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Predictive factors of immunogenicity to adalimumab in inflammatory bowel disease – a retrospective cohort study

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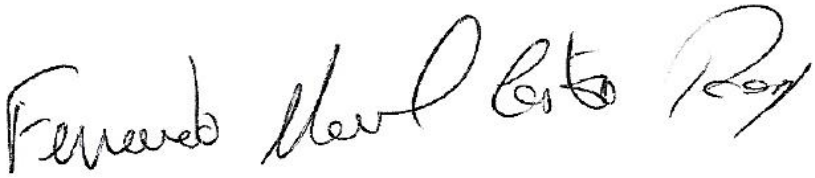

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Resumo

Introdução

O adalimumab tem eficácia comprovada no tratamento da doença inflamatória intestinal. Contudo, a imunogenicidade afeta esta eficácia, reduzindo a concentração sérica do fármaco e a probabilidade de resposta. Assim, a identificação de fatores preditores de imunogenicidade pode permitir uma abordagem individualizada e melhores resultados.

Objetivo

Identificação de fatores preditivos de imunogenicidade ao adalimumab na doença inflamatória intestinal. Secundariamente, perceber se o uso concomitante de imunomodulador influencia a concentração sérica de adalimumab, desenvolvimento de imunogenicidade e resposta bioquímica ao mesmo.

Métodos

Este estudo retrospetivo incluiu doentes adultos com doença inflamatória intestinal tratados com Adalimumab durante pelo menos um ano, no Centro Hospitalar Universitário do Porto, entre 1 de Janeiro de 2010 e 30 de Outubro de 2019. Foram excluídos pacientes sem doseamento de anticorpos anti-adalimumab e concentração sérica de adalimumab nos últimos 12 meses de tratamento. Os dados foram obtidos através dos registos clínicos eletrónicos. Foram considerados como níveis terapêuticos concentrações séricas de adalimumab entre 3,5 e 7µg/mL e o desenvolvimento de imunogenicidade foi definido como título de anticorpos superior a 10ng/ml, usando o método ELISA.

Resultados

Dos 191 doentes com doença inflamatória intestinal tratados com adalimumab, 71 cumpriam os critérios, dos quais, 5 (7%) desenvolveram imunogenicidade – maioritariamente durante o primeiro ano. Este grupo demonstrou maior uso de corticoides aquando do início do tratamento (60% vs. 15,2%; p=0,04) e níveis de calprotectina pré-tratamento mais elevados (1992µg/g vs. 265,50µg/g, IQR: 576,5; p=0,034) – com valor cut-off de 773,5 µg/g como um bom preditor de imunogenicidade (sensibilidade: 100%, especificidade: 72%). Foi encontrada uma correlação negativa entre os níveis séricos de adalimumab e os níveis de calprotectina pré-tratamento (p=0,030, CC: -0,367). O uso concomitante de imunomodulador não demonstrou impacto no desenvolvimento de imunogenicidade, mas o seu uso no início do tratamento foi

associado a menores níveis de calprotectina no follow-up (mediana: 154µg/g, IQR: 426,5 VS. mediana: 509µg/g, IQR: 1186; p=0,028).

Conclusões

Neste estudo, o principal fator preditor de imunogenicidade parece ser a atividade inflamatória da doença, sugerindo que doentes em que esta é maior podem beneficiar de uma monitorização mais apertada dos níveis séricos de adalimumab e anticorpos anti-adalimumab, especialmente no primeiro ano de tratamento, com uso precoce de dose otimizada ou suspensão precoce no caso de imunogenicidade. O uso de tratamento combinado no início do tratamento parece levar a menor atividade e melhor controlo da doença. Contudo, para confirmar esta conclusão, é necessário a realização de estudos prospetivos que incluam uma amostra maior e o uso de colonoscopia. Este estudo apresenta algumas limitações, nomeadamente a pequena amostra e o facto de ser retrospetivo.

Abstract

Introduction

Adalimumab has proven efficacy in the inflammatory bowel disease's treatment. Nevertheless, immunogenicity affects this efficacy, reducing the drug serum concentration and response odds. Therefore, the identification of predictive factors for immunogenicity can lead to an individualized management and improved outcome.

Aim

Identify predictive factors of immunogenicity to adalimumab in inflammatory bowel disease. Secondarily, find if the concomitant use of an immunomodulator impacts adalimumab serum concentration, development of immunogenicity and biochemical response to adalimumab.

Methods

This retrospective cohort study included adult patients with inflammatory bowel disease treated for at least a year with adalimumab at Centro Hospitalar Universitário do Porto between 1st of January of 2010 and 30th of October of 2019. Patients without titer of anti-adalimumab antibodies and adalimumab serum concentration in the last 12 months of treatment were excluded. The data was extracted from the patients' electronical medical records. The therapeutic range of adalimumab trough levels considered was 3,5 to 7µg/mL and development of immunogenicity was defined as a titer of anti-adalimumab antibodies over 10ng/ml, using the ELISA method.

Results

From the 191 patients with inflammatory bowel disease treated with adalimumab, 71 fulfilled inclusion and exclusion criteria, of which, 5 (7%) developed immunogenicity – mainly during the first year. This group demonstrated high usage of corticosteroids at the beginning of treatment (60% vs. 15,2%; p=0,04) and higher pre-treatment levels of calprotectin (1992µg/g vs. 265,50µg/g, IQR: 576,5; p=0,034) – with a cut-off value of 773,5µg/g as a good predictor of immunogenicity (sensitivity: 100%, specificity: 72%). We found a negative correlation between serum levels of adalimumab and the pre-treatment calprotectin level (p=0,030, CC: -0,367). The concomitant immunomodulator usage showed no impact in the development of immunogenicity, but its use at the beginning of treatment was associated with lower calprotectin levels at the follow-up (median: 154µg/g, IQR: 426,5 VS. median: 509µg/g, IQR: 1186; p=0,028).

Conclusions

In this study, the main predictive factor of immunogenicity seems to be the disease´s inflammatory activity, suggesting that patients with higher inflammatory activity may benefit from a strict monitorization of adalimumab levels and anti-adalimumab antibodies, especially during the first year of treatment, with early use of optimised dosing or early suspension in the event of immunogenicity. The use of combination therapy at the beginning of treatment seems to lead to a lower disease activity and better control. However, to confirm this conclusion, prospective studies are necessary, including a bigger sample and the use of colonoscopy. This study has some limitations as the sample is small and the study is retrospective.

Keywords

“Adalimumab”, “Inflammatory Bowel Disease”, “Colitis, ulcerative”, “Crohn disease”, “Retrospective Study”.

Abbreviations

AAA - Anti-adalimumab antibodies

ADA - Adalimumab

AZA – Azathioprine

CC - Correlation coefficient

CRP – C-reactive protein

IBD - Inflammatory bowel disease

IFX – Infliximab

IM – immunomodulator

IQR - Interquartil range

MTX – Methotrexate

SD – Standard deviation

TNFα - Tumor Necrosis Factor alpha

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Introduction/Aim

IBD is an idiopathic disease caused by a dysregulated immune response. This condition has developed a global distribution with an estimated incidence of 6,8 million cases in 2017[1], with an accelerating incidence especially in newly industrialised countries[2] and the highest prevalence seen in Europe.[3]

The impact of IBD in the patients' quality of life and the medical conditions associated with it, as intestinal occlusion, intestinal perforation and even the increased risk of gastrointestinal cancer, makes the correct treatment and the consequent control of the disease a major concern. Unfortunately, the treatment of IBD can be challenging, especially in more severe cases and in patients who have had the disease for many years. A huge breakthrough in the treatment was the use of biological agents, such as anti-TNFα agents (for example, IFX and ADA). These drugs are specially used in moderate to severe cases, permitting a decrease of inflammation and symptomatic control in states of disease that cannot be managed with other drugs[4]. Although anti-TNFα agents can significantly improve clinical outcomes and quality of life[5], a considerable portion of patients do not respond to anti-TNFα therapy or lose response with time. Some studies even demonstrate that this therapy is effective only on 30% to 50% of the patients, and that 40% to 50% of those who initially respond to therapy ultimately relapse within a year[6–8].

Specifically in the case of ADA it has been shown that this human monoclonal anti-TNFα antibody is associated with effective remission induction and maintenance in patients with IBD, reducing the frequency of hospitalizations, improving the quality of life, biochemical, endoscopic and histologic parameters[4,5,8–16]. However, as mentioned before, the loss of response to ADA is a reality and one of the main and known causes of it is the presence of immunogenicity, which consists of the formation of anti-drug antibodies by the patient's immune system.

This mechanism creates a barrier in the efficacy of these drugs by reducing drug concentration and the odds of response. Regarding the ADA, some studies show that up to 44% of patients with IBD treated with it develop anti-ADA antibodies and the presence of these decreases the odds of response by 87% [4,5,11,17,18].

The identification of the factors that influence the development of immunogenicity is of extreme importance, since it would permit predicting, preventing and controlling them while also identifying patients of risk who may benefit of, for example, closely monitoring and early usage of optimised dosing[19]. Various studies have tried to identify them, but there is still too much uncertainty about the topic especially in the case of ADA, as the majority of studies focus on IFX.

Some factors that have been suggested include sex, weight, age at the diagnosis, disease duration, Crohn's disease phenotype and location, concomitant use of immunosuppression, use of corticoids, dose and dose schedule and previous use of biological drugs[5,17].

Hence, this study aims to identify predictive factors of immunogenicity to ADA in IBD. Secondarily, it intends to find if the use of combination therapy with IM (as AZA or MTX) impacts 1) the titer of AAA and serum ADA concentration and 2) the IBD biological activity (calprotectin, CPR, albumin).

This secondary end point is based on the fact that combination therapy is one of the most studied influencer factors of immunogenicity. Initially it was thought that the simultaneous use of an anti-TNFα and an IM led to a reduction of anti-drug antibodies leading to this strategy being used at the beginning of treatment, to prevent immunogenicity, but also as strategy to overcome established immunogenicity. Several studies have proven this beneficial effect of concomitant therapy when using IFX, concluding that the use of IFX in conjunction with an IM is more effective than monotherapy with this anti-TNFα in the treatment of IBD [6,20–22]. However, concerning the use of ADA in combination therapy, the studies are controversial leading to much less certainty, with some findings pointing to the absence of any benefit.

Methods

The sampling frame for this retrospective cohort study was adult patients (18 years old or more) with IBD that were treated with ADA at Centro Hospitalar Universitário do Porto (CHUP) between the 1st of January of 2010 and the 30th of October of 2019. Patients who were treated with ADA for at least a year were eligible for the study. On the other hand, paediatric patients (under 18 years old), patients who initiated treatment with ADA before 2010 or without titer of AAA and ADA serum concentration in the last 12 months of treatment were excluded. Therefore, from the 191 patients with IBD treated with ADA in CHUP, 153 were eligible to the study. Of these, 82 were excluded (58 did not have titer of AAA and ADA serum concentration in the last 12 months of treatment; 18 started ADA before 2010; 4 did not have information about when ADA has started; 2 were under 18 years old), leading to a final sampling of 71 patients (Fig.1).

Demographic, clinical and analytical data was extracted from the patient electronic medical records. The variables used included: 1. Sex; 2. Age at diagnosis; 3. Type of IBD (DC/UC); 4. Disease location and behaviour; 5. Presence of perianal disease; 6. Reason that lead to starting ADA therapy

(a. AZA or MTX or CCT failure; b. Refractory, incomplete response, secondary loss of response (except if due to immunogenicity) or immunogenicity to biological drug; c. IFX and IM naïve (ADA as first therapy); d. Adverse effects to previous treatment); 7. Previous treatment with biological drug; 8. Age, weight and years of disease when ADA was started; 9. Concomitant use of CTT when ADA was started; 10. Concomitant use of IM when ADA was started - in those patients that started ADA in combination with an IM, the type of IM (MTX or AZA) was noted; 11. Concomitant use of IM started during the treatment – in those that started combination therapy with IM during the treatment, the type of IM (MTX or AZA) was noted; 12. ADA schedule administration scheme at the beginning of treatment (biweekly or weekly); 13. CPR, calprotectin and albumin levels at the time that ADA was initiated; 14. The most recent titer of AAA and serum ADA concentration in the last 12 months of treatment; 15. CPR, calprotectin and albumin in the last 12 months of therapy, at the end of follow-up (at the time that AAA and ADA levels were measured); 16. Time passed with ADA treatment until development of immunogenicity (in months); 17. Time under ADA therapy; 18. For those patients who stopped ADA, the reason that led to it (adverse effects, sustained remission, immunogenicity or secondary failure not due to immunogenicity) was noted.

The titer of AAA was obtained by ELISA technique. The development of immunogenicity was defined as a titer of AAA over 10ng/ml and the therapeutic range of ADA serum concentration considered was 3,5 to 7µg/mL before the new drug administration.

The statistical analysis of data was made using the IBM SPSS statistics software, version 26. The normal distribution of the quantitative variables was tested in general and within the different groups using the Shapiro-Wilk test. Median and standard deviation (SD) or median and interquartile ranges (IQR) were used for quantitative variables with and without normal distribution in general or within all groups, respectively. For the variables that had a normal distribution within all the groups, the latter were compared using the independent sample t-test (for two groups) or ANOVA (for more than two groups). When this condition was absent, the Man-Whitney U (for two groups) and the Kruskal-Wallis H (for more than two groups) tests were used to compare groups and, to study the correlations between two quantitative variables, the Spearman test was adopted. The qui-square or the Fisher´s exact test was used to compare groups relatively to qualitative variables, as appropriate. In order to find a possible cut-off value with adequate sensitivity and specificity we used the ROC curve analysis. Additionally, a 95% confidence interval was chosen, therefore, results were considered statistically significant when $p<0,05$.

Results

The description of the global sample is shown in table I. Of the 71 patients in the study 5 (7%) developed immunogenicity and the description of this group is also presented in table I. On average, the antibodies developed after 23,2 months of treatment (SD: 16,63), having developed at 12 (n=2), 13 (n=1), 29 (n=1) and 50 (n=1) months. The duration of ADA therapy was similar (12, 13, 33 and 52 months), with a median of 13 months (IQR 31). In the group that did not develop immunogenicity the duration of treatment ranged from 13 to 129 months, with a median of 46 months (IQR:41).

Comparing the group that developed immunogenicity and the one that did not (Table II), a significant difference was found between them regarding the use of corticosteroids at the beginning of treatment with ADA (p=0,04; OR: 8,4; 95% IC [1,242; 56,813]) with 60% of use in the immunogenicity group and only 15,2% in the other one. It was also found that the group that developed immunogenicity had significantly less time with ADA therapy (median 13 months, IQR 31) than the group that did not (median 47 months, IQR 48) (p=0,018). Besides that, the pre-treatment level of calprotectin in the immunogenicity group (median 1992µg/g) was significantly higher than the one in the non-immunogenicity group (median 442,50µg/g, IQR 626,75) (p=0,034). Regarding this last correlation, it was demonstrated that the cut-off of 773,5 µg/g of calprotectin had a good correlation to immunogenicity (100% of sensitivity and 72% of specificity). It also became apparent that the serum levels of ADA were significantly lower in the immunogenicity group (median 0,20, IQR 0,25 VS. median 11,75, IQR 8,22; p=<0,001). No other difference was found between the two groups, namely regarding weight, disease behaviour, presence of perianal disease, previous treatment with biological drugs and other analytical values besides calprotectin.

According to the obtained results, the combination therapy with IM did not affect the development of immunogenicity, the serum level of ADA or the last calprotectin, CRP and albumin values (Table II and III). Also, we found no significant difference regarding these same variables when comparing the group that did ADA in monotherapy for the entire treatment vs the ones that started ADA in combination with IM vs those who started combination therapy during the treatment (Table IV). On the other hand, compared to the rest of the sample, the subgroup that used IM since the beginning of treatment with ADA showed a significantly lower calprotectin level at the end of follow-up (median 154 µg/g, IQR 426,5 VS. median 509 µg/g, IQR 1186; p=0,028) but this difference was not found for CRP and albumin levels (Table V). Moreover, regarding the differences between the use of AZA or MTX, the combination therapy with the latter showed a significantly higher serum level of ADA (median 13,25 µg/mL, IQR 7,63 VS. median 10,10 µg/mL,

IQR 8,55; p= 0,036) (Table VI). This difference was not seen when considering only the ones that started the treatment with ADA in combination therapy, and no distinction was found regarding the other tested variables as well, including calprotectin levels. (Table VII)

Concerning the titer of AAA, a statistically meaningful, negative and weak correlation was discovered between this variable and the time with ADA therapy (p=0,014, CC: -0,289), however, this correlation was not found when considering only the ones that did not develop immunogenicity (p=0,272). A statistically meaningful, negative and weak correlation was also apparent between serum level of ADA and the pre-treatment calprotectin value (p=0,030, CC: -0,367) and the CRP value at the end of follow-up (p=0,012, CC: -0,3). No other significant correlation was found regarding the titer of AAA or the serum level of ADA (Table VIII), including comparing different possible cut-off points of serum level of ADA and calprotectin last follow-up values (Table IX).

Discussion and Conclusions

ADA is a subcutaneously administered, recombinant, fully human, immunoglobulin G1 monoclonal antibody that binds with high affinity and specificity to human TNF. It has been proven to be effective in the treatment of some inflammatory diseases, including IBD, being specially used when conventional treatment has failed and/or in moderate to severe cases. This drug is capable of inducing and maintaining remission, improving clinical outcomes and life quality, showing response in 72% of patients, after a year [4,5,8,9,11–16]. Though, this positive impact is threatened by the development of immunogenicity, which consists in the formation of antibodies against the drug. Initially it was thought that, for being fully human, ADA had a much inferior rate of immunogenicity than other anti-TNFα drugs. However, using different laboratory techniques, it is been pointed out that up to 44% of patients with IBD develop AAA[11,18].

In this retrospective cohort study, we found that the group that developed immunogenicity had lower serum level of ADA, which is in accordance with the well-studied relationship between the development of anti-drug antibodies and the decrease of drug concentration and the odds of response. In the case of treatment with ADA, some studies show that a considerable part of patients with IBD treated with ADA develop AAA and the presence of the anti-drug antibodies is associated with low serum ADA levels and decreased clinical response to the drug, reducing the odds of response by up to 87% [4,5,12–14,17,19].

The mechanism behind this impact of anti-drug antibodies is not well established, but it is known that there are two types of anti-drug antibodies – neutralizing and non-neutralizing - that act in different ways. Non-neutralizing antibodies lead to the formation of immune complexes, accelerating the clearance of the drug. On the other hand, neutralizing antibodies bind to the epitope binding (Fab')2 region of the anti-TNFα, preventing binding of the agents to target molecules and, consequently, reducing its therapeutic activity and efficacy. The latter are included in the so-called anti-idiotypic antibodies which represents the most significant part of the antibodies against ADA[4,11,13,23].

Normally, the development of antibodies occurs a few months after initiating the treatment. However there is some variability, with some studies pointing out that it can occur from as early as 14 days to several months post-dosing [4]. In this study we obtained a mean of 23,2 months (SD: 16,63) with a minimum of 12 months, with most patients developing immunogenicity in the first year of treatment. Even so, it is important to notice that one of the inclusion criteria in this study was that patients were treated with ADA for at least a year, and so, it was impossible to obtain a minimum lower than this value.

We also observed that the group that developed immunogenicity had significantly less time with ADA therapy. This can be justified by the fact that, in this sample, these patients had stopped the treatment short after developing immunogenicity, and so, as time passed, only those who had not developed it were left, leading to this result. The fact that the immunogenicity develops soon after initiating treatment emphasizes this discrepancy. This suggestion can be reinforced by the fact that the negative correlation found between the AAA titer and the time with ADA therapy (that is, the AAA decreases as the time with ADA increases) disappears when only patients who have not developed immunogenicity are considered. Although few studies have addressed this issue, it is thought that all biological drugs have the ability to induce immunogenicity over time[17].

Facing this problem led to the search for predictive factors of immunogenicity in order to prevent it or overcome it, as much as possible. Our study found that the use of corticosteroids at the beginning of treatment with ADA seems to influence the development of AAA, as the percentage of patients that used corticoids at the beginning of treatment was significantly higher in the group that developed immunogenicity. However, when analysing the impact of corticosteroids directly in the AAA titer and serum level of ADA, no correlation was found. Previous studies have pointed the concomitant use of corticosteroids as a predictive for loss of response, dose escalation and ADA immunogenicity [24–26], although others found no impact in the development of AAA or response to treatment[5,15]. As the CTT are normally used in patients with

higher inflammatory activity, this correlation may not be caused directly by the CTT but may be related to this higher inflammation. It is important to refer that this is not a very thoroughly studied correlation and this may be due to the fact that several studies exclude patients with concomitant use of corticosteroids due to the possibility that they underestimate the impact of combination therapy (ADA plus an IM), a frequently studied subject.

The group that developed immunogenicity was also associated with higher calprotectin values at the beginning of treatment with ADA, which in general clinical practice is associated with higher inflammatory activity. This could lead to the idea that the development of immunogenicity is more common when the initial inflammatory activity of the disease is higher. Nevertheless, there are several studies which refer that higher inflammatory activity, portrayed, for example, by greater baseline CDAI or markers of inflammation, is related to higher risk of loss of response or immunogenicity specifically. This impact may be related, at least partly, to the impact of it in the half-life of anti-TNFα, contributing to a faster drug elimination[8,11,17,19,24]. In fact, our study also showed that the serum level of ADA has a tendency to decrease as the calprotectin pre-treatment level increases, which may be associated to the direct impact of the increased inflammation status in ADA concentration and/or to the relation to the immunogenicity mentioned above. Concerning the latter, we found a cut-off value of 773,5 µg/g with sensitivity of 100% and specificity of 72%, with this meaning that pre-treatment calprotectin values greater than 773,5 µg/g are a good predictor of the development of immunogenicity. Nevertheless, it is important to mention the scarce number of patients with information about the pre-treatment calprotectin values in the group that developed immunogenicity, which could have influenced this result. Still, no correlation was found regarding the AAA titer directly, or the pre-treatment levels of CRP (other common marker if inflammation). About the CRP levels at the start of ADA treatment, a population-based cohort study[15] and the CLASSIC I trial[27] concluded that elevated CRP levels were associated with higher rates of response to induction therapy suggesting a better initial response when inflammatory disease activity is higher, which goes against what was mentioned previously about the impact of the baseline inflammation.

Even though in this study no additional relation was found, there are studies that point some of the variables tested here as possible predictive factors for immunogenicity, such as male sex, obesity and longer disease duration [8,15,17,19], although the mechanism behind it is not clear. For example, it is unknown if the impact of obesity is due to suboptimal dosing during induction or if it contributes directly to antibody formation.

Other studies address predictors of treatment failure in general, finding some of the factors mentioned above, but it is not possible to assure that the treatment failure is due to immunogenicity. Additionally, isolated colonic disease[24,25] and isolated ileum involvement[7] have been indicated as predictors of response loss and dose escalation, but no relation was found between any of these and immunogenicity in the studies mentioned. Regarding the structuring disease phenotype, a population-based cohort study[15] found it to have a negative effect in the induction phase response, but another study suggests it as a predictor of higher probability of disease remission[11].

Previous treatment with biological drug, namely IFX, is also commonly analysed and it has been associated with the development of AAA and loss of response, possibly in more than 50% of patients, affecting those who were refractory to the drug but also those who stopped it because of adverse effects. Despite that, it is known that, functional active antibodies against IFX are highly drug specific and do not cross react with ADA[5,24–26]. On the other hand, the GAIN study[28] concluded that ADA is effective in patients previously treated with IFX and the same conclusion has been found in other studies[15,16]. However, the absolute difference in remission induction obtained in the GAIN study was only 14% in comparison to the placebo group, which may suggest that ADA is less effective in these patients than in those who are IFX naïve[14,28]. It is noteworthy that in our study, the previous treatment with biological drug did not seem to affect the development of immunogenicity, titer of AAA or the ADA serum level.

One of the most relevant and controversial possible predictive factor is the concomitant use of an IM, such as AZA or MTX. This controversy is caused by the disparity of results found in different studies, both in relation to the clinical impact and in relation to the immunogenicity itself.

A population-based cohort study[15] concluded that the use of thiopurines, like AZA, in the first 6 months of treatment predicted an improved outcome of maintenance therapy but did not affect the induction phase. On the other hand, a systematic review and meta-analysis[18] demonstrated that combination therapy with ADA led to a mildly increased probability of induction of remission but did not alter the rate of clinical remission after one year or the need for dose escalation. Another study[29] raised the possibility of combination therapy being beneficial during the first semester of initiating ADA, leading to a slight decrease of failure and need for dose escalation but did not demonstrate any benefit in achieving clinical response and remission. However, other studies suggest that the concomitant use of IM does not improve the clinical response, chances of remission, serum levels of ADA or the development of AAA[14,18,20]. The CHARM trial showed that ADA was significantly superior to placebo for long-term treatment of CD independently of the use

of combination therapy and a stratified analysis of this data showed no relation between the use of AZA and the induction and maintenance of remission[16,30].

The DIAMOND study[30] indicated that combination therapy with ADA and AZA tends to lead to higher trough levels and lower positive rate of AAA, suggesting that thiopurines, such as AZA, are protective against immunogenicity of ADA. Still, this difference was not shown in maintaining clinical remission in patients with CD, although the combination therapy resulted in a better colonoscopic improvement at week 26 but not at week 52. This led the authors to suggest that combination therapy with ADA and AZA may be beneficial in CD patients with prominent mucosal damages. Other studies show a direct impact of IM in immunogenicity, leading to a lower frequency of antibodies by reducing the likelihood of their formation by 41% to 74%, mostly using MTX [13,17]. In contrast, a paediatric study demonstrated that this impact in drug concentration and AAA is significant only in patients who required the addition of IM due to low drug concentration with or without antibodies[31].

In our study, the concomitant use of IM (started at the beginning or during the treatment with ADA) did not have impact on the development of immunogenicity, the AAA titer, the serum level of ADA, or last calprotectin, CRP and albumin values, compared to the group that did ADA in monotherapy. Similarly, no difference was found between the 3 groups concerning the use of combination therapy (ADA in monotherapy for the entire treatment vs. combination therapy started at the beginning of treatment vs. combination therapy started during the treatment) regarding the same variables. However, comparing the group that did combination therapy at the beginning of treatment with the rest of the patients, we found significantly lower levels of calprotectin at the end of follow-up in the first group. This may show a positive impact of this strategy in the inflammatory activity of the disease, despite the lack of impact in the development of immunogenicity, as suggested above.

As seen above, as the controversy about the benefit of the combination therapy with IM remains, the doubt about the difference between the use of AZA and MTX persists as well. In our study we found a significantly higher serum level of ADA in the group that used MTX compared to the one that used AZA. This may show a positive impact of combination therapy with MTX at the expense of AZA. However, no difference was found regarding the last calprotectin value which possibly indicates that this higher level of ADA does not have influence in the inflammatory activity, and therefore may only contribute to higher toxicity. Nevertheless, when considering only the ones that used IMM at the beginning of treatment, no differences were found between the use of MTX and AZA.

While analysing comparisons within the group that initiated the treatment with ADA in combination with IM, it is important to remember that this only takes the use of combination therapy at the beginning of the treatment into consideration. This means that, in the period between the start of ADA therapy and the measurement of the AAA considered here (and the concomitant measurement of the other analytical values) these patients may have stopped the IM, changing the final results. The same reasoning can be applied to the group that started IM during treatment.

An additional limitation for the analysis of this topic is the fact that the decision between combination therapy or monotherapy was made by the responsible doctor for the patient at that time, having in consideration many factors, including the clinical manifestations of the disease and their own clinical judgment. This may lead to patients with more severe disease having a higher probability of starting combination therapy, leading to an unequal distribution regarding the patient´s condition and, possibly, to the absence of significant difference between these two groups. Moreover, it was not taken into account if the patients who did not do combination therapy had been treated with IM in the past.

Lastly, in our study the serum levels of ADA obtained when the AAA were measured exhibited a negative correlation with the CRP measured at that time, which means that a higher serum level of ADA is associated with lower CRP levels at the end of follow-up. Since the CRP is a commonly used inflammatory marker, this can possibly demonstrate the impact of ADA in the inflammatory activity of the disease. We also tested various possible cut-off values of serum level of ADA in order to try to find a value up to which there was a significant reduction of the calprotectin last follow-up value, and, therefore, possibly lower inflammation, however, no relation was found.

In fact, several studies have demonstrated that higher serum ADA concentrations are associated with biochemical, endoscopic and clinical remission. In this regard, optimal therapeutic cut-off points of serum ADA levels seems to range from 4,5 to 16 µg/mL, depending on the target outcome, with lower levels necessary to clinical remission and higher for endoscopic response[7,8,10,11,26,32]. However, this relation is complex since, as seen before, ADA is effective in the treatment of IBD and, therefore, its inflammation, but the inflamed tissue also influences drug concentration. Indeed, higher inflammation is associated with overexpression of biological drug targets, leading to more drug binging and so low blood levels. Additionally, it can accelerate drug clearance and intestinal inflammation specifically promotes drug loss into the lumen[11].

The present study has some limitations that are important to consider. Primarily, the fact that this is a retrospective cohort study which makes it only viable to establish association and not

causality between variables registered at the same time (in this case, titer of antibodies, serum level of ADA and albumin, CRP and calprotectin values at the end of follow-up) and makes it impossible to exclude possible confounders. Additionally, the therapeutic decisions and patient management were made by the patient's doctor at the time, being influenced by his personal judgement and possibly leading to a bias. Also, the small sample of our study, especially in some variables, makes it difficult to stagger the results, which must be done with caution. It is also worth mentioning that other variables that have been suggested as predictive of immunogenicity were not taken in account, in part, due to the study design, for example, smoking status, family history of IBD and ADA serum level during the induction phase. At last, it is important to remember that the method used to measure the titer of AAA (ELISA) is influenced by the concentration of the ADA itself, which means that high drug concentration can limit the detection of AAA, leading to a false low titer of it.

In conclusion, the concomitant use of corticosteroids and the baseline level of calprotectin were found as possible predictive factors of immunogenicity to ADA, which suggests that the initial inflammatory activity of IBD is a main factor for the development of immunogenicity. Hence, patients with higher inflammation may benefit from a stricter monitorization of serum levels of ADA and titer of AAA, especially during the first year of treatment, with early use of optimised dosing or early suspension in the event of immunogenicity. The use of combination therapy (ADA plus an IM) at the beginning of treatment, was associated with a lower calprotectin level at the end of follow-up, revealing a possible positive impact of this treatment strategy in decreasing inflammation and better disease control. However, in order to confirm this conclusion, there's a need for prospective studies with a bigger sample that use colonoscopy to evaluate inflammatory activity instead of only calprotectin levels. Still regarding the use of combination therapy, the use of MTX, at the beginning of treatment or started during it, exhibited a higher serum level of ADA. Yet, the lack of difference regarding the final calprotectin levels leaves the question of whether it really contributes to the reduction of inflammation or if it only increases toxicity of ADA by increasing its serum levels.

Appendices

Table I: Total sample and group that developed immunogenicity description.

	Total sample (n=71)	Group that developed immunogenicity[a] (n=5)
Gender: - Male - Female	 n=35 (49,3%) n=36 (50,7%)	 n=3 (60%) n=2 (40%)
Current age (years)	Mean: 42,39 (SD: 14,21)	Mean: 41,00 (SD: 14,21)
Age at diagnosis (years)	Median: 26,00 (IQR: 15)	Median: 33,00 (IQR: 19)
Age when ADA was started (years)	Median: 37,00 (IQR:20)	Median: 44,00 (IQR:26,50)
Years of disease when ADA was started	Median: 8,00 (IQR:11,00)	Median: 5,00 (IQR:10,50)
Weight when ADA was started [b] (Kg)	Mean: 64,70 (SD: 13, 90)	Mean: 64,10 (SD: 16,72)
Type of disease: - CD - UC - Undetermined colitis	 n=69 (97,2%) n=1 (1,4%) n=1 (1,4%)	 n=5 (100%) n=0 n=0
CD location: [c] - L1 - L2 - L3 - L1 and L4 - L2 and L4 - L3 and L4	 n=25 (36,2%) n=5 (7,2%) n=27 (39,1%) n=5 (7,2%) n=1 (1,4%) n=6 (8,7%)	 n=1 (20%) n=0 n=2 (40%) n=2 (40%) n=0 n=0
CD behaviour: [d] - B1 - B2 - B3 - B2 and B3	 n=27 (39,1%) n=25 (36,2%) n=13 (18,8%) n=4 (5,8%)	 n=4 (80%) n=1 (20%) n=0 n=0
Presence of perianal disease	n=22 (31,9%)	n=0
Previous treatment with biological drug	n=28 (39,4%)	n=1 (20%)

	Total sample (n=71)	Group that developed immunogenicity[a] (n=5)
Reason to start ADA: - AZA failure - MTX failure - Corticosteroid failure - Refractory to biological drug - Incomplete response to biological drug - Secondary loss of response to biological drug (not due to immunogenicity) - Immunogenicity to biological drug - Adverse effects to previous treatment - IFX and IM naive	 n=16 (22,5%) n=3 (4,2%) n=7 (9,9%) n=1 (1,4%) n=1 (1,4%) n=8 (11,3%) n=11 (15,5%) n=19 (26,8%) n=5 (7,0%)	 n=1 (20%) n=0 n=1 (20%) n=0 n=0 n=0 n=0 n=2 (40%) n=1 (20%)
Regularity of ADA administration at beginning of treatment: - Weekly - Biweekly	 n=17 (23,9%) n=54 (76,1%)	 n=1 (20%) n=4 (80%)
Combination therapy with IM started at the beginning of treatment with ADA: - AZA - MTX	n=41 (82%) n=25 (61%) n=16 (39%)	n=2 (40%) n=1 (50%) n=1 (50%)
Combination therapy with IM started during the treatment with ADA: - AZA - MTX	n=9 (18%) n=1 (11,1%) n=8 (88,9%)	n=0
Combination therapy with IM (started at the beginning or during the treatment with ADA): - AZA - MTX	n=50 (29,6%) n=26 (52%) n=24 (48%)	n=2 (40%) n=1 (50%) n=1 (50%)
Use of corticosteroids at the beginning of treatment with ADA	n=13 (18,3%)	n=3 (60%)
AAA titer (ng/mL) [e]	Median: 0,4 (IQR: 0)	Median: 84,20 (IQR: 247,95)
Serum level of ADA (µg/mL) [e]	Median: 11,20 (IQR: 8,70)	Median: 0,20 (IQR: 0,25)
Time until the development of immunogenicity [a] (months)	-	Mean: 23,20 (SD:16,63)
Present treatment with ADA: - Yes - No, due to adverse effects - No, due to immunogenicity - No, due to secondary failure - No, due to sustained remission	 n=51 (71,8%) n=5 (7,0%) n=5 (7,0%) n=9 (12,7%) n=1 (1,4%)	 n=0 n=0 n=5 (100%) n=0 n=0
Time with ADA therapy (months)	Median: 46 (IQR: 41)	Median: 13,00 (IQR: 31)

	Total sample (n=71)	Group that developed immunogenicity[a] (n=5)
Pre-treatment analytical values		
- CRP[f](mg/dL)	Median: 6,38 (IQR: 14,12)	Median: 5,13 (IQR:49,35)
- Calprotectin[g](µg/g)	Median: 503,00 (IQR: 800)	Median: 1992,00 (-)
- Albumin[h](g/dL)	Median: 4,30 (IQR: 0,68)	Median: 4,05 (-)
Analytical values when measuring AAA/drug level		
- CRP[i] (mg/dL)	Median: 2,57 (IQR: 5,89)	Median: 8,99 (IQR: 35,53)
- Calprotectin[j](µg/g)	Median: 276,00 (715)	Median: 788,00 (IQR: 1351)
- Albumin[k](g/dL)	Median: 4,44 (0,79)	Median: 4,07 (-)

a. Immunogenicity was defined as anti-ADA antibodies titer > 10ng/mL.
b. Total sample: n=68.
c. L1- ileum; L2- colon; L3- ileum-colon; L4- upper GI tract.
d. B1- non-stenosing, non-penetrating; B2- stenosing; B3- penetrating.
e. The most recent measurement in the last 12 months of treatment.
f. Total sample: n=69.
g. Total sample: n=35 and group that developed immunogenicity: n=3.
h. Total sample: n=30 and group that developed immunogenicity: n=2.
i. Total sample: n=70 and group that developed immunogenicity: n=4.
j. Total sample: n=59.
k. Total sample: n=26 and group that developed immunogenicity: n=2.

Table II. Comparison between the group that developed immunogenicity and the group that did not.

	Immunogenicity Negative (n=66)	Immunogenicity Positive [a] (n=5)	p
Male gender	n=32 (48,5%)	n=3 (60,0%)	0,620
Age when ADA was started (years)	Median: 36,50 (IQR:19,25)	Median: 44,00 (IQR:26,50)	0,910
Age at diagnosis (years)	Median: 25,50 (IQR:16)	Median: 33,00 (IQR:19)	0,451
Years of disease when ADA was started	Median: 8,00 (IQR:12,00)	Median: 5,00 (IQR:10,50)	0,265
Weight when ADA was started [b] (Kg)	Mean: 64,76 (SD:13,82)	Mean: 64,10 (SD:16,72)	0,920
B1-Non-stenosing, non-penetrating [c]	n=23 (35,9%)	n=4 (80,0%)	0,052
Absence of perianal disease[c]	n=42 (65,6%)	n=5 (100%)	0,112
Previous treatment with biological drug	n=27 (40,9%)	n=1 (20,0%)	0,356
Reasons to start ADA			0,679
- Immunotherapy or corticoid failure	n=24 (36,4%)	n=2 (40,0%)	
- Adverse effects	n=17 (25,8%)	n=2 (40,0%)	
- Other	n=25 (37,9%)	n=1 (20,0%)	
ADA dose at the beginning of treatment (mg)	Median: 40,00 (IQR:0)	Median: 40,00 (IQR:0)	0,574
Weekly administration of ADA	n=16 (24,2%)	n=1 (20,0%)	0,830
Time with ADA therapy (months)	Median:47,00 (IQR: 48)	Median:13,00 (IQR: 31)	0,018
Use of corticosteroids at the beginning of treatment with ADA	n=10 (15,2%)	n=3 (60,0%)	0,040[d] OR:8,4; 95% CI [1,242; 56,813]
Combination therapy with IM	n=48 (72,7%)	n=2 (40,0%)	0,122
- Combination therapy with immunomodulator at the beginning of treatment with ADA	n=39 (59,1%)	n=2 (40,0%)	0,405
- Combination therapy with IM initiated during the treatment with ADL	n=9 (13,6%)	n=0 (0%)	1,000[d]

	Immunogenicity Negative (n=66)	Immunogenicity Positive [a] (n=5)	p
Pre-treatment analytical values			
- CRP [c](mg/dL)	Median: 6,48 (IQR:13,49)	Median: 5,13 (IQR:49,35)	0,594
- Calprotectin[e](µg/g)	Median: 442,50 (IQR: 626,75)	Median: 1992,00 (IQR: -)	0,034
- Albumin[f](g/dL)	Median: 4,30 (IQR: 0,64)	Median:4,05 (IQR: -)	0,506
Analytical values when measuring AAA			
- CRP[g](mg/dL)	Median: 2,26 (IQR: 5,38)	Median: 8,99 (IQR: 35,53)	0,054
- Calprotectin[h](µg/g)	Median: 265,50 (IQR: 576,50)	Median: 788,00 (IQR: 1351)	0,097
- Albumin[i](g/dL)	Median: 4,44 (IQR: 0,75)	Median: 4,07 (IQR: -)	0,736
Serum level of ADA [j] (µg/mL)	Median: 11,75 (IQR: 8,22)	Median: 0,20 (IQR: 0,25)	<0,001

a. Immunogenicity titer > 10ng/mL.
b. Immunogenicity Negative: n=63.
c. Immunogenicity Negative: n=64.
d. Fisher test.
e. Immunogenicity Negative: n=32 and Immunogenicity positive: n=3.
f. Immunogenicity Negative: n=28 and Immunogenicity positive: n=2.
g. Immunogenicity positive: n=4.
h. Immunogenicity Negative: n=54.
i. Immunogenicity Negative: n=24 and Immunogenicity positive: n=2.
j. The most recent measurement in the last 12 months of treatment.

Table III: Comparison between the group that used combination therapy and the group that did not.

	ADA in monotherapy during the entire treatment (no combination therapy with immunomodulator) (n=21)	ADA in combination therapy with immunomodulator (started in the beginning or during the treatment) (n=50)	p
Development of immunogenicity[a]	n=3 (14,3%)	n=2 (4%)	0,122
Serum level of ADA [b](µg/mL)	Median: 10,30 (IQR: 8,15)	Median: 11,55 (IQR: 9,30)	0,391
AAA titer [b] (ng/mL)	Median: 0,40 (IQR: 0,15)	Median: 0,40 (IQR: 0)	0,193
Analytical values when measuring AAA			
- CRP[c](mg/dL)	Median: 2,13 (IQR: 10,27)	Median: 2,67 (IQR: 5,10)	0,944
- Calprotectin[d](µg/g)	Median: 509,00 (IQR: 1388,00)	Median: 230,50 (IQR: 523,25)	0,107
- Albumin[e](g/dL)	Median: 4,45 (IQR: 0,93)	Median: 4,44 (IQR: 0,70)	0,597

a. Immunogenicity was defined as anti-ADA antibodies titer > 10ng/mL.
b. The most recent measurement in the last 12 months of treatment.
c. ADA in combination therapy with immunomodulator (started in the beginning or during the treatment): n=49.
d. ADA in monotherapy during the whole treatment (no combination therapy with immunomodulator): n= 15 and ADA in combination therapy with immunomodulator (started in the beginning or during the treatment): n= 44.
e. ADA in monotherapy during the whole treatment (no combination therapy with immunomodulator): n= 8 and ADA in combination therapy with immunomodulator (started in the beginning or during the treatment n=18.

Table IV: Comparison between different groups regarding the use of combination therapy: ADA in monotherapy during the entire treatment vs. ADA initiated in combination therapy with IM vs. IM started during ADA therapy.

	ADA in monotherapy during the entire treatment (n=21)	ADA initiated in combination therapy with IM (n=41)	IM started during ADA therapy (n=9)	p
Serum level of ADA [a] (µg/mL)	Median: 10,30 (IQR: 8,15)	Median: 10,90 (IQR: 9,05)	Median: 16,00 (IQR: 5,90)	0,181
AAA titer [a] (ng/mL)	Median: 0,40 (IQR: 0,15)	Median: 0,40 (IQR: 0)	Median: 0,40 (IQR: 0)	0,242
Analytical values when measuring AAA				
- CRP[b](mg/dL)	Median:2,13 (IQR: 10,27)	Median: 2,66 (IQR: 5,15)	Median: 2,67 (IQR: 6,27)	0,991
- Calprotectin[c](µg/g)	Median: 509,00 (IQR: 1388,00)	Median: 154,00 (IQR: 426,50)	Median: 460,00 (IQR: 824,25)	0,090
- Albumin[d](g/dL)	Median: 4,45 (IQR: 0,93)	Median: 4,44 (IQR:0,72)	Median: 3,69 (IQR: -)	0,592

a. The most recent measurement in the last 12 months of treatment.
b. ADA initiated in combination therapy with IM: n=40.
c. ADA in monotherapy during the entire treatment: n=15; ADA initiated in combination therapy with IM: n=36; IM started during ADA therapy; n=8.
d. ADA in monotherapy during the entire treatment: n=8; ADA initiated in combination therapy with IM: n=16; IM started during ADA therapy; n=2.

Table V: Comparison between the group that used combination therapy since the beginning of treatment and the group that did not.

	ADA initiated in monotherapy (no combination therapy with immunomodulator) (n=30)	ADA initiated in combination therapy with immunomodulator (n=41)	p
Development of immunogenicity[a]	n=3 (10)	n=2 (4,9)	0,405
Serum level of ADA [b](µg/mL)	Mean: 12,07 (SD: 8,04)	Mean: 10,87 (SD: 6,06)	0,475
AAA titer [b] (ng/mL)	Median: 0,4 (IQR: 0)	Median: 0,4 (IQR: 0)	0,774
Analytical values when measuring AAA			
- CRP[c](mg/dL)	Median: 2,46 (IQR: 9,81)	2,66 (5,15)	0,981
- Calprotectin[d] (µg/g)	Median: 509,00 (IQR: 1186)	154,00 (426,50)	0,028
- Albumin[e](g/dL)	Mean: 4,17 (SD: 0,70)	Mean: 4,25 (SD: 0,51)	0,731

a. Immunogenicity was defined as anti-ADA antibodies titer > 10ng/mL.
b. The most recent measurement in the last 12 months of treatment.
c. ADA initiated in combination therapy with immunomodulator: n=40.
d. ADA initiated in monotherapy: n= 23 and ADA initiated in combination therapy with immunomodulator: n=36.
e. ADA initiated in monotherapy: n= 10 and ADA initiated in combination therapy with immunomodulator: n=16.

Table VI: Comparison between the group that used combination therapy with AZA and the group that used combination therapy with MTX.

	ADA in combination therapy with AZA (n=26)	ADA in combination therapy with MTX (n=24)	p
Serum level of ADA [a] (µg/mL)	Median: 10,10 (IQR: 8,55)	Median: 13,25 (IQR: 7,63)	0,036
AAA titer [a] (ng/mL)	Median: 0,40 (IQR: 0)	Median: 0,40 (IQR: 0)	0,931
Analytical values when measuring AAA			
- CRP[b] (mg/dL)	Median: 1,65 (IQR: 4,03)	Median: 3,64 (IQR: 6,00)	0,071
- Calprotectin[c] (µg/g)	Median: 64,50 (IQR: 469,75)	Median: 286,00 (IQR: 520,50)	0,121
- Albumin[d](g/dL)	Mean: 4,17 (0,54)	Mean: 4,19 (0,65)	0,947

a. The most recent measurement in the last 12 months of treatment.
b. ADA in combination therapy with MTX: n=23.
c. ADA in combination therapy with AZA: n=22 and ADA in combination therapy with MTX: n=22.
d. ADA in combination therapy with AZA: n=9 and ADA in combination therapy with MTX: n=9.

Table VII: Comparison between the group that used combination therapy with AZA and the group that used combination therapy with MTX within the patients that initiated ADA in combination therapy.

	ADA initiated in combination therapy with AZA (n=25)	ADA initiated in combination therapy with MTX (n=16)	p
Serum level of ADA [a] (µg/mL)	Mean: 9,66 (SD: 5,04)	Mean: 2,76 (SD: 7,14)	0,110
AAA titer [a] (ng/mL)	Median: 0,40 (IQR: 0)	Median: 0,40 (IQR: 0)	0,572
Analytical values when measuring AAA			
- CRP[b] (mg/dL)	Median: 1,68 (IQR: 4,31)	Median: 3,78 (IQR: 6)	0,158
- Calprotectin[c] (µg/g)	Median: 64,00 (IQR: 376,50)	Median: 199,00 (IQR: 399)	0,162
- Albumin[d](g/dL)	Mean: 4,18 (SD: 0,54)	Mean: 4,34 (SD: 0,51)	0,545

a. The most recent measurement in the last 12 months of treatment.
b. ADA initiated in combination therapy with MTX: n=15.
c. ADA initiated in combination therapy with AZA: n=21 and ADA initiated in combination therapy with MTX: n=15.
d. ADA initiated in combination therapy with AZA: n=9 and ADA initiated in combination therapy with MTX: n=7.

Table VIII: Correlation between different variables and the most recent anti-ADA antibodies titer and serum ADA concentration in the last 12 months of treatment with ADA.

	AAA titer (ng/mL) [a]		Serum level of ADA (µg/mL) [a]	
		p		p
Gender: Male (n=35) / Female (n=36)	Median (IQR): 0,40 (74,06) / 0,40 (0)	0.869	Median (IQR): 10,90 (9,30) / 11,50 (9,13)	0,927
Age when ADA was started (years) (n=71)	-	0,713	-	0,911
Age at diagnosis (years) (n=71)	-	0,997	-	0,785
Years of disease when ADA was started	-	0,369	-	0,918
Weight when ADA was started (Kg) (n=68)	-	0,828	-	0,421
Type of IBD: CD (n=69) /Other (n=2)	Median (IQR): 0,40 (0) / 0,40 (0)	0,544	Median (IQR): 11,20 (8,50) / 8,20 (-)	0,702
Disease location[b] : L1 (n=25) / L2 (n=5) / L3 (n=27) / L4 component (n=11)	Median (IQR): 0,40 (0) / 0,40 (0) / 0,40 (0) / 0,40 (0,60)	0,436	Median (IQR): 12,70 (8,75) / 11,80 (6,40) / 10,30 (6,60) / 13,00 (15,30)	0,524
Disease Behaviour [c]: B1 (n=27) / B2 (n=25) / B3 (n=13) / B2 and B3 (n=4)	Median (IQR): 0,40 (0) / 0,40 (0) / 0,40 (0) / 0,40 (0)	0.590	Median (IQR): 11,20 (6,60) / 11,90 (10,65) / 12,00 (11,05) / 10,45 (7,20)	0,778
Perianal disease: Present (n=22) / Absent (n= 47)	Median (IQR): 0,40 (0) / 0,40 (0)	0,241	Median (IQR): 10,55 (7,83) / 11,30 (8,50)	0,471
Previous treatment with biological drug: Yes (n=28) / No (n=43)	Median (IQR): 0,40 (0) / 0,40 (0)	0.365	Median (IQR): 12,05 (8,15) / 10,50 (8,50)	0.337
Reason to start ADA: AZA, MTX or CCT failure (n=26) /Adverse effects (n=19) / IFX and IMM naïve (n=5) / Other [d] (n=21)	Median (IQR): 0,40 (0) / 0,40 (0) / 0,40 (65,55) / 0,40 (0)	0,717	Median (IQR): 10,00 (7,23) / 11,80 (7,90) / 6,70 (10,75) / 13,50 (8)	0,182
Start of ADA due to immunogenicity [e]: Yes (n=11) / No (n=60)	Median (IQR): 0,40 (0) / 0,40 (0)	0,496	Median (IQR): 13,50 (6,10) / 10,75 (8,25)	0,131

	AAA titer (ng/mL) [a]	Serum level of ADA (µg/mL) [a]	AAA titer (ng/mL) [a]	Serum level of ADA (µg/mL) [a]
		p		p
ADA dose at the beginning of treatment (mg) (n=71)	-	0,387	-	0,912
Regularity of ADA administration at the beginning of treatment: Weekly (n=17) / Biweekly (n=54)	Median (IQR): 0,40 (0) / 0,40 (0)	0,797	Mean (SD): 13,06 (8,33) / 10,84 (6,44)	0,253
Use of corticosteroids when ADA was started: Yes (n=13) / No (n=58)	Median (IQR): 0,40 (22,85) /0,40 (0)	0,277	Median (IQR): 9,70 (13,15) /11,50 (9,30)	0,209
Time with ADA therapy (months) (n=71)	-	0,014 CC: -0,289 (weak)	-	0,765
- Within the group that did not develop immunogenicity [e]	-	0,272	-	0,439
Pre-treatment analytical values				
-CRP (mg/dL) (n=69)	-	0,591	-	0,853
- Calprotectin (µg/g) (n=35)	-	0,257	-	0,030 CC: -0,367 (weak)
-Albumin(g/dL) (n=30)	-	0,532	-	0,961
Analytical values when measuring AAA				
-Albumin (g/dL) (n=26)	-	0,903	-	0,105
-CRP (mg/dL) (n=70)	-	0,137	-	0,012 CC: -0,300 (weak)
- Calprotectin (µg/g) (n=59)	-	0,137	-	0,969

	AAA titer (ng/mL) [a]	Serum level of ADA (µg/mL) [a]	AAA titer (ng/mL) [a]	Serum level of ADA (µg/mL) [a]
		p		p
Analytical values when measuring AAA within the group that did not develop immunogenicity [e]				
-Albumin (g/dL) (n=24)	-	0,865	-	0,073
-CRP (mg/dL) (n=66)	-	0,801	-	0,062
- Calprotectin (µg/g) (n=54)	-	0,680	-	0,415

a. The most recent measurement in the last 12 months of treatment.
b. L1- ileum; L2- colon; L3- ileum-colon; L4- upper GI tract.
c. B1- non-stenosing, non-penetrating; B2- stenosing; B3- penetrating.
d. Includes: Refractory to IFX, secondary loss of response to biologic drug, immunogenicity and incomplete response to biologic drug.
e. Immunogenicity was defined as anti-ADA antibodies titer > 10ng/mL.

Table IX: Correlation between different groups of serum level of ADA (µg/mL) and calprotectin last follow-up value.

Serum level of ADA (µg/mL)[a]	Calprotectin level when measuring AAA (µg/g) - Median (IQR)	p	Calprotectin level >250 µg/g when measuring AAA	p
- <3,5 (n=7) - ≥ 3,5 and ≤ 7 (n=8) - > 7 and ≤ 10 (n=8) - > 10 (n= 36)	- 788,00 (2008,00) - 233,50 (498,75) - 45,00 (356,00) - 286,00 (832,75)	0,130	- n=5 (15,6%) - n=4 (12,5%) - n=2 (6,3%) - n=21 (65,6%)	0,274
- <3,5 (n=7) - ≥ 3,5 and ≤ 7 (n=8) - > 7 (n=44)	- 788,00 (2008,00) - 233,50 (498,75) - 265,50 (667,50)	0,164	- n=5 (15,6%) - n=4 (12,5%) - n=23 (71,9%)	0,619
- ≤ 5 (n=11) - > 5 (n=48)	- 435,00 (976,00) - 265,50 (663,50)	0,161	- n=7 (21,9%) - n=25 (78,1%)	0,488

a. The most recent measurement in the last 12 months of treatment.

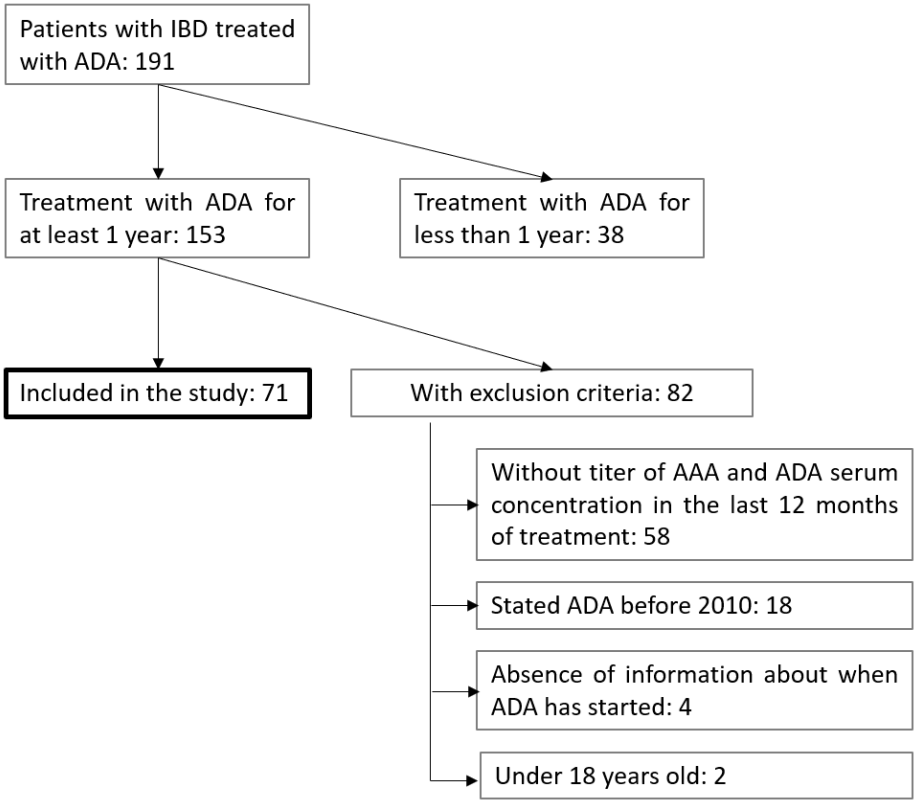

Figure 1: Patient selection scheme.

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