Prospective Observational Evaluation of Time-Dependency of Adalimumab Immunogenicity and Drug Concentrations: The POETIC Study

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OBJECTIVES: **Adalimumab is usually self-injected at home, making prospective serial-sampling studies challenging and scarce. This has led to a gap in knowledge about evolution of anti-adalimumab antibodies (AAAs) over time and its correlation with clinical and inflammatory outcomes.**

METHODS: **A program for home visits by physicians at induction, every 3 months and at event of relapse, was established prospectively for Crohn's disease (CD) patients. At each visit, patients' clinical scores were determined and sera were obtained for C-reactive protein, drug, and AAA levels. This cohort was compared to a parallel prospective cohort of infliximab-treated CD patients. In a subgroup of 29 patients, trough and in-between-trough levels were compared, to elucidate the importance of timing of sampling during the injection cycle.**

RESULTS: **Ninety-eight CD patients starting adalimumab were prospectively followed (median follow-up 44 weeks) and 621 serum samples were analyzed. Thirty-three patients (32%) developed AAA; 18/33 (55%) of them as early as week 2, and 26/33 (79%) by week 14. Induction period AAAs were strongly associated with primary non-response (odds ratio (OR) = 5.4, 95% confidence interval (CI): 1.6–17.8, $p = 0.005$). As compared to antibodies-to-infliximab (ATI), AAA formation rate over time was significantly lower ($p = 0.01$) and AAA were much more specific—85% of AAA events were associated with loss-of-response compared with 58% rate for ATI ($p = 0.01$). In 29 patients sampled serially during an injection cycle, levels of drug and AAA seemed comparable between four time-points during a single cycle both in patients with or without AAA ($n = 8$, $n = 21$, respectively).**

CONCLUSIONS: **When followed prospectively and serially, AAAs are found to arise earlier than previously appreciated and their impact may be more pronounced for primary rather than secondary, non-response. Drug and AAA levels were similar both at trough and in-between injections, enabling to simplify therapeutic drug monitoring of adalimumab.**

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INTRODUCTION

Higher adalimumab trough levels have been associated with favorable clinical outcome in CD, and anti-adalimumab antibodies (AAAs) have been linked with clinical deterioration [1–4]. In a recent sub-analysis of adalimumab pharmacokinetics in a CD cohort, AAAs were detected in 20% of patients. AAAs were also strongly associated with subsequent higher C-reactive protein (CRP) levels and discontinuation of adalimumab [5]. In another sub-analysis of the CLASSIC trials, a positive association between serum adalimumab concentrations and early clinical remission

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was identified [6]. However, a general cutoff concentration associated with clinical remission could not be defined. Most other currently available data are derived from cross-sectional studies, reporting a single time-point measurement of adalimumab drug and AAA levels during either induction or maintenance treatment, which limits generalization of their findings [7–9].

Adalimumab and infliximab are both first-line biologics for moderate-to-severe CD, with comparable efficacy [10, 11]. As reported by a recent meta-analysis, the overall rate of AAA seems lower than that reported for antibodies-to-infliximab (ATI) [12], but this widely held view is limited by the absence of direct comparisons, as well as by the fact that different studies use dissimilar and mostly drug-sensitive assays and employ a sporadic single-time non-standardized sampling rather than serial-sampling methodology. Moreover, adalimumab therapeutic drug monitoring (TDM) is currently performed at trough, encumbering clinical follow-up and/or yielding hard-to-interpret results if trough-sampling times are not adhered to. Whether in-between trough levels differ from trough drug/AAA levels remains to be determined. Thus, in the current study, we investigated temporal aspects of adalimumab drug and AAA levels in relation to clinical and inflammatory outcomes in a prospectively followed CD cohort.

METHODS

Patient population

This was a prospective observational multi-center cohort study of CD patients receiving scheduled adalimumab therapy at five Israeli medical centers between April 2014 and February 2017. Patients' demographics and clinical characteristics were recorded before drug initiation. Each patient, enrolled at the study, was visited at home regularly by a physician at weeks 0, 2, and 14 of adalimumab therapy and every 12 weeks thereafter. Additional visits were performed upon clinical worsening or change in therapy. Each visit took place before adalimumab administration (at trough) and included an update on medications and clinical interventions, clinical score evaluation, and drawing of blood for CRP, adalimumab, and AAA levels. For each patient enrolled, the home-visit program continued until adalimumab therapy cessation or end of study period (February 2017).

Eligible patients were individuals older than 18 years. Patients who have previously received adalimumab were eligible if presently starting the drug after at least 6 months elapsed from previous adalimumab administration, and if receiving the standard induction 160–80 mg course. The final analysis was a per-event analysis, so that a patient, who had received two courses of adalimumab during the study period as per the above criteria, was analyzed as two events. The study was approved by the ethics committees of the participating centers and all patients signed an informed consent.

Among a subgroup of patients ($n = 29$) serum samples were drawn for drug and AAA levels at 3-day intervals from one trough level to the next (during one "adalimumab therapy cycle"). Sera were obtained at four time-points per a four quartile division of the injection cycle: 1–4 days—early cycle, 5–9 days—mid-cycle, 10–13 days—late cycle, in addition to trough levels (day 14). Only patients receiving adalimumab every fortnight were included in this analysis.

Comparative analysis with infliximab

Comparative data regarding infliximab pharmacokinetics was retrieved for CD patients included in our previously reported prospective study of infliximab pharmacokinetics and immunogenicity, in which infliximab levels were gauged using a similar enzyme-linked immunosorbent assay (ELISA) technique at similar time-points [13]

Clinical scores

Clinical status was determined by HBI (Harvey–Bradshaw index) [14, 15]. Clinical remission was defined as HBI <5. Clinical response was defined as drop of ≥3 points of the HBI [16]. Primary non-response was defined as cessation of adalimumab therapy by week 14, due to the lack of clinical response as defined above [17].

Therapeutic drug monitoring

Serum samples were routinely and systematically collected at trough, before adalimumab injections. Adalimumab and AAA levels were measured by a previously described drug-tolerant assay at Sheba Medical Center [18, 19]. Transient AAAs were defined as AAA that appeared during the course of adalimumab therapy, were not associated with clinical worsening, and disappeared after no more than two consecutive measurements. AAAs which disappeared upon therapy optimization were not considered transient [20].

Biomarker analysis

CRP serum levels were measured with the use of the CardioPhase hsCRP particle-enhanced immunonephelometric assay on the BN ProSpec® analyzer (Siemens Medical Solutions Diagnostics, Malvern, PA, USA).

Statistical analysis

Continuous variables were expressed as the median and interquartile range (IQR). Mann–Whitney test was used to compare continuous variables and Fischer's exact test was used for categorical data. Wilcoxon's test was used to compare paired samples. A receiver-operating characteristic (ROC) analysis was performed for adalimumab trough levels using CRP normalization and clinical remission as classification variables. ROC analyses cutoffs were determined by the Youden most accurate point. One-way analysis of variance and Cochran's Q test were used to test the differences between quartiles. Multivariable analysis was performed using backward logistic regression. Kaplan–Meier curves were plotted to assess the temporal rate of events and log-rank test was computed for the comparison between survival-free durations.

All reported p values were two sided, and a p value <0.05 was considered statistically significant. All statistics were performed with MedCalc software (version 12.2.1.0, Mariakerke, Belgium).

RESULTS

Demography and clinical outcomes

Ninety-eight CD patients were included and followed prospectively. Four patients had two adalimumab courses during the study period (three stopped the therapy before intestinal resection and resumed it postoperatively more than 1 year later, and one patient stopped therapy against medical advice and resumed it 9 months later). Hence, the per-event analysis included a total of 102 adalimumab induction events followed prospectively thereafter. The patients' clinical and demographic characteristics are depicted in Table 1.

Median follow-up period was 44 weeks (IQR 16–79, range 2–140 weeks). Eight out of 102 (7.8%) stopped adalimumab therapy due to adverse events. The most common reason for drug discontinuation due to an adverse event was psoriasis or other type of rash in five out of these eight patients. One patient treated with adalimumab and azathioprine developed basal cell carcinoma, which was treated with excision and did not necessitate therapy cessation. Supplementary Table **1** describes all cases of adverse events.

Fourteen (14%) patients experienced primary non-response and 20 (20%) lost response to adalimumab therapy during maintenance, which necessitated therapy cessation. Thirty-five patients (35.7%) underwent therapy escalation. Of these, 10 (28%) were added an immunomodulator and 25 (72%) underwent interval shortening. The response was recaptured in 7/10 in whom an immunomodulator was added (70%) and in 16/25 in whom adalimumab therapy was escalated (60%). Out of 46 patients who completed 1 year of therapy (54 weeks), 36 (78%) were in clinical remission, whereas out of 18 patients who completed 2 years of therapy (108 weeks), 16 (90%) were in clinical remission.

Predictors of clinical and biological remission

Clinical and demographic factors were assessed for association with clinical remission by the end of induction and during maintenance (weeks 14 and 26, respectively). In addition to week 2 adalimumab trough levels, only Jewish Ashkenazi ethnicity (as opposed to Jewish Sephardic ethnicity, $p = 0.05$) was associated with clinical remission at both weeks 14 and 26 ($p = 0.01$, 0.05, respectively). Anti-tumor necrosis factor-naive status had a trend for higher rate of week 26 remission ($p = 0.09$), but was not associated with week 14 remission. Supplementary Table **2** depicts all parameters analyzed. On multivariable analysis, which included the mentioned parameters, as well as week 2 and week 14 trough adalimumab levels alternately, only week 2 and 14 drug levels remained significantly associated with clinical remission at both end points (Supplementary Table **3**).

Temporal evolution of adalimumab immunogenicity

A total of 621 serum samples were analyzed. Thirty-three (32.3%) of the patients developed AAA during follow-up. Of these, AAA developed as early as week 2 in 18/33 (55%) of AAA-positive patients and by the end of induction (week 14) in 26/33 (79%). Twenty-four of 33 AAA-positive patients (73%)

Table 1 Background disposition and clinical characteristics

Parameter	*N*=98[a]
Gender	
Male (*n*, %)	59 (60.2)
Age (median ± IQR, years)	35 (27.3–42)
Disease duration (median ± IQR, years)	8 (3–15)
Median age at diagnosis (median ± IQR, years)	23 (18–36)
Median weight at induction (median ± IQR, kg)	67 (59.3–79)
Median BMI at induction (median ± IQR)	22.8 (20.4–25.3)
Concomitant immunomodulator therapy (*n*, %)[b]	24 (24.5)
Concomitant steroid therapy (*n*, %)	14 (14.3)
Disease location	
Ileal (*n*, %)	48 (49)
Ileo-colonic (*n*, %)	36 (36.7)
Colonic (*n*, %)	14 (14.3)
Upper GI (*n*, %)	5 (5.1)
CD—disease behavior	
Non-stricturing and non-penetrating (*n*, %)	45 (45.9)
Stricturing (*n*, %)	21 (21.4)
Penetrating (*n*, %)	30 (30.6)
Perianal disease (*n*, %)	24 (23.8)
Extra-intestinal manifestations (*n*, %)	30 (30.6)
Smoking at induction (*n*, %)	20 (20.4)
Smoking at diagnosis (*n*, %)	21 (21.4)
Comorbidities (*n*, %)	21 (21.4)
Prior immunomodulator therapy (*n*, %)	61 (62)
Prior infliximab therapy (*n*, %)	35 (35.7)
Reason for infliximab discontinuation	
Primary non-response	3 (9)
Secondary loss of response	19 (54)
Remission	6 (17)
Adverse event/infusion reaction	7 (20)
Previous episode of adalimumab therapy (*n*, %)	8 (8.2)
Reason for adalimumab discontinuation	
Primary non-response	2 (25)
Secondary loss of response	3 (38)
Remission	2 (25)
Adverse event	1(12)
History of intestinal resection (*n*, %)	34 (34.7)
First-degree family history of IBD (*n*, %)	27 (27.5)

IQR inter-quartile range, *BMI* body mass index, *CD* Crohn's disease, *GI* gastrointestinal, *Tx* therapy, *IBD* inflammatory bowel disease

[a] Ninety-eight CD patients were included with a total of 102 adalimumab therapy cases (four of the patients had two episodes of adalimumab therapy during the study period)

[b] Concomitant immunomodulator therapy—immunomodulator therapy was started before/concomitantly with adalimumab, and was continued throughout adalimumab therapy

were never exposed to adalimumab before. Most of them (20 patients, 83.3%) also developed AAA by week 14. The appearance of AAA during induction was significantly more frequent among primary non-responders compared to responders (9/14, 64% vs. 22/88, 25%, respectively, $p=0.005$). Similar results was obtained when patients previously exposed to adalimumab ($n=9$) were excluded and when per-patient analysis, rather than per-event analysis, was performed. Thus, AAA formation during induction was associated with significantly increased risk for primary non-response compared to patients without AAA during induction (odds ratio (OR) = 5.4, 95% confidence interval (CI): 1.6–17.8, $p=0.005$; Fig. 1). Moreover, levels of AAA were significantly higher among primary non-responders than among responders (median week 2 AAA levels 43.9, 7.5 μg/ml-eq, IQR 3.1–55, 2.5–35.6 μg/ml-eq, respectively, $p=0.01$, Supplementary Figure **1a**). A significant association between AAA formation and secondary loss of response (LOR) was also found (OR = 4, 95% CI: 1.5–1.5, $p=0.007$). AAA preceded or occurred simultaneously with therapy failure (i.e., primary non-response or secondary loss-of-response) in 26/28 of cases (96%, median interval between AAA appearance and adverse clinical outcome: 6, IQR 0–26 weeks).

In addition to AAA-positive patients, 8 out of 102 (7.8%) patients developed transient AAA. Transient AAA levels were significantly lower than AAA levels (median levels 5.9, 3.2 μg/ml, IQR 2.3–90.8, 2.6–20.8 μg/ml, respectively, $p=0.05$). Transient AAA formation was distributed throughout the time course of adalimumab therapy, similarly to persistent AAA (median time to transient AAA—2, IQR, 2–96 weeks, median time to persistent AAA—2 weeks, IQR 2–70 weeks, respectively, $p=0.79$, Supplementary Figure **1b**).

There was no significant difference in time to development of AAA or time to therapy failure (primary or secondary) between patients who received combination therapy with an immunomodulator and adalimumab monotherapy patients ($p=0.28$, $p=0.24$, respectively, log-rank test, Fig. 2a, b). In addition, combination therapy was not more frequent among primary responders (20/88, 22.7%), than among primary non-responders (4/14, 28.6%, $p=0.6$).

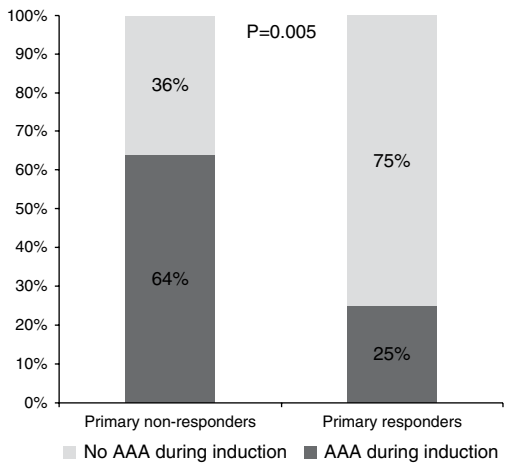

Fig. 1 AAA levels among primary non-responders were significantly higher than among responders to induction therapy. *AAA* anti-adalimumab antibodies

Using a drug-tolerant assay, 18 patients were found to develop AAA in the presence of adalimumab (i.e., "double-positive status"). However, only six of them (33%) became "AAA positive/drug negative" in subsequent sera measurements, and contrary to originally AAA-positive/adalimumab-negative sera, double-positive status was not significantly associated with therapy failure (OR = 1.6, 95% CI: 0.6–4.7, $p=0.32$).

TDM: early prediction of clinical and inflammatory outcome

Week 2 adalimumab levels were significantly associated with clinical remission by the end of induction (week 2 median levels of 6.8 vs. 4.85 μg/ml, IQR 1.5–19.2, 0–8.2 μg/ml, among those in clinical remission vs. those clinically active at week 14, $p=0.0005$, Fig. 3a). Primary responders had significantly higher week 2 adalimumab levels than primary non-responders (defined as therapy cessation due to clinically active disease by week 14, median levels: 6.2 μg/ml, IQR 4.5–7.6, vs. 3.1 μg/ml, IQR 0.04–5, respectively, $p=0.0008$). Moreover, on ROC curve analysis, week 2 adalimumab levels >6.7 μg/ml were significantly associated with clinical remission by the end of induction ($p<0.0001$, AUC = 0.73, sensitivity 85% specificity 54%, Fig. 3b). Week 2 adalimumab levels were also associated with clinical remission at six months of therapy (6.4 μg/ml vs. 4.45 μg/ml, IQR 0–11.4, 3.1–9.7 μg/ml, among those in clinical remission vs. those clinically active at week 26, $p=0.04$). No association between week 2 drug levels and clinical remission at 1 year of therapy (week 54) was detected ($p=0.66$). Adalimumab levels at the end of induction (week 14) were similarly associated with clinical remission at week 26 (4.75 μg/ml vs. 3 μg/ml, IQR 0–35, 0–10 μg/ml, among those in clinical remission vs. those clinically active at week 26, $p=0.03$), and had a borderline association with week 54 status (5.3 μg/ml vs. 3.25 μg/ml, IQR 0–35, 0–8 μg/ml, among those in clinical remission vs. those clinically active at week 54, $p=0.12$). All associations are detailed at Supplementary Table **4**. Week 2 adalimumab level quartiles were also significantly associated with clinical outcome. There was a negative association between increasing drug level quartiles and primary non-response/LOR (OR = 10.5, 95% CI: 2.5–44.8, $p=0.0015$ for Q1 vs. Q4, Fig. 3c).

An additional outcome of drug retention was examined. Drug retainment was defined as therapy discontinuation due to either LOR or adverse events. A survival analysis was performed based on the identified cutoff of adalimumab trough levels at week 2. These two analyses showed that adalimumab >6.7 μg/ml at week 2 was indeed correlated significantly with longer drug retainment ($p=0.0004$), but drug level was not associated with retainment free of adverse events ($p=0.97$, Supplementary Figures **2a, b**).

The outcome of inflammatory marker (CRP) normalization was also explored. Adalimumab trough levels were significantly, although modestly, correlated with CRP values for all maintenance period sera ($\rho=-0.27$, $p<0.0001$). Trough levels were signifi-

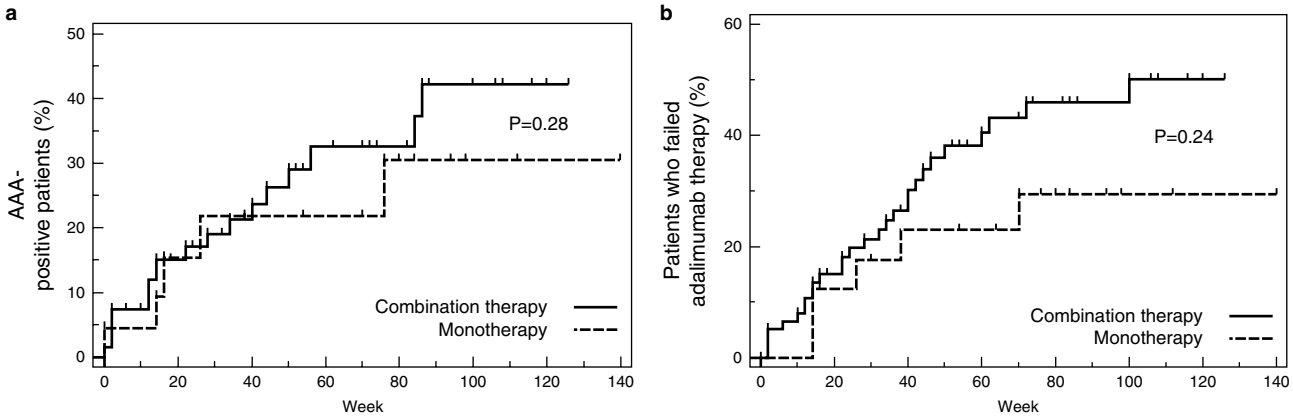

Fig. 2 **a** There was no significant difference in time to AAA formation in patients receiving combination therapy with an immunomodulator, vs. monotherapy patients. AAA anti-adalimumab antibodies. **b** There was no significant difference in time to therapy failure (primary or secondary), in patients receiving combination therapy with an immunomodulator, vs. monotherapy patients. LOR loss of response

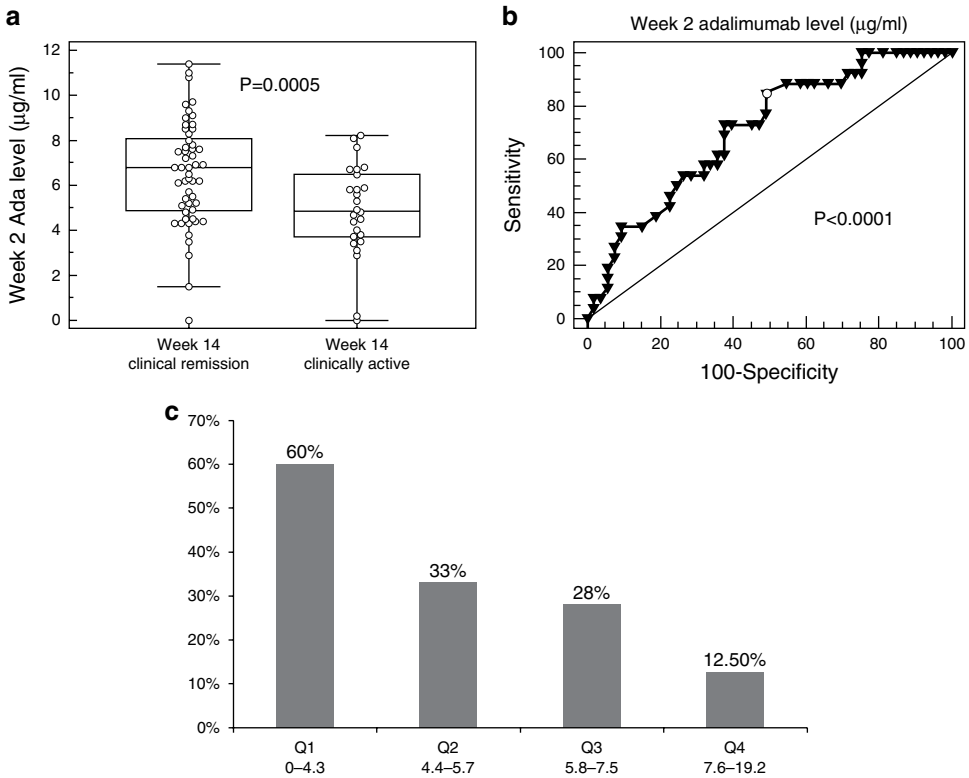

Fig. 3 **a** Week 2 adalimumab levels were significantly higher among patients in clinical remission by the end of induction, than among those clinically active. Ada adalimumab. **b** On ROC curve analysis, week 2 adalimumab levels >6.7 μg/ml were significantly associated with clinical remission by the end of induction ($p<0.0001$, AUC = 0.73, sensitivity 85%, specificity 54%). **c** Association between adalimumab level quartiles (Q1–Q4) at week 2 and rate of primary non-response/loss of response

cantly higher for CD patients with normal, rather than elevated CRP values (5.3 vs. 3.85 μg/ml, 95% CI 4.8–5.7, 3.1–4.6 μg/ml, among those with normal vs. elevated CRP, $p=0.001$, Fig. 4a). Similar findings were demonstrated for week 14 only sera ($n=85$, $\rho=-0.29$, $p=0.009$). ROC curve analysis demonstrated that adalimumab levels above 3.65 μg/ml at week 14 were associated with CRP normalization (AUC = 0.65, $p=0.0001$, sensitivity = 59%, specificity = 70%, Fig. 4b). Nevertheless, adalimumab levels at either week 2 or 14 were not predictive of CRP normalization at weeks 14, 28, 54, and 28, 54, respectively (data not shown).

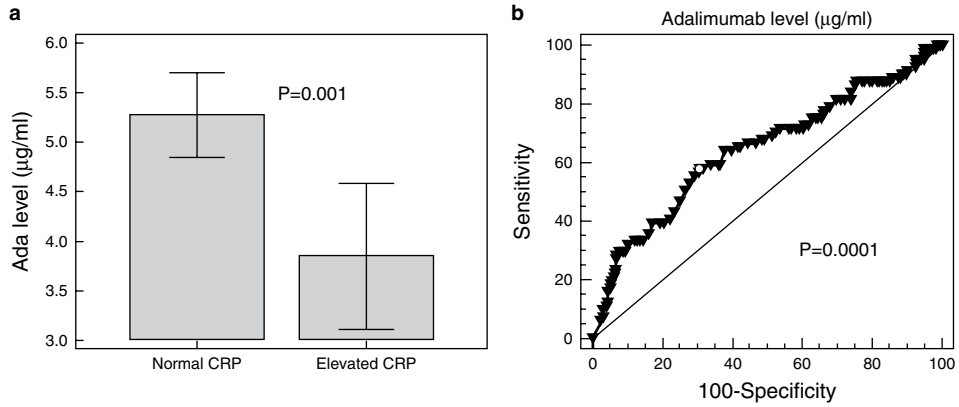

Fig. 4 **a** Patients with normal CRP values had significantly higher adalimumab trough levels than those with elevated CRP values. CRP C-reactive protein, Ada adalimumab. **b** On ROC curve analysis, week 14 adalimumab levels >3.65 μg/ml were significantly associated with CRP normalization at the same time-point ($p = 0.0001$, AUC = 0.65, sensitivity 58%, specificity 70%)

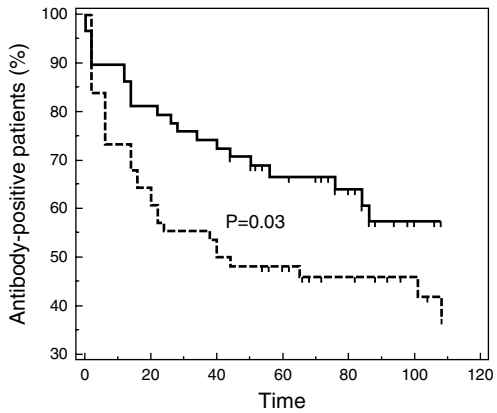

Fig. 5 AAA formation rate over time was significantly lower among adalimumab therapy patients than ATI formation among infliximab therapy patients. Solid line - adalimumab therapy patients without AAA, Dashed line - infliximab therapy patients without ATI. *AAA* anti-adalimumab antibodies, *ATI* anti-infliximab antibodies

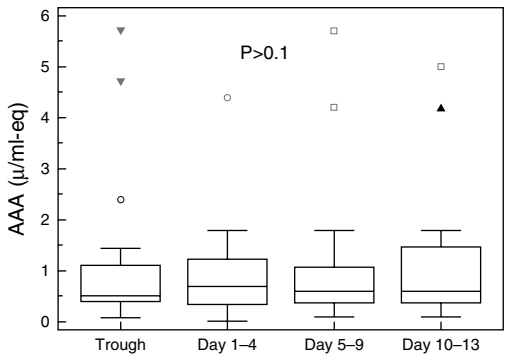

Fig. 6 No significant difference in AAA levels was identified between the four time-points. *AAA* anti-adalimumab antibodies

Comparison of adalimumab vs. infliximab immunogenicity

Anti-drug–antibody formation rate was compared with a previously published cohort of 100 CD patients treated with infliximab, who were followed at our center prospectively for development of ATI using the same assay and similar protocol and time-points [13]. All patients treated with infliximab/adalimumab for at least 1 year, or who developed anti-drug antibodies beforehand, were included in the analysis. Episodic therapy patients were excluded from both cohorts. Anti-drug–antibodies formation rate over time was significantly lower among adalimumab therapy patients than among infliximab therapy patients ($p = 0.03$, log-rank test, Fig. 5). However, 85% of AAA events were associated with therapy failure compared with 58% rate for ATI ($p = 0.01$). Transient AAAs were also much less common than transient ATI (7% vs. 32%, $p < 0.0001$).

Comparison of serum adalimumab and AAA levels measured at trough, with levels measured in-between injections

To investigate the importance of sampling adalimumab levels at trough, 29 patients were prospectively followed for trough and in-between troughs adalimumab levels by repeated sampling during a single two-week injection cycle. Twenty-nine patients (72%) had positive drug levels and negative AAA, while 8/29 (28%) had positive AAA and low drug levels. For the purpose of comparative analysis, results were grouped into samples obtained at 1–4 days (early cycle), 5–9 days (mid-cycle), and 10–13 days (late cycle) after adalimumab injection, and compared to levels obtained at trough (day 14). No significant difference in drug levels was identified between the four time-points (median 4.4, 4.15, 4.1, 3.8 μg/ml respectively, $p > 0.1$ for all six comparisons). AAA levels were also similar for all four time-points (median 0.6, 0.8, 0.7, 0.6 μg/ml-eq, respectively, $p = 0.96, 0.64, 0.77, 0.33, 0.35, 0.46$, Fig. 6). Additional sub-analyses demonstrated that drug levels did not significantly differ during the cycle either within the sub-groups of patients with or without AAA ($p > 0.05$ for all comparisons, Supplementary Figures **3a, b**). Moreover, there was no difference in AAA levels among AAA-positive patients ($p > 0.1$ for all comparisons).

DISCUSSION

Despite ample research on adalimumab immunogenicity, the prognostic value of AAA for adalimumab primary and secondary failure has not been precisely defined and scarce data exist as to the temporal aspects of AAA appearance and their chronologic evolution throughout therapy. This is partly due to paucity of prospective serial-sampling pharmacokinetic/immunogenicity studies of adalimumab. In the present study, 32% of the patients developed AAA. AAA appeared as early as week 2 in 55% of ultimately AAA-positive patients, and in 79% of them by the end of induction (median time to AAA—2 weeks). AAAs were associated more often with primary non-response rather than with secondary LOR to adalimumab and conferred a fivefold higher risk for primary non-response. Baert et al. [5] recently reported AAA formation in 20% of patients, somewhat comparable albeit lower than the rate in the present study. However, they reported a longer time till AAA detection—a median of 34 weeks. The difference in time-to-AAA detection between the studies might stem from different sera sampling intervals and from different assays' sensitivities. At any rate, the present findings are more congruent with known kinetics of B-cell immune response to a cognate antigen, adalimumab in this case, which usually start to become evident at 10–14 days after primary exposure to the antigen in most individuals [21]. The present observations indicating a possible impact of immunogenicity already during induction, and earlier than previously appreciated are resonant with recent findings with infliximab, whereby two studies showed that early ATI may be responsible for a non-negligible portion of acute UC patients experiencing primary non-response to infliximab [22, 23]. These observations might suggest the importance of a tighter TDM during induction and perhaps early combination therapy, with sparser monitoring of AAA once adequate adalimumab levels with lack of immunogenicity are witnessed later on. In our study, in 26/28 of cases (96%), AAA preceded or occurred simultaneously with primary non-response/loss-of-response. This temporal relationship, which to our knowledge has not been previously demonstrated with adalimumab therapy, is of clinical importance. It signifies that TDM might provide us with a "therapeutic window" for pre-emptive interventions, such as immunomodulator addition, in order to prevent future LOR [24].

An association between higher adalimumab trough levels and lower clinical score has been previously demonstrated in most [25–27], but not all studies [28]. Cutoffs of 6–7.5 μg/ml for adalimumab trough levels have been associated with clinical, endoscopic and even histological remission [2, 8]. However, most studies were cross-sectional analyses and predictive value of early induction adalimumab levels has scarcely been evaluated in Crohn's disease and even more so in ulcerative colitis. In the current study, week 2 and 14 adalimumab levels were positively associated with remission by the end of induction (week 14) and during maintenance (week 26). Moreover, week 2 adalimumab levels >6.7 μg/ml were significantly associated with clinical remission at week 14. This emphasizes the notion that adalimumab levels >5–7 μg/ml are sufficient, not only during stable therapy, but already at week 2 of induction, for prediction of future response.

Previous studies have associated lower trough levels and AAA with elevated CRP [4, 5]. In our study, a significant association has been similarly observed between trough levels and CRP values. However, early induction drug and AAA levels did not predict future CRP normalization, perhaps due to limited sample size in this temporal analysis (23% of patients were excluded due to normal baseline CRP values).

Several studies have compared efficacy outcomes of infliximab vs. adalimumab [10, 11, 29, 30]. Although rates of immunogenicity of adalimumab are generally reported to be lower than those reported for infliximab, direct comparisons using similar (drug-tolerant) assays, and using serial consecutive sampling over time are absent. In the current study, two prospective cohorts treated in the same center, assayed by the same drug-tolerant technique and followed serially in a similar manner, were compared for infliximab vs. adalimumab immunogenicity features. In the absence of head-to-heard trials of these two drugs, this comparative analysis is likely to be the most validated one possible. It showed that as compared to ATI, AAA cumulative formation rate was significantly lower. Transient AAA were also less common than transient ATI [13]. Thus, once formed, AAA were more specific for loss of clinical response than ATI.

As opposed to the reduced immunogenicity rate for infliximab-treated patients receiving combination immunomodulators [31], previous studies demonstrate conflicting findings as to immunogenicity rate with combination therapy in adalimumab-treated patients. Combination therapy has not been associated with improved clinical outcome in most studies [1, 6, 11, 12, 32]. Similarly, in our study, concomitant immunomodulator therapy affected neither immunogenicity nor treatment outcome. In a recent randomized controlled trial, adalimumab monotherapy had similar week 26 clinical efficacy to combination therapy, but AAA formation rate was numerically lower and endoscopic outcome was superior in the latter group [10]. These somewhat conflicting findings might stem from a selection bias possible in observational studies, where patients with higher disease scores may be more likely to receive combination therapy. As recently published by Kariyawasam et al. [33], they might also stem from variability in thioguanine nucleotide levels in patients receiving combination thiopurine therapy, a parameter which has not been explored in most previous studies, including ours [33]. This should be clarified in future randomized studies.

In our study, in-between trough levels did not differ from trough levels, both for patients with adequate and low adalimumab levels, and both for patients with negative and positive AAA. Ward et al. [34] have recently performed an analysis of inter-trough adalimumab levels in comparison with trough levels. Similarly to our finding, drug levels did not fluctuate significantly between different time-points within an injection cycle ($p = 0.542$). Nevertheless, in that study, AAA-positive patients were not assessed, and these are the patients who are most of concern when the best timing for sampling is considered. The present study shows, for the first time, that the pattern of AAA detection during injection cycle is also stable, providing re-assurance about non-trough-sampling feasibility also for these patients. This significantly simplifies monitoring for both patient and physician in clinical practice.

Our study has several limitations. First, the complete study population consisted of 98 CD patients (102 adalimumab treatment events). Even though this is a large-scale prospective longitudinal "real-life" cohort, the follow-up time was variable, which should be borne in mind when interpreting the results. Second, the outcomes evaluated in this study in relation to pharmacokinetics included clinical scores and inflammatory markers, but not routine endoscopies. Further, because treating physicians were not kept blinded to these serial adalimumab and AAA results, one cannot exclude that the assays' results may have influenced clinical management. Finally, although TDM was performed using our extensively validated drug-tolerant ELISA assay, corroborating studies using other assays would be beneficial.

In conclusion, in the present prospective study, 32% of 102 CD patients developed AAA. The majorities (80%) of AAA appeared during induction period and were associated with primary non-response. There was no difference in AAA formation or therapy failure rate between patients on monotherapy and combination therapy. Drug and AAA levels were similar both at trough and in-between injections, enabling to simplify the logistics of blood level monitoring. As compared to ATI, AAA formation rate over time was significantly lower, but they were more specific for LOR. Taken together, these findings signify that adalimumab immunogenicity may be elicited and become clinically relevant earlier than appreciated, already during induction. This impacts primary response to adalimumab and highlights the importance of tighter TDM during the first months of therapy.

CONFLICT OF INTEREST

Guarantor of the article: Bella Ungar.

Specific author contributions: SB-H conceived the study and drafted the manuscript; BU was involved in study conception, analysis and interpretation of data and manuscript drafting; UK, TE, DY, AL, AL, BA, OH-N, TN, EB, ES, and DC participated in acquisition of data; LS, NL, SN, MY, EF, and OP took part in data analysis; RE and YC participated in data interpretation and in critical revision of the manuscript for important intellectual property. All authors have approved the final draft submitted.

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Study Highlights

WHAT IS KNOWN

- ✓ Adalimumab has been shown to induce and maintain remission in CD patients.
- ✓ Lower trough adalimumab and higher trough anti-adalimumab antibody levels have been associated with LOR to adalimumab therapy.

WHAT IS NEW HERE

- ✓ Adalimumab drug and antibody levels measured in-between troughs were similar to those measured at trough.
- ✓ Anti-adalimumab antibodies appear mostly during induction and are especially associated with primary non-response to therapy.
- ✓ Anti-adalimumab antibodies are rarer than anti-infliximab antibodies, but more specific for LOR.
- ✓ There was no difference in antibody formation or LOR between patients who received combination therapy with an immunomodulator in comparison to monotherapy patients.

POTENTIAL FUTURE IMPACT

- ✓ Adalimumab drug and antibody levels can probably be measured anytime during the injection cycle, facilitating simpler adalimumab TDM at non-trough time-point clinic visits.
- ✓ The risk for anti-adalimumab antibodies formation is greatest during the first months of therapy. Hence, monitoring adalimumab drug and antibody levels also during that period—using a drug-tolerant assay—may allow early interventions to reduce immunogenicity and drug failure.
- ✓ Given an overall similar immunogenicity rate in adalimumab monotherapy and combotherapy, more data are needed as to the patient sub-populations that will benefit from combination immunomodulator with adalimumab.

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