a single-centre cohort. Gut. 2009;58:492–500.
4. Gisbert JP, Panes J. Loss of response and requirement of infliximab dose intensification in Crohn's disease: a review. Am J Gastroenterol. 2009;104:760–767.
5. Ben-Horin S, Chowers Y. Review article: loss of response to anti-TNF treatments in Crohn's disease. Aliment Pharmacol Ther. 2011; 33:987–995.
6. Baert F, Noman M, Vermeire S, Van Assche G, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. N Engl J Med. 2003;348:601–608.
7. Ben-Horin S, Yavzori M, Katz L, et al. The immunogenic part of infliximab lies within the Fab fragment, but measurement of antibodies against the intact infliximab molecule shows superior clinical usefulness. Gut. 2011;60:41–48.
8. Colombel JF, Sandborn WJ, Reinisch W, et al. SONIC Study Group. Infliximab, azathioprine, or combination therapy for Crohn's disease. N Engl J Med. 2010;362:1383–1395.
9. Seow CH, Newman A, Irwin SP, et al. Trough serum infliximab: a predictive factor of clinical outcome for infliximab treatment in acute ulcerative colitis. Gut. 2010;59:49–54.
10. Ainsworth MA, Bendtzen K, Brynskov J. Tumor necrosis factor-alpha binding capacity and anti-infliximab antibodies measured by fluid-phase radioimmunoassays as predictors of clinical efficacy of infliximab in Crohn's disease. Am J Gastroenterol. 2008;103:944–948.
11. Wolbink GJ, Vis M, Lems W, Voskuyl AE, et al. Development of anti-infliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis. Arthritis Rheum. 2006;54:711–715.
12. Klotz U, Teml A, Schwab M. Clinical pharmacokinetics and use of infliximab. Clin Pharmacokinet. 2007;46:645–660.
13. Maser E, Villela R, Silverberg M, et al. Association of trough serum infliximab to clinical outcome after scheduled maintenance treatment for Crohn's disease. Clin Gastroenterol Hepatol. 2006;4:1248–1254.
14. Kopylov U, Mantzaris GJ, Katsanos KH, et al. The efficacy of shortening the dosing interval to once every six weeks in Crohn's patients losing response to maintenance dose of infliximab. Aliment Pharmacol Ther. 2011;33:349–357.