



Observational Study

Significance of low level infliximab in the absence of anti-infliximab antibodies

Bella Ungar, Adi Anafy, Henit Yanai, Yulia Ron, Miri Yavzori, Orit Picard, Ella Fudim, Ronen Loebstein, Uri Kopylov, Yehuda Chowers, Iris Dotan, Rami Eliakim, Shomron Ben-Horin

Bella Ungar, Adi Anafy, Miri Yavzori, Orit Picard, Ella Fudim, Uri Kopylov, Rami Eliakim, Shomron Ben-Horin, Department of Gastroenterology, Sheba Medical Center Tel Hashomer and Sackler School of Medicine, Tel-Aviv University, Tel-Aviv 6997801, Israel

Henit Yanai, Yulia Ron, Iris Dotan, IBD Center, Department of Gastroenterology, Tel-Aviv Sourasky Medical Center and Sackler School of Medicine, Tel-Aviv University, Tel-Aviv 6997801, Israel
Ronen Loebstein, Institute of Clinical Pharmacology, Sheba Medical Center Tel Hashomer and Sackler School of Medicine, Tel-Aviv University, Tel-Aviv 6997801, Israel

Yehuda Chowers, Rambam Health Care Campus and Bruce Rappaport School of Medicine, Technion Institute of Technology, Haifa 31999, Israel

Author contributions: Ungar B, Anafi A and Yanai H analyzed the data; Ungar B drafted the manuscript; Yavzori M, Fudim E and Picard O performed the laboratory research; Kopylov U, Ron Y, Loebstein R, Dotan I and Eliakim R assisted in data acquisition and drafted the manuscript; Ben-Horin S conceived the study, designed the research and drafted the manuscript; all authors finally approved the manuscript.

Supported by (in part) "Talpiot" Medical Leadership program of the Sheba Medical Center (to SBH) and the Helmsley Charitable Trust (To SBH, RE, ID and YC).

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Correspondence to: Bella Ungar, MD, Department of Gastroenterology, Sheba Medical Center Tel Hashomer and Sackler School of Medicine, Tel-Aviv University, Tel-Aviv 6997801, Israel. bellageyshis@gmail.com

Telephone: +972-3-5302694

Fax: +972-3-5303160

Received: August 8, 2014

Peer-review started: August 9, 2014

First decision: August 27, 2014

Revised: September 8, 2014

Accepted: October 14, 2014

Article in press: October 15, 2014

Published online: February 14, 2015

Abstract

AIM: To evaluate the prevalence of double negative (DN) sera and the mechanisms responsible for DN status.

METHODS: Sera of inflammatory bowel disease patients treated with infliximab (IFX) were tested for drug/antibodies to infliximab (ATI) trough levels and the proportion of DN results was compared between a commercially available double antigen ELISA (with labeled IFX as the detection antibody) and an anti-lambda ELISA (with anti-human lambda chain detection antibody). Repeat testing with lower than customary serum dilution (1:10) was performed. Patients with DN status were matched with IFX+/ATI- controls and were followed-up for subsequent development of non-transient ATI to investigate if DN status precedes ATI.

RESULTS: Of 67 sera obtained at time of loss of response, only 6/67 (9%) were DN by anti-lambda ELISA compared to 27/67 (40%) with double antigen ELISA ($P < 0.001$, Fisher's Exact test). Of the latter 27 sera, 22% were also DN by anti-lambda ELISA, whereas 44% were actually IFX positive (IFX+ATI-), 30% were ATI positive (IFX-ATI+) and 4% were double positive (IFX+ATI+). Re-testing using a 1:10 dilution converted most DN results into IFX+ and /or ATI+ status. Patients with DN status had shorter survival free of non-transient ATI compared with matched controls (log rank test, $P < 0.001$). In 9/30 (30%) of these patients, non transient ATI occurred before and after the event at which the DN serum was obtained, supporting the view that a DN result may represent a

particular time-point along the two curves of ATI titer rise and infliximab drug level decline.

CONCLUSION: DN status may result from false negative detection of IFX or ATI by double antigen ELISA, suggesting a transitional state of low-level immunogenicity, rather than non-immunological clearance.

Key words: Inflammatory bowel disease; Biological therapy; Infliximab; Immunology; Drug response

© **The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Among patients who lose response to infliximab (IFX) 10%-60% have low IFX levels in the absence of antibodies to infliximab (ATI) - double negative (DN) status. We explored the prevalence and the mechanisms responsible for DN status. The prevalence of DN sera varied with the assay and dilution used. Patients with DN status had shorter survival free of ATI compared with matched controls ($P < 0.001$). We believe that DN status may result from false negative detection of IFX or ATI by a conventional ELISA assay, suggesting a transitional state of low-level immunogenicity, rather than non-immunological drug clearance.

Ungar B, Anafy A, Yanai H, Ron Y, Yavzori M, Picard O, Fudim E, Loebstein R, Kopylov U, Chowers Y, Dotan I, Eliakim R, Ben-Horin S. Significance of low level infliximab in the absence of anti-infliximab antibodies. *World J Gastroenterol* 2015; 21(6): 1907-1914 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v21/i6/1907.htm> DOI: <http://dx.doi.org/10.3748/wjg.v21.i6.1907>

INTRODUCTION

Infliximab (IFX) is a chimeric mouse - human monoclonal immunoglobulin G1 (IgG1) antibody against tumor necrosis factor α (TNF α). It is effective in inducing and maintaining remission in crohn's disease (CD) and ulcerative colitis (UC)^[1-3]. Between 30%-70% of patients who initially respond to IFX subsequently lose their response and experience exacerbation of symptoms, necessitating either dose escalation, switch to another anti-TNF agent, concomitant immunomodulator therapy or surgical intervention^[4-6]. Antibodies to infliximab (ATI) develop in approximately 40% of IFX treated patients and correlate with lower IFX trough levels and clinical loss of response (LOR)^[7,8]. In 10%-60% of LOR patients, pharmacokinetic tests reveal low IFX trough levels and absence of detectable ATI, designated double negative (DN) status (IFX-/ATI-)^[5,9]. Furthermore, several studies, including the SONIC trial, demonstrated that among patients with LOR, the DN status was in fact the more common scenario rather than

the expected IFX-/ATI+ status^[7,10].

There is a lack of data regarding the mechanisms responsible for the DN status and its consequence. DN status has been attributed to both immune and non-immune clearance of anti-TNF, as well as to technical limitations, such as non-uniform timing of measurement (trough levels are more sensitive than in-between infusions)^[5,11]. The uncertainty about the causes and implications of an IFX-/ATI- status makes it hard to establish optimal strategies to prevent and/or manage LOR events in the presence of such a pharmacokinetic situation.

The aims of the present study were to evaluate the frequency and clinical significance of DN status among IFX-treated IBD patients (both in general and at time of LOR) and to investigate the impact of the diagnostic technique on the incidence of this phenomenon.

MATERIALS AND METHODS

Study design and patient population

The study population included IBD patients treated with IFX at the gastroenterology departments of Sheba medical center and the Tel-Aviv Sourasky Medical Center between February 2009 and October 2013, who had available sera stored. All participants provided written informed consent and the ethics committees of the two medical centers approved the study. Pre-infusion sera were obtained and analyzed for trough IFX and ATI levels. Sera of patients whose infusions were delayed for over 2 wk from the scheduled date were excluded.

The study consisted of two separate parts: (1) an analytical part, which targeted differences between assays and technical limitations; and (2) a clinical part, aiming to study the natural history of the DN phenomenon (Figure 1). In the analytical part of the study, IFX and ATI trough levels of patients experiencing LOR were evaluated using two different ELISA assays: double antigen and anti-lambda ELISA. Subsequently, the fraction of IgG4 ATI was measured and compared in a sample of patients with discrepant results between the two ELISA assays to investigate if the conflicting results stemmed from a predominant monovalent IgG4 ATI response. Finally, to investigate the analytical accuracy of the anti-lambda ELISA, this assay was repeated in 45 randomly selected DN sera using a serum dilution of 1:10 (rather than the conventional 1:100 dilution). Patients' sera in this analysis were tested regardless of response status, and sera of healthy volunteers unexposed to IFX served as controls.

The clinical part was a case-control study; cases were patients with IBD who had at least one DN (IFX-/ATI-) measurement during routine follow-up (not necessarily at a point of LOR) and controls were IBD patients with positive drug levels without ATI (IFX+/ATI-). The starting point of the analysis was defined as the month of the DN event in the

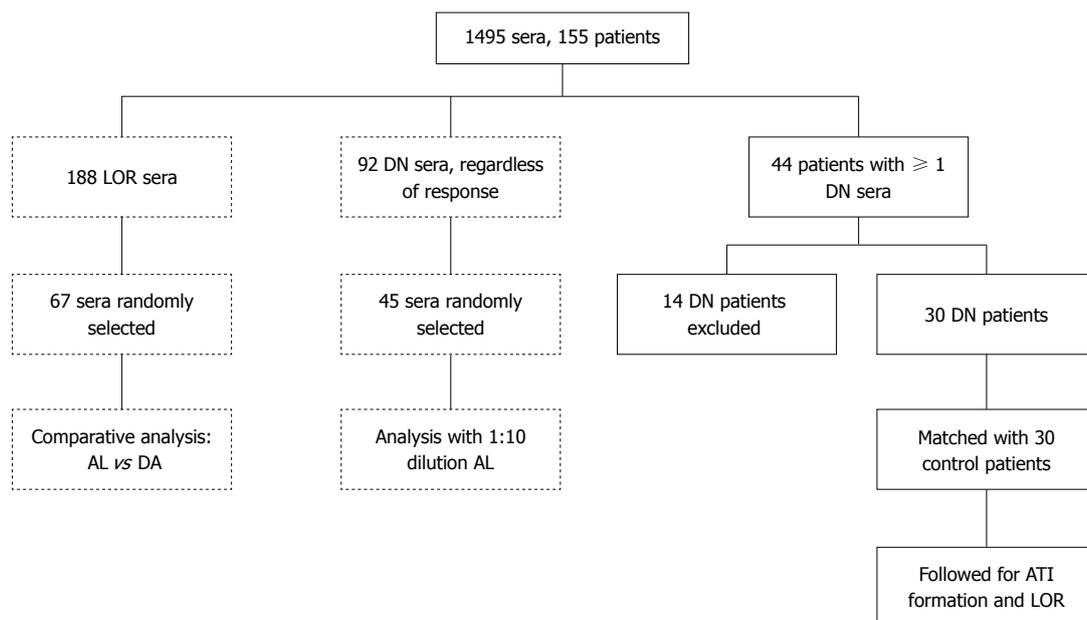


Figure 1 Flow chart of the patients included in the two parts of this study. The analytical part (dashed lines) comprised a comparison of two different assays and of two different serum dilutions; the clinical part (solid lines) followed up, in a case-control study, double negative patients versus patients with adequate infliximab levels for subsequent antibodies to infliximab formation and clinical outcome. DN: Double negative; LOR: Loss of response; AL: Anti-lambda; DA: Double-antigen; IFX: Infliximab; ATI: Antibodies to infliximab.

cases and the matching month in the controls. The pharmacokinetics at the end of follow-up were correlated to clinical outcome. Cases and controls were matched according to the duration of IFX therapy. Patients with unavailable subsequent measurements were excluded. Antibody formation was defined as positive when a patient tested positive for ATI during follow-up on more than two consecutive time points. Transient antibodies were defined as measurable ATI on up to two consecutive infusions, which disappeared on subsequent infusions without any alteration of therapy^[8,12,13]. Permanent, non-transient, ATI comprised the primary end point, while transient ATI were disregarded. Clinical response was defined by an improvement in disease activity indexes, the Harvey-Bradshaw index (HBI) and the simple clinical colitis activity index (SCCAI) for CD and UC patients, respectively, coupled with a treating physician's decision to continue IFX therapy without alteration. Clinical response was evaluated on the day of IFX infusion. Secondary LOR was defined as increased disease activity (a rise of > 3 points in HBI score or of > 2 points in SCCAI for CD and UC, respectively) after achieving an appropriate induction response^[14-16]. When unavailable, clinical response was determined by the documented physician's global assessment.

Measurement of IFX by TNF- α -blocker-monitoring infliximab drug level ELISA

IFX levels were measured by a commercially available quantitative ELISA, TNF- α -blocker-monitoring (Immundiagnostik, Bensheim, Germany), following the manufacturer's instructions. The assay's detection

threshold was IFX > 1 μ g/mL.

Measurement of ATI by TNF- α -blocker-ADA double antigen ELISA

ATI levels were measured by a commercially available qualitative TNF- α -blocker-ADA (antibodies against infliximab, Immundiagnostik, Bensheim, Germany), following the manufacturer's instructions. The assay's detection threshold was ATI > 10 AU/mL, which was standardized in our laboratories to 1 AU/mL.

In house determination of IFX levels

A volume of 100 μ L of 1:100 diluted serum was added to pre-plated 750 ng/mL TNF α (Peprotech, Rocky Hill, NJ, United States) and incubated for 90 min. Following washing, horseradish peroxidase (HRP) labeled goat anti-human Fc fragment antibody (MP Biomedicals, Solon, OH, United States) at a concentration of 0.62 μ g/mL was added for 60 min and reacted with the tetramethylbenzidine (TMB) substrate. The results were then read on an ELISA reader. Quantification of the measured IFX concentration was done by calibration to a standard curve in which exogenous IFX (Schering Plough, NJ, United States) was added at concentrations between 3 and 200 ng/mL. The assay's detection threshold was IFX > 0.6 μ g/mL.

Measurement of ATI by anti-human lambda chain detection antibody ELISA

ATI were determined as previously described^[11,17]. Briefly, IFX (0.1 mg/mL) was added to pre-plated TNF α (500 ng/mL) in 100 μ L wells of ELISA plates

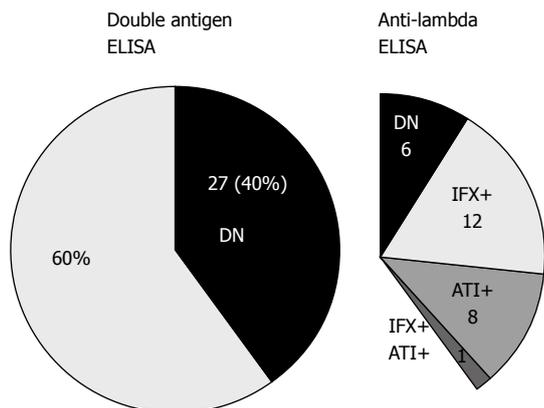


Figure 2 ELISA tests of patients with loss of response. Out of 67 patients with loss of response, 27 (40%) were double negative with double antigen ELISA. Of those, only 6 (9%) were DN using anti-lambda ELISA. LOR: Loss of response; DN: Double negative; IFX: Infliximab; ATI: Antibodies to infliximab.

(Nunc, Roskilde, Denmark). After drying, 100 µL of serum (1:100 dilution) was added and incubated for 90 min at room temperature. Plates were then washed and goat anti-human λ chain HRP-labeled antibody (Sertec, Oxford, United Kingdom) was added at a dilution of 2.5×10^4 for 60 min and reacted with the TMB substrate. The results were read by an ELISA reader EL-800 (Biotek Instruments, Winooski, VT, United States) and expressed as mcg/mL-equivalent (mcg/mL-e) after normalization vs results obtained using additions of graded concentrations between 9 and 600 ng/mL of HRP labeled goat anti-human F(ab')₂ fragment antibody (MP Biomedicals). The assay's detection threshold was ATI > 2.5 µg/mL-eq.

Measurement of IgG4 fraction of ATI

IFX (0.1 mg/mL) was added to pre-plated TNFα (500 ng/mL) in 100 µL wells of ELISA plates (Nunc). After drying, 100 µL of diluted serum (1:100) was added and incubated at room temperature. Plates were then washed and an HRP-labeled monoclonal antibody to human IgG4 (fc-specific, Acris antibodies CN AM20252HR-N) was added and reacted with the TMB substrate. The results were read by an ELISA reader EL-800 (Biotek Instruments) and expressed as mcg/mL. Normalization was obtained using graded concentrations of Human IgG4 Kappa (Millipore CN AG508).

Statistical analysis

Categorical variables were analyzed by Fisher's exact test. Kaplan-Meier survival curves were plotted to assess the temporal rate of events and the log rank test was computed for the comparison between survival free durations. Odds ratio and 95%CI were computed for all compared variables. The analysis was performed using MedCalc software (version 12.2.1.0, Mariakerke, Belgium). A two-tailed *P* < 0.05

was considered statistically significant.

RESULTS

Prevalence of the DN status using two ELISA assays

Out of 188 sera obtained from patients with LOR during regular IFX therapy, 67 were randomly selected for comparative analysis using the two techniques (anti-lambda and double antigen ELISA, Figure 1). In this analysis, 27/67 sera (40%) tested IFX-/ATI- with double antigen-ELISA compared to 6/67 (9%) with anti-lambda-ELISA (*P* < 0.001, Fisher's exact test). The calculated number needed to test (NNT) for a false-negative DN result by double-antigen ELISA was 3.2. As depicted in Figure 2, when applying anti-lambda-ELISA to the 27 sera that were IFX-/ATI- with the double antigen assay, only 6 (22%) remained DN, while 12 (44%) were actually IFX positive (IFX+ATI-), 8 (30%) were ATI positive (IFX-ATI+) and one serum (4%) was double positive (IFX+ATI+).

Double negativity on 1:10 dilution anti-lambda ELISA

When investigating the occurrence of double negativity regardless of patients' response status, we found that only 92 of the 1495 sera (6%) analyzed at our center between 2009-2013 were DN by anti-lambda ELISA (Figure 1). To examine whether some of these DN sera represented low-titer ATI or low level IFX, we randomly selected 45 DN sera and re-tested them at a 1:10 dilution to increase analytical sensitivity (compared with standard anti-lambda testing using 1:100 serum dilution). Upon this 1:10 dilution test, 24 (53%) DN sera became IFX positive (IFX+ATI-), 15 (33%) were double positive (IFX+ATI+) and 5 (11%) were ATI positive (IFX-ATI+). Only one serum (2%) retained its double negativity on 1:10 dilution (Figure 3). This transformation into detectable levels on 1:10 dilution was primarily caused by the fact that all 30 sera of healthy controls unexposed to IFX remained DN when tested by 1:10 dilution, but with lower detection cut-off levels.

Determination of IgG4 vs IgG1 ATI

IgG4 are monovalent antibodies (as opposed to the bivalent IgG1), and are thereby detectable by the anti-lambda ELISA, rather than by the double antigen assay^[18]. Therefore, we assumed that a DN status on double antigen ELISA might be a result of non-detection of IgG4 ATI. To test this, we analyzed five sera that were DN by the double antigen ELISA and ATI positive by the anti-lambda ELISA (IFX-ATI-), as well as five sera that were ATI positive on both assays (IFX-ATI+). Contrary to our assumption, IgG4 levels were higher among the double antigen ELISA ATI+ positive sera (Median 6.6, IQR 0.9-7.4 vs median 0.5, IQR 0.07-0.97, *P* = 0.047, respectively).

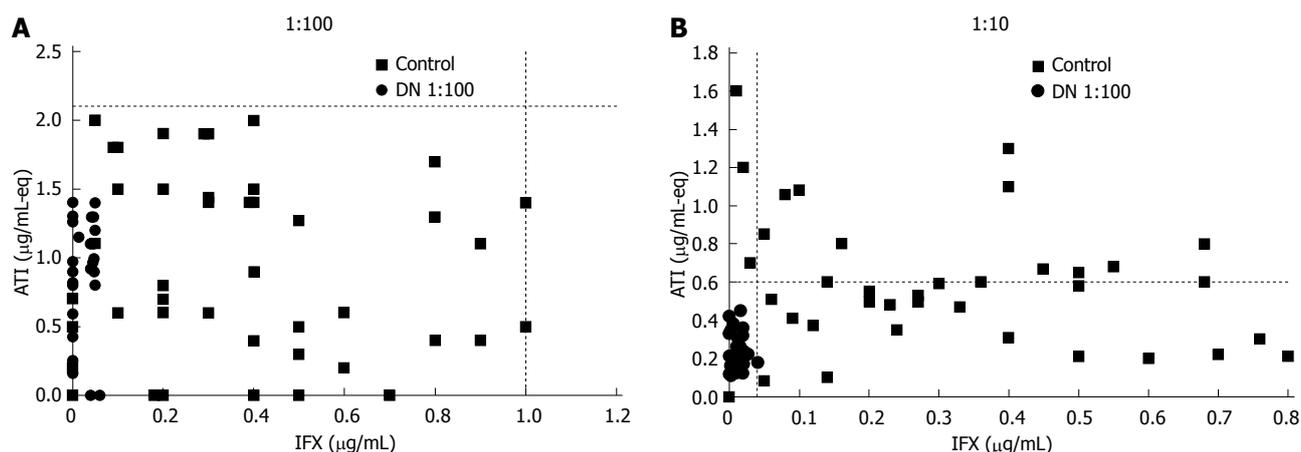


Figure 3 Re-testing of double negative sera to increase analytical sensitivity. To examine whether some of the double negative sera represented low-titer antibodies to infliximab or low-level infliximab, we randomly selected 45 double negative sera and re-tested them at a 1:10 dilution to increase analytical sensitivity (compared with standard anti-lambda testing using 1:100 serum dilution). A: IFX and ATI values of double negative vs healthy controls' sera, analyzed at 1:100 dilution anti-lambda ELISA. Cut off values for double negativity: IFX < 1 µg/mL, ATI < 2.1 µg/mL-eq. DN sera - black squares, healthy controls - circles; B: IFX and ATI values of sera DN on 1:100 dilution vs healthy controls analyzed at 1:10 dilution anti-lambda ELISA. Cut off values for double negativity: IFX < 0.04 µg/mL, ATI < 0.6 µg/mL-eq. Previously DN sera (on 1:100 dilution) - black squares, healthy controls - circles. DN: Double negative; IFX: Infliximab; ATI: Antibodies to infliximab.

Subsequent ATI formation and clinical response rate in DN patients

To investigate whether DN status is a harbinger of pending immunogenicity, we sought to determine whether patients with DN sera were predisposed to develop ATI compared with patients with measurable IFX (IFX+ATI-). During the study period, 44 out of 155 patients on standard IFX regimen had at least one DN serum sample determined by anti-lambda ELISA (Figure 1). Fourteen of them were excluded from analysis because of missing data (10 were inconsistently followed, two were lost to follow up after the DN event and two received infusions outside our center). Thus, 30 patients (25 CD, 5 UC) were included and matched with 30 controls (27 CD, 3 UC). Median follow up time was 21 ± 25.1 mo vs 20.75 ± 25.5 mo, respectively, $P = 0.97$. The patients' demographic and clinical characteristics are presented in Table 1.

ATI formation was significantly more frequent among the DN group compared with controls (OR = 11, 95%CI: 3.3-36.8, $P \leq 0.001$). In 9/30 (30%) DN patients who developed non-transient ATI, ATI formation occurred both before and after the event at which the DN serum was obtained (Figure 4), supporting the fact that a DN result may represent a particular time-point along the two curves of ATI titer rise and IFX drug level decline.

To investigate the temporal evolution of immunogenicity in DN patients, Kaplan-Meier analysis was performed. ATI free survival was significantly longer among controls (log rank test, $P < 0.001$, Figure 5A) than among DN cases. Of note, ATI appearance prior to the DN status was disregarded in this analysis. Nevertheless, when considering ATI existence before the starting point as positive, similar results were obtained (log rank test, $P < 0.001$). Secondary LOR

was also more frequent among DN cases (OR = 4.66 95%CI: 1.57-13.86, $P = 0.006$) and survival free of secondary LOR was significantly shorter than among controls ($P = 0.02$, log rank test, Figure 5B).

DISCUSSION

A substantial portion of IFX treated patients develop low trough levels of IFX in the absence of measurable ATI (IFX-ATI-), *i.e.*, a DN status. Several studies have demonstrated that among patients with LOR, a DN result is more prevalent than antibody positive sera^[10,19]. The actual mechanism of LOR remains unclear in most such cases, and the role - if any - of immunogenicity in instigating this phenomenon remains to be determined. In addition to assay limitations and irregular sampling time-points, DN status has been attributed to non-immune clearance of anti-TNF, high tissue inflammatory burden "absorbing" anti-TNF drug and temporal "window phenomenon", which refers to sampling when all drug-ATI complexes have been cleared^[5,11,20].

Studies that employed double antigen ELISA assay reported 20%-40% of the patients as DN, regardless of response status^[7,9,21,22]. Vande Castele *et al*^[12] recently demonstrated a prevalence of only 11% using the homogeneous mobility shift assay (HMSA). Little data exists comparing different methods for IFX and ATI level measurement. Steenholdt *et al*^[23] recently demonstrated a lower detection rate of ATI using double-antigen ELISA than by other assays. In the current study, double negativity was significantly more prevalent when using the double antigen ELISA compared with the anti-lambda ELISA (40% vs 9%, $P < 0.001$). Furthermore, when applying anti-lambda ELISA to the sera that were IFX-/ATI- by the double antigen assay, only six (22%) remained DN.

Table 1 Background disposition and clinical characteristics n (%)

Parameter	Cases	Controls	P value	OR (95%CI)
Gender			0.19	2.0 (0.70-5.70)
Male	15 (50)	10 (33.3)		
Female	15 (50)	20 (66.6)		
Type of IBD			0.45	0.56 (0.12-2.57)
CD	25 (83.3)	27 (90.0)		
UC	5 (16.7)	3 (10.0)		
Duration of IFX therapy ¹ (mo)	21 ± 25.1	20.75 ± 25.5	0.97	
Concomitant therapy	11 (36.7)	11 (36.7)	1.00	1.00 (0.35-2.86)
Episodic therapy	6 (20)	6 (20.0)	1.00	1.00 (0.28-3.54)
Median age (yr)	33 ± 15.2	28.5 ± 10.7	0.24	
Median disease duration (yr)	10.5 ± 9	9 ± 7.7	0.65	
Median age at diagnosis (yr)	22 ± 12.4	20 ± 9.7	0.19	
CD - disease location				
Ileal	9 (36)	8 (29.7)	0.62	1.30 (0.41-4.30)
Ileo-colonic	8 (32)	11 (40.7)	0.51	0.68 (0.22-2.10)
Colonic	8 (32)	8 (29.6)	0.85	1.10 (0.34-3.63)
CD - upper GI involvement	2 (8)	2 (7.4)	0.93	1.08 (0.14-8.40)
CD - anal/perianal involvement	13 (52)	14 (51.8)	0.99	1.00 (0.34-3.00)
CD - disease behavior				
Non stricturing non penetrating	12 (48)	12 (44.5)	0.79	1.15 (0.38-3.43)
Stricturing	8 (32)	10 (37.0)	0.70	0.80 (0.25-2.50)
Penetrating	5 (20)	5 (18.5)	0.89	1.10 (0.28-4.40)
UC - disease location				
Proctitis	0	0		
Left-sided colitis	2 (40)	1 (33.3)		
Extensive colitis	3 (60)	2 (66.7)		
Extra-intestinal manifestations	15 (50)	15 (50.0)	1.00	1.00 (0.36-2.75)
Smoking	2 (6.7)	5 (16.7)	0.24	0.30 (0.06-2.00)
Immunomodulator therapy prior to infliximab therapy	26 (86.7)	23 (76.7)	0.32	1.97 (0.51-7.63)
Adalimumab therapy prior to infliximab therapy	4 (13.3)	0	0.12	10.30 (0.53-201.00)
Surgery prior to infliximab therapy	8 (26.7)	7 (23.3)	0.76	1.19 (0.37-3.85)

¹All patients received infliximab infusions at a standard protocol of 5 mg/kg at 0, 2, 6 and every 8 wk. IBD: Inflammatory bowel disease; CD: Crohn's disease; UC: Ulcerative colitis; GI: Gastro-intestinal.

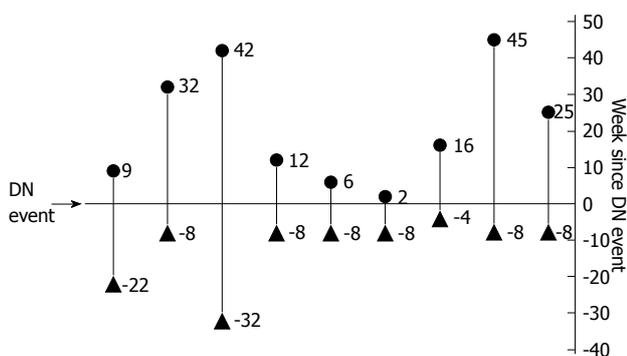


Figure 4 Temporal formation of antibodies to infliximab in nine patients in whom antibodies to infliximab positive sera preceded double negative sera. ATI formation before DN event (triangles), ATI formation after DN event (circles). ATI: Antibodies to infliximab; DN: Double negative.

We assumed that the higher frequency of double negativity using double antigen ELISA stems at least partly from false negative detection of IFX or ATI. As IgG4-ATI levels were not higher among the double antigen ELISA DN sera, double negativity cannot be attributed to the technical inability of double antigen ELISA to detect ATI in patients with a predominance of IgG4-ATI.

Interestingly, only one serum out of 45 examined

with 1:100 dilution anti-lambda ELISA retained its double negativity at 1:10 dilution anti-lambda. The other sera became mostly IFX+ATI- or IFX-ATI+. The fact that almost all sera "lost" their DN status at 1:10 dilution implied that at least part of this phenomenon probably arises from low drug and ATI levels close to the detection threshold of the more sensitive anti-lambda assay. These sera may reflect a transitional state of immunological equilibrium between antibody-mediated IFX clearance and endogenous ATI production, rather than genuine non-immunological drug clearance. Notably, in clinical practice, physicians should be aware that some patients may present with DN status at trough merely because of arriving late for a delayed infusion. Such cases were excluded from the present work.

Few studies have addressed the question of subsequent ATI development in patients with DN sera. Hanauer *et al*^[7] demonstrated that only 2.5% of DN patients turned ATI+ at week 76, although IFX infusions were halted at week 46. By contrast, Seow *et al*^[9] showed that 77% of DN UC patients later developed ATI, regardless of response status. In our study, double negativity was also predictive of future non-transient ATI formation. This re-enforced our conclusion that DN status is an immunologically

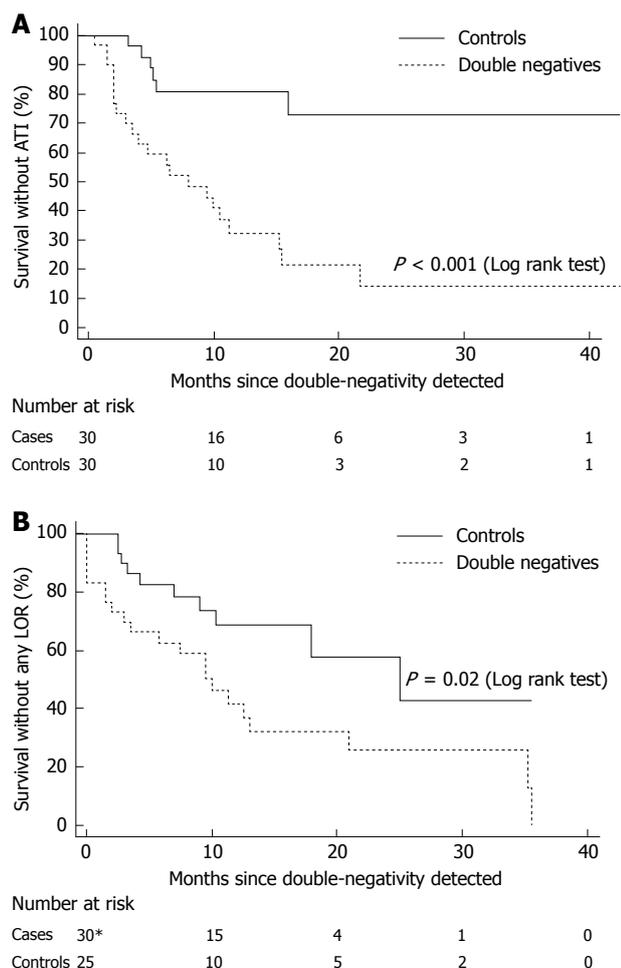


Figure 5 Survival free of antibodies to infliximab and loss of response. A: ATI development in cases vs controls; B: Secondary LOR in cases vs controls. The asterisk shows that five of the controls experienced secondary LOR at a time-point comparable to the double negative event. ATI: Antibodies to infliximab; LOR: Loss of response.

mediated phenomenon, albeit with low titer antibodies close or below the detection level of the assay when employed by certain specifications. As previously demonstrated, transient ATI had little clinical and immunological significance^[8,12,13].

There are several limitations to our study. Primarily, the results were obtained with the double antigen ELISA and the anti-lambda ELISA; however, corroborating studies using other assays, such as HMSA, are pertinent. Secondly, because treating physicians were not blinded to the results, one cannot exclude that the DN status of the sera analyzed may have influenced clinical management. However, LOR was defined per clinical indexes and constituted only the secondary outcome. Finally, previous events of positive ATI might influence future ATI formation. To neutralize such past effects, we performed an analysis incorporating former ATI events as if they occurred at time zero, which yielded similar results.

In conclusion, the type of assay employed influences the occurrence of DN status. DN is rarely observed when LOR patients' sera are analyzed by

the sensitive anti-lambda assay, and in many cases it probably results from low-level immunogenicity rather than elusive non-immunogenic mechanisms. Further studies are required to better assess the immunological processes leading to the absence of both drug and ATI, to investigate possible drug clearance pathways and to define appropriate interventions in these patients.

COMMENTS

Background

Antibodies to infliximab (ATI) correlate with lower infliximab (IFX) trough levels and loss of response (LOR). However, 10%-60% of LOR patients have low IFX levels in the absence of ATI, which are designated double negative (DN) status.

Research frontiers

The clinical and immunological significance of the DN status is currently unknown.

Innovations and breakthroughs

Only 9% of sera obtained at time of LOR were DN by anti-lambda ELISA compared with 40% with double antigen ELISA ($P < 0.001$, Fisher's Exact test). Re-testing with 1:10 dilution converted most of the DN results into IFX+ and/or ATI+ status. Patients with DN status had shorter survival free of non-transient ATI compared with matched controls (log rank test, $P < 0.001$).

Applications

The type of assay employed influences the occurrence of DN status. It is rarely observed when LOR patients' sera are analyzed by the sensitive anti-lambda assay, and in many cases it probably results from low-level immunogenicity rather than non-immunogenic mechanisms. Further studies are required to better assess the immunological processes leading to the absence of both drug and ATI, to investigate possible drug clearance pathways and to define appropriate interventions in these patients.

Terminology

DN status: A serum sample that is negative for both infliximab and ATI (IFX-ATI-). Double antigen ELISA: A commercially available ELISA assay for the detection of ATI, which incorporates infliximab as the detection antibody. Anti-lambda ELISA assay: An in-house developed ELISA assay for the detection of ATI, which incorporates an anti-human λ chain antibody as the detection antibody. LOR: Loss of clinical response to infliximab therapy.

Peer-review

This manuscript evaluates the prevalence and clinical significance of DN status among infliximab-treated inflammatory bowel disease patients. The article is well written and is suitable to be published.

REFERENCES

- 1 **Hanauer SB**, Feagan BG, Lichtenstein GR, Mayer LF, Schreiber S, Colombel JF, Rachmilewitz D, Wolf DC, Olson A, Bao W, Rutgeerts P; ACCENT I Study Group. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. *Lancet* 2002; **359**: 1541-1549 [PMID: 12047962 DOI: 10.1016/S0140-6736(02)8512-4]
- 2 **Sands BE**, Anderson FH, Bernstein CN, Chey WY, Feagan BG, Fedorak RN, Kamm MA, Korzenik JR, Lashner BA, Onken JE, Rachmilewitz D, Rutgeerts P, Wild G, Wolf DC, Marsters PA, Travers SB, Blank MA, van Deventer SJ. Infliximab maintenance therapy for fistulizing Crohn's disease. *N Engl J Med* 2004; **350**: 876-885 [PMID: 14985485 DOI: 10.1056/NEJMoa030815]
- 3 **Rutgeerts P**, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johans J, Travers S, Rachmilewitz D, Hanauer SB, Lichtenstein GR, de Villiers WJ, Present D, Sands BE, Colombel JF. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2005; **353**: 2462-2476 [PMID: 16339095 DOI: 10.1056/NEJMoa050516]
- 4 **Kopylov U**, Mantzaris GJ, Katsanos KH, Reenaers C, Ellul P, Rahier JF, Israeli E, Lakatos PL, Fiorino G, Cesarini M, Tsianos

- EV, Louis E, Ben-Horin S. 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 [PMID: 21118397 DOI: 10.1111/j.1365-2036.2010]
- 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 [PMID: 21366636 DOI: 10.1111/j.1365-2036.2011.04612]
- 6 **Gisbert JP**, Panés J. Loss of response and requirement of infliximab dose intensification in Crohn's disease: a review. *Am J Gastroenterol* 2009; **104**: 760-767 [PMID: 19174781 DOI: 10.1038/ajg.2008.88]
- 7 **Hanauer SB**, Wagner CL, Bala M, Mayer L, Travers S, Diamond RH, Olson A, Bao W, Rutgeerts P. Incidence and importance of antibody responses to infliximab after maintenance or episodic treatment in Crohn's disease. *Clin Gastroenterol Hepatol* 2004; **2**: 542-553 [PMID: 15224278 DOI: 10.1016/S1542-3565(04)00238-1]
- 8 **Ungar B**, Chowers Y, Yavzori M, Picard O, Fudim E, Har-Noy O, Kopylov U, Eliakim R, Ben-Horin S; ABIRISK consortium. The temporal evolution of antidrug antibodies in patients with inflammatory bowel disease treated with infliximab. *Gut* 2014; **63**: 1258-1264 [PMID: 24041539 DOI: 10.1136/gutjnl-2013-305259]
- 9 **Seow CH**, Newman A, Irwin SP, Steinhart AH, Silverberg MS, Greenberg GR. Trough serum infliximab: a predictive factor of clinical outcome for infliximab treatment in acute ulcerative colitis. *Gut* 2010; **59**: 49-54 [PMID: 19651627 DOI: 10.1136/gut.2009]
- 10 **Colombel JF**, Sandborn WJ, Reinisch W, Mantzaris GJ, Kornbluth A, Rachmilewitz D, Lichtiger S, D'Haens G, Diamond RH, Broussard DL, Tang KL, van der Woude CJ, Rutgeerts P. Infliximab, azathioprine, or combination therapy for Crohn's disease. *N Engl J Med* 2010; **362**: 1383-1395 [PMID: 20393175 DOI: 10.1056/NEJMoa0904492]
- 11 **Kopylov U**, Mazor Y, Yavzori M, Fudim E, Katz L, Coscas D, Picard O, Chowers Y, Eliakim R, Ben-Horin S. Clinical utility of antihuman lambda chain-based enzyme-linked immunosorbent assay (ELISA) versus double antigen ELISA for the detection of anti-infliximab antibodies. *Inflamm Bowel Dis* 2012; **18**: 1628-1633 [PMID: 22038899 DOI: 10.1002/ibd.21919]
- 12 **Vande Castele N**, Gils A, Singh S, Ohrmund L, Hauenstein S, Rutgeerts P, Vermeire S. Antibody response to infliximab and its impact on pharmacokinetics can be transient. *Am J Gastroenterol* 2013; **108**: 962-971 [PMID: 23419382 DOI: 10.1038/ajg.2013.12]
- 13 **Steenholdt C**, Al-khalaf M, Brynskov J, Bendtzen K, Thomsen OØ, Ainsworth MA. Clinical implications of variations in anti-infliximab antibody levels in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2012; **18**: 2209-2217 [PMID: 22344964 DOI: 10.1002/ibd.22910]
- 14 **Higgins PD**, Schwartz M, Mapili J, Krokos I, Leung J, Zim-mermann EM. Patient defined dichotomous end points for remission and clinical improvement in ulcerative colitis. *Gut* 2005; **54**: 782-788 [PMID: 15888785 DOI: 10.1136/gut.2004.056358]
- 15 **Harvey RF**, Bradshaw JM. A simple index of Crohn's-disease activity. *Lancet* 1980; **1**: 514 [PMID: 6102236 DOI: 10.1016/S0140-6736(80)92767-1]
- 16 **Vermeire S**, Schreiber S, Sandborn WJ, Dubois C, Rutgeerts P. Correlation between the Crohn's disease activity and Harvey-Bradshaw indices in assessing Crohn's disease severity. *Clin Gastroenterol Hepatol* 2010; **8**: 357-363 [PMID: 20096379 DOI: 10.1016/j.cgh.2010.01.001]
- 17 **Ben-Horin S**, Yavzori M, Katz L, Kopylov U, Picard O, Fudim E, Coscas D, Bar-Meir S, Goldstein I, Chowers Y. The immunogenic part of infliximab is the F(ab')₂, but measuring antibodies to the intact infliximab molecule is more clinically useful. *Gut* 2011; **60**: 41-48 [PMID: 20519742 DOI: 10.1136/gut.2009.201533]
- 18 **Nirula A**, Glaser SM, Kalled SL, Taylor FR. What is IgG4? A review of the biology of a unique immunoglobulin subtype. *Curr Opin Rheumatol* 2011; **23**: 119-124 [PMID: 21124094 DOI: 10.1097/BOR.0b013e3283412fd4]
- 19 **Afif W**, Loftus EV, Faubion WA, Kane SV, Bruining DH, Hanson KA, Sandborn WJ. Clinical utility of measuring infliximab and human anti-chimeric antibody concentrations in patients with inflammatory bowel disease. *Am J Gastroenterol* 2010; **105**: 1133-1139 [PMID: 20145610 DOI: 10.1038/ajg.2010.9]
- 20 **Ordás I**, Feagan BG, Sandborn WJ. Therapeutic drug monitoring of tumor necrosis factor antagonists in inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2012; **10**: 1079-187; quiz 1079-187; [PMID: 22813440 DOI: 10.1016/j.cgh.2012.06.032]
- 21 **Maser EA**, Villela R, Silverberg MS, Greenberg GR. Association of trough serum infliximab to clinical outcome after scheduled maintenance treatment for Crohn's disease. *Clin Gastroenterol Hepatol* 2006; **4**: 1248-1254 [PMID: 16931170 DOI: 10.1016/j.cgh.2006.06.025]
- 22 **Pariente B**, Pineton de Chambrun G, Krzysiek R, Desroches M, Louis G, De Cassan C, Baudry C, Gornet JM, Desreumaux P, Emilie D, Colombel JF, Allez M. Trough levels and antibodies to infliximab may not predict response to intensification of infliximab therapy in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2012; **18**: 1199-1206 [PMID: 22127789 DOI: 10.1002/ibd.21839]
- 23 **Steenholdt C**, Ainsworth MA, Tovey M, Klausen TW, Thomsen OO, Brynskov J, Bendtzen K. Comparison of techniques for monitoring infliximab and antibodies against infliximab in Crohn's disease. *Ther Drug Monit* 2013; **35**: 530-538 [PMID: 23765033 DOI: 10.1097/FTD.0b013e31828d23c3]

P- Reviewer: De Silva AP, Trifan A **S- Editor:** Ma YJ
L- Editor: Stewart GJ **E- Editor:** Ma S





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: bpgoffice@wjgnet.com

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



ISSN 1007-9327

