CLINICAL STUDY PROTOCOL

Grupo de Estudo da Doença Inflamatória Intestinal (GEDII)

Title: Study to investigate the correlation of fecal calprotectin with serum Drug levels and development of antI-dRug antibodiEs among Pediatric patients with inflammatory bowel disease reCeiving anti-TNF-alfa treatment – P-Direct Study

Study code: **P- DIRECT**

Type of study: Observational

Date of protocol: 07 March 2015

Version no.: 1

Study Sponsor:	Grupo de Estudo da Doença Inflamatória Intestinal (GEDII)
Name of Scientific Coordinator	Dra. Eunice Trindade
Signature and Date	_____ _____
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PRINCIPAL INVESTIGATOR SIGNATURE PAGE *(to be signed by the PI from each participating center)*

Study Title: Study to investigate the correlation of fecal calprotectin with serum Drug levels and development of antI-dRug antibodiEs among pediatric patients with inflammatory bowel disease reCeiving anti-TNF-alfa treatment –P-Direct Study

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Center Name: _____

Principal Investigator:_____

Name:

Academic degree:

Address:

Phone:

Email:

I, the undersigned, am responsible for the conduct of the study at this site and affirm that:

I understand and will conduct the study according to the protocol, any approved protocol amendments, and all applicable Health Authority requirements and national laws.

I will not deviate from the protocol without prior written permission from the GEDII, except where necessary to prevent immediate danger to the subject.

Signature Date of Signature

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1. Sinopse

Title:	**Study to investigate the correlation of fecal calprotectin with serum Drug levels and development of antI-dRug antibodiEs among pediatric patients with inflammatory bowel disease reCeiving anti-TNF-alfa treatment –P-DIRECT Study**
Study Code:	**P-DIRECT**
Scientific Coordinator:	Dra. Eunice Trindade
Disease/Condition	Crohn's disease (CD) and Ulcerative Colitis (UC)
Rational	Thus far, there is no evidence if fecal calprotectin, a recognized biomarker in the context of IBDs, correlates with serum levels of anti-TNF-alfa therapies and with the development of antibodies against this class of drugs. Taking advantage of the valuable tool such as the GEDII Registry, the GEDII promoted a research to increase this knowledge. This study will follow a cohort of patients with CD or UC registered in the GEDII database and who are receiving infliximab according to the approved label in Portugal. All patients, regardless of the anti-TNF-alfa treatment phase (induction or maintenance) at study inclusion, will be followed throughout a period of approximately 2 years. In addition, this study will investigate the correlation of serum drug levels with development of anti-drug antibodies among different subsets of the eligible population and the association of these indicators with physician reported clinical outcomes.
Research hypothesis:	We hypothesize that fecal calprotectin levels will significantly correlate with serum levels of infliximab (IFX) and with the development of antibodies against these drug.
Primary Objectives:	**Among biologic-naïve and non-naïve patients with CD or patients with UC registered in the GEDII Registry:** • To explore the association of fecal calprotectin levels with serum IFX levels throughout a period of 2 years since the start of observation period. • To explore the association of fecal calprotectin levels with the development of anti-IFX antibodies throughout a period of 2 years since the start of observation period.
Secondary Objective(s):	**Among biologic-naïve and non-naïve patients with DC or patients with UC registered in the GEDII Registry:** • To explore the association of serum IFX levels with the development of anti-IFX antibodies • To explore the association of serum IFX levels with clinical activity throughout a period of 2 years since the start of observation period. • To explore the association of development of anti-IFX antibodies with clinical activity throughout a period of 2 years since the start of observation period. • To explore the association of therapeutic attitude with serum IFX levels throughout a period of 2 years since the start of observation period. • To explore the association of therapeutic attitude with development of anti-IFX antibodies throughout a period of 2 years since the start of observation period • To explore the association of calprotectin levels with clinical activity among the subgroup of patients who were co-medicated with azathioprine (AZA). • To explore the association of calprotectin levels with clinical activity among the subgroup of patients who were co-medicated with methotrexate (MTX).
Study Design:	Multicenter, prospective, observational study designed to gather and analyze data on a consecutive cohort of subjects with CD or UC treated with infliximab. There is no imposed experimental intervention and infliximab will be managed taking into account the label approved in Portugal.

	The overall duration of observation for each patient is approximately two years from the time patient initiated the participation in the study (start of observation period), regardless of the anti-TNF-alfa treatment phase (induction or maintenance) at the time of inclusion in the study. Basal socio-demographic and clinical characteristics will be collected as well as physician-reported clinical outcomes (Pediatric ulcerative colitis activity index – PUCAI or Pediatric Crohn´s disease activity index - PCDAI, according to the IBD studied) throughout several data collection time points. The data collection time points in this study will reflect the routine scheduled appointments, and will take into account the condition being studied (CD or UC) and the anti-TNF-alfa received. Faecal samples will be collected at the same time points as for the collection of blood samples (serum levels and anti-drug antibodies)
Inclusion Criteria:	Study subjects must fulfil the following criteria: 1. Male or female patients, <18 years 2. Patients who are registered in the GEDII Registry 3. Patients with moderate to severe active Crohn's disease or moderate to severe active Ulcerative Colitis; 4. Patients receiving infliximab according to the local approved label, including: o Biologic-naïve patients initiating induction with infliximab at time of inclusion in the study or; o Patients already under maintenance treatment with infliximab at time of inclusion in the study; 5. Patients who gave their informed consent.
Exclusion Criteria:	Subjects will be excluded if at least one of the following criteria is met: 1.Patients who are not eligible for therapy with anti-TNF-alfa 2.Patients who are being treated with any investigational agent; 3.Patients who are not willing to comply with routine clinical appointments.
Expected number of subjects:	100 patients
Expected number of sites:	Approximately 11 centers are expected to participate.
Subject selection:	All subjects who meet eligibility criteria and give their written informed consent to participate will be consecutively enrolled.
Exposure of interest:	Infliximab (Remicade) is the exposure of interest. The product will be used according to the approved label.
Main data collected:	Socio-demographic and medical history and UC/CD related data will be collected for all eligible patients. Clinical activity of disease, serum IFX, anti-drug antibodies and fecal calprotectin levels will be measured at first appointment and throughout the data collection time points of the follow up period (up to month 24).
Endpoints	**Primary endpoints:** **In the subset of biologic-naïve and non-naïve patients with active, moderate to severe CD receiving IFX:** •Fecal calprotectin levels and serum IFX levels at each data collection time points •Fecal calprotectin levels and presence of anti-IFX antibodies (+ or -) at each data collection time points **In the subset of biologic-naïve and non-naïve patients with active, moderate to severe UC receiving IFX:** •Fecal calprotectin levels and serum IFX levels at each data collection time points •Fecal calprotectin levels and presence of anti-IFX antibodies (+ or -) at each data collection time points **Secondary endpoints:** **In the subset of biologic-naïve and non-naïve patients with active, moderate to severe CD receiving IFX:** •Serum IFX levels and anti-IFX antibodies (+ or -) at each data collection time points •Serum IFX levels and clinical activity (PCDAI) at each data collection time points. •Anti-IFX antibodies (+ or -) and clinical activity (PCDAI) at each data collection

	time points. •Therapeutics administered for CD (name and dose) and serum IFX levels. •Therapeutics administered for CD (name and dose) and anti-IFX antibodies (+ or -). **In the subset of biologic-naïve and non-naïve patients with active, moderate to severe UC receiving IFX:** •Serum IFX levels and anti-IFX antibodies (+ or -) at each data collection time points •Serum IFX levels and clinical activity (PUCAI) at each data collection time points. •Anti-IFX antibodies (+ or -) and clinical activity (PUCAI) at each data collection time points.. •Therapeutics administered for UC (name and dose) and serum IFX levels. •Therapeutics administered for UC (name and dose) and anti-IFX antibodies (+ or -) **In the subsets of biologic-naïve and non-naïve patients patients with active, moderate to severe CD receiving IFX + azathioprine or IFX + methotrexate:** •Fecal calprotectin levels and serum IFX levels at each data collection time points •Fecal calprotectin levels and presence of anti-IFX antibodies (+ or -) at each data collection time points **In the subsets of biologic-naïve and non-naïve patients with active, moderate to severe UC receiving IFX + azathioprine or IFX + methotrexate:** •Fecal calprotectin levels and serum IFX levels at each data collection time points •Fecal calprotectin levels and presence of anti-IFX antibodies (+ or -) at each data collection time points
Statistical methods	The association between two quantitative variables will be performed through Pearson correlation coefficient or Spearman correlation coefficient, in case the normality assumption is not verified. The association of two categorical variables will be tested through the Chi-Square test or Fisher Exact test (if applicable). The comparison of two independent samples in respect to quantitative variables will be performed through t-test for independent samples or the Mann-Whitney non-parametric test, according to the assumption validations of the statistical test (if applicable). The association between quantitative variables and a categorical binary variable will be analyzed by Area Under the ROC Curve. The concordance kappa will be used binary variables, if applicable. For each subset, the proportion of patients with active disease, clinical response, clinical remission (physician reported PUCAI or PCDAI), presence of anti-drug antibodies at each data collection time points will be summarized using 95% confidence intervals. Exploratory Generalized Estimated Equations with AR1 correlation structure in time, to account for the within-subject correlations, will be used to explore the association between fecal calprotectin levels and serum drug levels (dependent variable) as well as the association of fecal calprotectin levels with the presence of antidrug antibodies (dependent variable), throughout the follow up period. The impact of immunogenicity on the efficacy of IFX will also be exploratory assessed by associating serum drug levels and anti-drug antibodies in respect to the outcomes, need for dose escalation, and discontinuation rate at the predetermined time points.
Overall Study Duration:	The overall duration of the study is three years (1 year of recruitment + 2-year observation period).
Study timelines:	The study is expected to start during the 2nd Quarter of 2016. Study closure is expected to occur 3th Quarter of 2019.

2 INTRODUCTION

2.2 INFLAMMATORY BOWEL DISEASE AND ANTI-TNF-ALFA THERAPIES

Inflammatory bowel disease (IBD) is a chronic and disabling condition with an increasing incidence in southern Europe. The etiology of IBD remains unknown, but the characteristic disproportionate inflammatory response in the gut may develop through various mechanisms at the cellular and subcellular level.[1] Ulcerative colitis (UC) and Crohn's disease (CD) represent the two main types of IBD.

Ulcerative colitis is a relapsing non-transmural inflammatory disease that is restricted to the colon. Patients typically have bloody diarrhoea (often nocturnal and postprandial), passage of pus, mucus, or both, and abdominal cramping during bowel movements and children usually present with severe disease (extensive colitis or pancolitis)[2,3]. Crohn's disease is a relapsing, transmural inflamatory disease of the gastrointestinal mucosa that can affect the entire gastrointestinal tract from the mouth to the anus. Typical presentations include the discontinuous involvement of various portions of the gastrointestinal tract and the development of complications including strictures, abscesses, or fistulas. Pediatric patients may present with a variety of phenotypes at diagnosis and treatment can be very challenging[2,4].

In Portugal, the prevalence of IBDs increased from 86 to 146 persons per 100 000 inhabitants between 2003 and 2007. Within the same timeframe, the prevalence of UC and CD increased from 42 to 71 persons per 100 000 inhabitants and from 43 to 73 persons per 100 000 inhabitants, respectively. IBDs are more prevalent among women[5]. There are no portuguese pediatric data about the incidence of IBD but it is increasing worldwide, ranging from 2.5 to 11.4 per 100,000, with an estimated prevalence of 58/100,000[6,7].

Tumor necrosis factor α (TNF) is a proinflammatory cytokine that plays a central role in the pathogenesis of CD and UC[8]. Abundantly expressed in the gastrointestinal tracts of patients with IBD[9], TNF is believed to contribute to intestinal mucosal inflammation through several mechanisms, including disruption of the epithelial barrier, induction of apoptosis of the villous epithelial cells, and secretion of chemokines from intestinal epithelial cells[8].

Over the past decade, anti-TNF agents have dramatically influenced the treatment of patients with refractory IBD. These agents have been developed as monoclonal antibodies (mAbs) and are directed against TNF molecules.

Infliximab (IFX, Remicade) is a chimeric immunoglobulin G (IgG) human (75%)/murine (25%) mAb administered by intravenous infusion (5 mg/kg), indicated for induction and maintenance of remission in adult and pediatric CD and for induction and maintenance of remission of UC.[10,11,12] IFX is also approved for other chronic inflammatory conditions such as rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, and plaque psoriasis.

2.3 IMMUNOGENICITY AND SERUM ANTI-TNF-ALFA

Despite the proven efficacy of anti-TNF-alfa agents in the maintenance of remission in IBDs, a significant proportion of patients lose their clinical response over time despite maintenance treatment.[13,14] This loss of response occurs in up to 70% of patients treated with IFX, and usually requires escalation of dosing or change in anti-TNF-alfa agent to re-capture clinical remission.[15,16]

There are several explanatory mechanisms to loss of response to anti-TNF-alfa agents. However, immunogenicity to the antibody itself appears to be a commonly identified factor.[10,17,18]

Since IFX is a chimeric mouse–human IgG1 molecule, antibodies to IFX are primarily directed against the murine F(ab)2 fragment of the agent.[19,20] Anti-drug antibodies are reported to develop in 8–60%

of patients with inflammatory bowel disease (IBD), depending on IFX dosing schedule, administration of concomitant steroids or immunomodulators and the method of measuring anti-drug antibodies in the blood.[18,19,21] These antibodies can appear as soon as after the first infusion of the drug, and can persist in the blood stream for up to 1–4.5 years even after discontinuation of therapy.[22,23] Until present there are few studies in pediatric population with limited number of patients but apparently with results comparable to those observed in adult patients[24,25].

Higher IFX trough levels have been associated with sustained clinical remission in several studies.[23,26,27] Seow et al. (2010)[27] showed that in their UC population treated with scheduled IFX dosing, detectable IFX trough was associated with significant positive predictive value for clinical remission, endoscopic improvement, and avoidance of colectomy. Similarly, Maser et al. (2006)[26] showed that in a subgroup of patients on maintenance therapy, higher IFX levels were associated with a significant improvement of clinical remission, CRP, and endoscopic healing. Steenholdt et al. (2012)[23] investigated a mixed population of IBD patients and found that higher IFX trough levels were associated with maintenance of response. They were also able to determine clinically relevant threshold values for trough IFX and anti-IFX antibodies concentrations.
The association of development of antibodies against anti-TNF-alfa therapies with trough serum drug levels and response to these drugs has been inconsistent due to a lack of standardization of methods of measurement of serum drug levels or antidrug antibodies. For instance, the presence of detectable drug in the serum typically impairs the performance of a solid-phase enzyme-linked immunosorbent assay (ELISA) and western blot.[19] With classic Enzyme-linked immunosorbent assay (ELISA), antibodies remain undetectable as long as the drug is present in the blood. The type of detection assays also affects the reported incidence of antidrug antibodies.[28]
Studies have shown that clearance of IFX is greatly increased in the presence of antibodies to infliximab, and results in low IFX trough levels.[19,29,30] Low serum IFX levels have been associated with a lack of clinical response in both CD and UC (16,21,22).[23,26,31] Multiple studies in IBD patients have linked the development of antidrug antibodies with loss of treatment response, shorter duration of response, and infusion reactions.[19,21,27,31,32] Conversely, others have shown no difference in clinical outcomes between antidrug antibodies-positive or antidrug antibodies-negative patients.[26]

2.4 FAECAL CALPROTECTIN

Given the invasive nature of endoscopy, the implementation of an easy, non-invasive method to support the pre-diagnostic screening and monitoring of disease activity is essential in children.
Fecal calprotectin has been shown to be useful in the diagnosis of IBD, correlates with mucosal disease activity and can help to predict response to treatment or relapse. In IBD, the presence of active gut inflammation is associated with migration of leucocytes, including neutrophils, to the gut mucosa.[33] As a result the faecal stream contains increased levels of these inflammatory proteins including calprotectin. Faecal calprotectin has been shown to differentiate quiescent from active disease in both patients with CD and UC.[34,35]
A meta-analysis including 30 prospective studies in adults showed that the sensitivity and specificity of fecal calprotectin in diagnosing IBDs could reach up to 95% and 91%, respectively.[36] Fecal calprotectin also showed to be a reliable surrogate marker of treatment response.[37] Calprotectin levels decreases significantly after IFX treatment for 12 weeks, and it correlates with endoscopic index of severity (CDEIS).[38] Røseth et al. show that fecal calprotectin levels correlated with endoscopic mucosal healing.[39] A meta-analysis focusing on fecal calprotectin in IBD relapse showed that the sensitivity and specificity when predicting the relapse are 78% and 73%, separately.[40]

In children a meta-analysis including only 8 studies (six prospective and two retrospective) showed that pooled sensitivity and specifity for the diagnosis utility of fecal calprotectin during the investigation of suspected pediatric IBD were 0,978 (95%CI,0.947-0.996) and 0,682 (95%CI, 0.502-0.863)[41]. Hamalain et al show in 36 children that fecal calprotectin levels rapidly decreases by week 2 in one third of patients treated with infliximab[42]. In a preliminary study Kolho et al measured fecal calprotectin level during the induction and maintenance treatment with infliximab in 76 pediatric patients and concluded that the long- term prognosis was related to the response to induction therapy and reflected in low fecal calprotectin values between weeks 2 and 6 and clinical remission [43].

To our knowledge, there is no evidence if fecal calprotectin levels correlate with serum levels of anti-TNF-alfa drugs or with the development of anti-drug antibodies.

2.5 THE GEDII REGISTRY

Patient registries are a powerful tool to observe the course of disease, understand variations in treatment and outcomes, to assess effectiveness, to monitor safety and harm and to examine factors that influence prognosis and quality of life. It also allows to describe care patterns and to measure quality of care.

From a clinician's perspective, registries can collect data about disease presentation and outcomes on large numbers of patients rapidly, thereby producing a real-world picture of disease, current treatment practices, and outcomes. A registry might also provide data that can be used to assess the degree to which clinicians are managing a disease in accordance with evidence based guidelines, focus attention on specific aspects of a particular disease that might otherwise be overlooked, or provide data for clinicians to compare themselves with their peers. Overall, the use of patient registries appears to be active and growing.[12]

The "Grupo de Estudo da Doença Inflamatória Intestinal" (GEDII) Registry was created on 2005 and allows the regular and systematic capture of socio-demographic and clinical characteristics of patients diagnosed with IBDs (CD, UC and indeterminate UC). The Registry also captures the clinical and safety outcomes, treatments and the use of health resources.

So far, the Registry covers 20 gastroenterology departments of public hospitals. As of April 2014, the Registry comprised a total of 2500 patients.

2.6 RATIONALE

Thus far, there is no evidence if fecal calprotectin, a recognized biomarker in the context of IBDs, correlates with serum levels of anti-TNF-alfa therapies and with the development of antibodies against this class of drugs. Taking advantage of the valuable tool such as the GEDII Registry, the GEDII promoted a research to increase this knowledge. This study will follow a cohort of patients with CD or UC registered in the GEDII database and who are receiving infliximab according to the approved label in Portugal. All patients, regardless of the anti-TNF-alfa treatment phase (induction or maintenance) at study inclusion, will be followed throughout a period of approximately 2 years. In addition, this study will investigate the correlation of serum drug levels with development of anti-drug antibodies among different subsets of the eligible population and the association of these indicators with physician reported clinical outcomes.

2.7 RESEARCH HYPOTHESIS

We hypothesize that fecal calprotectin levels will significantly correlate with serum levels of IFX and with the development of antibodies against these drug.

3 OBJECTIVES

3.2 PRIMARY OBJECTIVES

Among biologic-naïve and non-naïve patients with CD or patients with UC registered in the GEDII Registry:

- To explore the association of fecal calprotectin levels with serum IFX levels throughout a period of 2 years since the start of observation period.
- To explore the association of fecal calprotectin levels with the development of anti-IFX antibodies throughout a period of 2 years since the start of observation period.

3.3 SECONDARY OBJECTIVES

Among biologic-**naïve** and non-naïve patients with CD or patients with UC registered in the GEDII Registry:

- To explore the association of serum **IFX levels** with the development of **anti-IFX antibodies**
- To explore the association of serum **IFX levels** with **clinical activity** throughout a period of 2 years since the start of observation period.
- To explore the association of development of anti-**IFX antibodies** with **clinical activity** throughout a period of 2 years since the start of observation period.
- To explore the association of **therapeutic attitude** with **serum IFX levels** throughout a period of 2 years since the start of observation period.
- To explore the association of **therapeutic attitude** with development of **anti-IFX antibodies** throughout a period of 2 years since the start of observation period
- To explore the association of **calprotectin** levels with **clinical activity** among the subgroup of patients who were co-medicated with **azathioprine (AZA)**.
- To explore the association of **calprotectin** levels with **clinical activity** among the subgroup of patients who were co-medicated with **methotrexate (MTX**).

4 STUDY DESIGN

This is a multicenter, prospective, observational study designed to gather and analyze data on a consecutive cohort of subjects with CD or UC treated with infliximab. There is no imposed experimental intervention and infliximab will be managed taking into account the label approved in Portugal.

A prospective, observational study is considered an appropriate tool to evaluate the impact of exposures of interest in real-world outcomes and an opportunity to explore biomarkers that can potentially predict clinical response in these settings.
The study will analyze a consecutive sample of 100 patients who are registered in the GEDII Registry and who fulfill the protocol's eligibility criteria. The recruitment period is 12 months but may be extended if the target number of participants is not achieved within the defined timeframe.
The overall duration of observation for each patient is approximately two years from the time patient initiated the participation in the study (start of observation period), regardless of the anti-TNF-alfa treatment phase (induction or maintenance) at the time of inclusion in the study. Basal socio-demographic and clinical characteristics will be collected as well as physician reported clinical outcomes (PCDAI or PUCAI according to the IBD studied) throughout several data collection time points [44,45]. The data collection time points in this study will reflect the routine scheduled appointments, and will take into account the condition being studied (CD or UC) and the anti-TNF-alfa received - See Cronogram A in Appendix.
Blood samples for assessment of serum levels of IFX and antibodies against these drug will be collected during scheduled appointments to the hospital as follows:

- Patients initiating induction with infliximab at study inclusion: Day 1, week 6 and then every 8 weeks up to week 102 (or every 6 weeks, if required);
- Patients in maintenance with infliximab at study inclusion: Week 'number' (number of weeks since patient started infliximab) and every 8 weeks thereafter, until completing the 24-month follow up period (or every 6 weeks, if required);

Faecal samples will be collected at the same time points as for the collection of blood samples (serum levels and anti-drug antibodies). Fecal and blood samples should always be collected prior to infusion of IFX during the scheduled appointment.

A total of 11 pediatric centers are expected to participate.

5 STUDY TIMELINES

The study is expected to start during the 2nd Quarter of 2016.

The overall duration of the study is three years (1 year of recruitment + 2 year observation period).

6 STUDY POPULATION

6.2 INCLUSION CRITERIA

Study subjects must fulfill the following criteria:

1. Male or female patients, <18 years older;
2. Patients who are registered in the GEDII Registry
3. Patients with moderate to severe active Crohn's disease or moderate to severe active Ulcerative Colitis;
4. Patients receiving infliximab according to the local approved label, including:

- Biologic-naïve patients initiating induction with infliximab at time of inclusion in the study **or;**
- Patients already under maintenance treatment with infliximab at time of inclusion in the study;

5. Patients who gave their informed consent.

6.3 EXCLUSION CRITERIA:

Subjects will be excluded if at least one of the following criteria is met:

1. Patients who are not eligible for therapy with infliximab;
2. Patients who are being treated with any investigational agent;
3. Patients who are not willing to comply with routine clinical appointments.

6.4 DISCONTINUATION FROM OBSERVATION PERIOD

In this study, patients will be followed up to a maximum period of 24 months. However, observation may be stopped prior to the 24 months for different reasons including, but not limited to:

- Patient discontinues the exposure of interest (infliximab)
- Protocol violation
- Lost to follow up
- Patient withdrawal of consent
- Pregnancy
- Death

In the case observation period is stopped prior to the 24 months, the date of study discontinuation, last intake of IFX, and the reason for discontinuation should be recorded in the electronic CRF. In addition, all efforts should be made to assess calprotectin levels, serum drug levels and antidrug antibodies.

7 INFORMATION TO BE COLLECTED

7.1 VARIABLES CAPTURED BY THE GEDII REGISTRY

The GEDII Registry allows the collection of socio-demographic, clinical characteristics and outcomes of patients diagnosed with IBDs.

For the purpose of this study the following variables will be obtained from the Registry:

Basal characteristics

- Date of birth
- Sex
- Height
- Weight
- BMI
- Smoking status
- Pregnancy status (if applicable)
- Diagnosis (UC, CD) and date
- Date of start of symptoms
- Familial history of IBDs
- Disease presentation (abdominal, constitutional, abdominal + constitutional, anal disease, acute abdomen, fever, anemia, extra-intestinal manifestation, abdominal mass, similar presentation to UC)
- Clinical course
- PCDAI includes (CD activity): Subjective reporting of the degree of abdominal pain; Presence of extraintestinal manifestations, such fever, arthritis, rash, and uveitis; Physical examinations findings; Weigth and height; Hematocrit, erythrocyte sedimentation rate, and serum albumin. The scale can be assessed at: http://www.gedii.pt/_scores_online
- PUCAI (UC activity): abdominal pain, stool frequency, rectal bleeding, stool consistency, nocturnal stools, activity level.
  The scale can be assessed at: http://www.gedii.pt/_scores_online
- Routine laboratory parameters (hemoglobin, leukocytes, neutrophils, eosinophiles, basophiles, monocytes, lymphocytes, erythrocyte sedimentation rate, CRP, ferritin, iron and transferrin)
- First episode - Paris classification for CD: age at diagnosis, location, behavior, growth .[46]
  The scale can be assessed at: http://www.gedii.pt/_scores_online
- First episode - Paris classification for UC: extent, severity.[46]
  The scale can be assessed at: http://www.gedii.pt/_scores_online
- Anal lesion (strictures, abscesses, or fistulas)
- Extra-abdominal manifestations
- IFX dose, date of start (first infusion - Day 1)
- Concomitant therapies (since the first infusion - Day 1)
- Assessment of serum levels of IFX (prior to infusion) - µg/mL
- Assessment of anti-drug antibodies of IFX (prior to infusion)
- Assessment of fecal calprotectin levels (µg/g)

Information to be collected during induction therapy with IFX (week 2 and 6) and maintenance therapy (every 8 weeks up to week 102 or until completion of the 24-month follow up period)* - see Cronogram A:

- Weight, Height and BMI
- Comorbidities
- Clinical activity (physician reported PCDAI - CD or PUCAI – UC)
- Dose of IFX, if changed since previous infusion.
- Concomitant therapies, if changed since previous appointment

- Routine laboratory parameters (hemoglobin, leukocytes, neutrophils, eosinophiles, basophiles, monocytes, lymphocytes, erythrocyte sedimentation rate, CRP, ferritin, iron and transferrin)
- Assessment of serum levels of IFX (prior to infusion)
- Assessment of anti-drug antibodies of IFX (prior to infusion)
- Assessment of fecal calprotectin levels (prior to infusion)
- Status: ongoing/discontinuation. If discontinued, reason.

* data collection is expected every 6 weeks, if required.

7.2 ASSESSMENT OF SERUM IFX LEVELS AND ANTIDRUG ANTIBODIES

Serum IFX levels and antidrug antibodies will be assessed immediately prior to drug infusion and at the scheduled appointment to the hospital. Overall, each patient will have a maximum of 15 determinations. The number of determinations of serum drug levels and antidrug antibodies will depend on the treatment phase of the patients at the time of inclusion:

- Patients initiating induction with infliximab at study inclusion: 15 determinations up to week 102;
- Patients in maintenance with infliximab at study inclusion: 14 determinations until completing the 24-month follow up period;

Infliximab and anti-infliximab levels

Blood from infliximab treated patients will be collected prior to infliximab infusion. Blood will be collected to blood clotting tubes. One hour after collection, blood will be centrifuged at 10 min at 3000 RPM. Sera will be transferred into fresh tubes and stored at -20C.
Infliximab and anti-infliximab levels will be determined with an ELISA assay. Both assays employ the quantitative enzyme immunoassay technique. A monoclonal antibody specific for IFX has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IFX present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IFX is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IFX bound in the initial step. The color development is stopped and the intensity of the color is measured by a spectrophotometer.

7.3 ASSESSMENT OF BIOLOGICAL MARKER - FECAL CALPROTECTIN

In this study, a stool sample will be collected during the scheduled appointment at the same time points as the collection of blood samples for serum drug levels and antidrug antibodies. Similarly, the fecal sample should be collected prior the administration of infliximab.
The collected stools sample will be sent immediately to the laboratory at room temperature for extraction. Stools can be store up to 6 days at room temperature. After 6 days, stools must be extracted. One gram of stool is enough for calprotectin detection.
Stools will be weighted and extracted with extraction buffer delivered with commercial kit.
After extraction and before storage, samples will be centrifuged (5 min at 3000xg). Once centrifuged, supernatant must be transferred into a fresh tube. Undiluted extracts can be stored at -20°C for at least 4 months. Extracts must be diluted prior to analysis.
Calprotectin from diluted samples will be determined with an ELISA assay. Calprotectin wells are coated with monoclonal antibodies against calprotectin. Calprotectin present in patient samples binds

to calprotectin antibody in the coating. After washing the unbound components, an anti-human calprotectin enzyme-labeled (calprotectin conjugate) is added to give a complex conjugate calprotectin antibody. After incubation, unbound conjugate is washed and removed, and the complex is incubated with a developing solution. Stops solution ends reaction and fluorescence in the wells is read. A standard curve allows the determination of calprotectin concentration in the sample.

The fecal samples will be analyzed by a Central Laboratory (Dept. de Farmacologia FMUP). Report with the results will be provided to Investigators by mail.

8 EXPOSURE OF INTEREST

Infliximab (Remicade) is the exposure of interest. These product will be used according to the approved label.

Infliximab (Remicade) is administered by intravenous infusion:

- **Crohn's disease (induction):** 5 mg/kg at 0, 2 and 6 weeks, then every 8 weeks.
- **Ulcerative Colitis (induction):** 5 mg/kg at 0, 2 and 6 weeks, then every 8 weeks.
- **Crohn's disease (maintenance):** 5 mg/kg or 10 mg/kg every 8 weeks or every 6 weeks (if required)
- **Ulcerative colitis (maintenance):** 5 mg/kg or 10 mg/kg every 8 weeks or every 6 weeks (if required)

9 ENDPOINTS

9.2 PRIMARY ENDPOINT

In the subset of biologic-naïve and non-naïve patients with active, moderate to severe CD receiving IFX:

- **Fecal calprotectin** levels and **serum IFX levels** at each data collection time points
- **Fecal calprotectin** levels and presence of **anti-IFX antibodies (+ or -)** at each data collection time points

In the subset of biologic-naïve and non-naïve patients with active, moderate to severe UC receiving IFX:

- **Fecal calprotectin** levels and **serum IFX levels** at each data collection time points
- **Fecal calprotectin** levels and presence of **anti-IFX antibodies (+ or -)** at each data collection time points

9.3 SECONDARY ENDPOINTS

In the subset of biologic-naïve and non-naïve patients with active, moderate to severe CD receiving IFX:

- Serum **IFX levels** and **anti-IFX antibodies (+ or -)** at each data collection time points
- Serum **IFX levels** and **clinical activity (PCDAI)** at each data collection time points.
- **Anti-IFX antibodies (+ or -)** and **clinical activity (PCDAI)** at each data collection time points.
- **Therapeutics administered** for CD (name and dose) and serum **IFX levels.**
- **Therapeutics administered** for CD (name and dose) and **anti-IFX antibodies** (+ or -).

In the subset of biologic-naïve and non-naïve patients with active, moderate to severe UC receiving IFX:

- Serum **IFX levels** and **anti-IFX antibodies (+ or -)** at each data collection time points
- Serum **IFX levels** and **clinical activity (PUCAI)** at each data collection time points.
- **Anti-IFX antibodies (+ or -)** and **clinical activity (PUCAI)** at each data collection time points.
- **Therapeutics administered** for UC (name and dose) and serum **IFX levels.**
- **Therapeutics administered** for UC (name and dose) and **anti-IFX antibodies** (+ or -)

In the subsets of biologic-naïve and non-naïve patients patients with active, moderate to severe CD receiving IFX + azathioprine or IFX + methotrexate:

- **Fecal calprotectin** levels and **serum IFX levels** at each data collection time points
- **Fecal calprotectin** levels and presence of **anti-IFX antibodies (+ or -)** at each data collection time points

In the subsets of biologic-naïve and non-naïve patients with active, moderate to severe UC receiving IFX + azathioprine or IFX + methotrexate:

- **Fecal calprotectin** levels and **serum IFX levels** at each data collection time points
- **Fecal calprotectin** levels and presence of **anti-IFX antibodies (+ or -)** at each data collection time points

9.4 DEFINITIONS OF INTEREST

Clinical activity

Crohn's disease

- Clinical remission among patients with CD: PCDAI≤10
- Clinically active disease among patients with CD:
  - Mildly active disease: PCDAI ≥11≤30
  - Moderately/severely active disease: ≥31

- Clinical response in patients with CD: 15 points reduction in the PCDAI at week 2 after induction therapy
- Clinical relapse: an increase in the PCDAI of 15 points during maintenance therapy with IFX.

Ulcerative colitis

- Clinical remission among patients with UC: PUCAI<10
- Clinically active disease among patients with UC:
  - Mildly active disease: PUCAI >11<30
  - Moderately active disease PUCAI >30<64
  - Severely active disease: >65

- Clinical response in patients with UC : defined as an improvement of PUCAI≥ 20 points at week 2 after induction therapy

- Clinical relapse: PUCAI ≥11 during maintenance therapy with IFX.

Biologic naïve patients:

Patients who had never received biologic therapy prior to study inclusion in the study.

Biologic non-naïve patients:

Patients who are receiving maintenance therapy with infliximab at the time of study inclusion.

Definition of Observation period

- Start of observation period – corresponds to the date of the first appointment of the study.

- End observation period – 24 months after the start of the observation period.

PCDAI (assessment of Crohn's Disease activity)

- PCDAI includes (CD activity): Subjective reporting of the degree of abdominal pain; Presence of extraintestinal manifestations, such fever, arthritis, rash, and uveitis; Physical examinations findings; Weigth and height; Hematocrit, erythrocyte sedimentation rate, and serum albumin. The scale can be assessed at: http://www.gedii.pt/_scores_online

PUCAI (assessment of ulcerative colitis clinical activity)

- PUCAI (UC activity): abdominal pain, stool frequency, rectal bleeding, stool consistency, nocturnal stools, activity level.

Paris classification (first episode)

- CD: age at diagnosis, location, behavior, growth
- UC: extent, severity

10 STATISTICAL ANALYSIS

10.2 GENERAL CONSIDERATIONS

All quantitative variables will be summarized through descriptive statistics namely mean, median, standard deviation and range (minimum and maximum) and qualitative variables though absolute (n) and relative frequencies (%) and 95% confidence intervals (if applicable). The statistical analysis will be performed through frequency tables for qualitative variables and tables with descriptive statistics for quantitative variables.

The association between two quantitative variables will be performed through Pearson correlation coefficient or Spearman correlation coefficient, in case the normality assumption is not verified.
The association of two categorical variables will be tested through the Chi-Square test or Fisher Exact test (if applicable).
The comparison of two independent samples in respect to quantitative variables will be performed through t-test for independent samples or the Mann-Whitney non-parametric test, according to the assumption validations of the statistical test (if applicable).

The association between quantitative variables and a categorical binary variable will be analyzed by Area Under the ROC Curve.
The concordance kappa will be used for binary variables, if applicable.

For each subset, the proportion of patients with active disease, clinical response, clinical remission (PUCAI, PCDAI report), presence of anti-drug antibodies at each data collection time points will be summarized using 95% confidence intervals.

Results for hemoglobin, leukocytes, neutrophils, eosinophiles, basophiles, monocytes, lymphocytes, erythrocyte sedimentation rate, CRP, ferritin, iron, transferrin, fecal calprotectin, serum levels of IFX, will be summarized by total number of observations (n), mean, median, standard deviation, minimum and maximum.

Exploratory Generalized Estimated Equations with AR1 correlation structure in time, to account for the within-subject correlations, will be used to explore the association between fecal calprotectin levels and serum drug levels (dependent variable) as well as the association of fecal calprotectin levels with the presence of antidrug antibodies (dependent variable), throughout the follow up period.

The impact of immunogenicity on the efficacy of IFX will also be exploratory assessed by associating serum drug levels and anti-drug antibodies in respect to the outcomes, need for dose escalation, and discontinuation rate at the predetermined time points.

Therapeutics used will be compared with mean serum drug levels and presence/absence of anti-drug antibodies at each data collection through a descriptive analysis.

10.3 SAMPLE SIZE

The sample size is not based on formal statistical assumptions. This exploratory study will analyze all patients who are registered in the GEDII Registry and meet this study's eligibility criteria, during a recruitment period of 12 months. In this circumstance, it is expected to analyze a total of 100 patients. This sample will allow a descriptive analysis of clinical characteristics among pre-defined subsets. Furthermore, this sample will allow the exploratory analysis of correlations between independent variables and outcomes of interest within subsets during the follow period.

11 PHARMACOVIGILANCE

New safety findings that can potentially affect the risk/benefit profile of a medicinal product identified during the conduct of epidemiological studies will be reported promptly to the Health Authorities, according to local pharmacovigilance regulations.

12 ETHICAL AND LEGAL ASPECTS

12.2 ETHICS

The study will be conducted according to the ethics principles originated from the Declaration of Helsinki and the Portuguese Clinical Research law (Law #21/2014, 16th April 2014).[44]
A copy of the protocol, proposed informed consent form and other written subject information will be submitted to the competent Ethics Committee for written approval. A copy of the written approval of the protocol and informed consent form must be received by the Investigator before recruitment of subjects and data collection.
The investigator will submit and, where necessary, obtain approval from the competent Ethics Committee for all subsequent protocol amendments and changes to the informed consent document.

12.3 INFORMED CONSENT

Before any protocol specific procedures are performed, the investigator is responsible for obtaining written informed consent from the subject (or authorized representative) and after an adequate and clear explanation of the aims, methods, anticipated benefits, and potential hazards of the study.
The informed consent process should be documented in the subject's medical charts, and the informed consent form should be signed and personally dated by the subject (or authorized representative, if applicable) and by the person who conducted the informed consent discussion (not necessarily an investigator). The original signed informed consent form should be retained in

accordance with institutional policy, and a copy of the signed consent form should be provided to the subject or authorized representative.

12.4 STUDY DISCONTINUATION CRITERIA

Premature termination of this study may occur because of a regulatory authority decision, change in opinion of the ethics committee, or at the discretion of the GEDII. If GEDII decides to terminate prematurely the study, GEDII or designee will promptly notify the appropriate IEC and regulatory authority (if applicable).

13 QUALITY CONTROL

The study will involve a GEDII monitor who will be responsibility to ensure that the study is conducted according to the protocol and to the local regulatory/ethical requirements.
Before the start of activities a study monitor will conduct initiation Visits at the sites in order to train the investigational team on the protocol and other protocol-related procedures.
During the study the monitor will also be responsible for conducting periodic monitoring visits at the sites to ensure that the protocol is being followed and the data recorded is accurate and collected according to the defined procedures.
The sites may be subject to review by the Independent Ethics Committee and/or to quality assurance audits performed by GEDII designated representative, and/or to inspection by appropriate regulatory authorities. The Investigator(s) and their relevant staff should be available during the monitoring visits and possible audits or inspections. The investigator and institution will allow the appropriate regulatory authorities direct access to source documents to perform this verification.
Prior to study start, the investigator is required to sign a protocol signature page confirming his/her agreement to conduct the study in accordance with this protocol and to give access to all relevant data and records to monitors, auditors, Ethics Committees, and regulatory authorities, as required.

All investigators and study staff will receive training on the protocol and other protocol-related procedures prior to start of activities. The training will be provided by the GEDII or its representative.

14 DATA HANDLING

14.2 CONFIDENTIALITY

The investigator is responsible for ensuring that the subject's confidentiality is maintained.
Questionnaires, database and other documents generated in this study will be identified by a unique subject identification number only. This 2-digit number will be assigned sequentially by each investigator, based on subject's recruitment schedule (e.g.: first subject will be assigned No. 01, the second subject will be No. 02 and so on). Each center will also be assigned a predefined two-digit number.
The GEDII Registry received the authorization of Comissão Nacional para Protecção de Dados for the purpose of data processing under the scope of "Lei de Protecção de Dados de Carácter Pessoal Dec. 67/98 de 26 de Outubro".

14.3 DATA COLLECTION

All study data will be obtained from the GEDII Registry, laboratory reports and, when relevant, will be complemented by subject's interview or other medical sources (as appropriate).

The investigator will be responsible for ensuring that all findings and data are accurately and reliably recorded in the case report form.

All eligible subjects who are not enrolled in the study will be recorded in a specific form. No personal data will be collected in this form, only the date of assessment of eligibility criteria and reason for non-enrollment. This form will be kept exclusively at each site.

14.4 STUDY ARCHIVE

The investigator will keep an adequate archive of all study documentation with access restricted to study team. The study archive will be kept at each site for at least 15 years from the study close out.

14.5 PUBLICATION POLICY

All documents and results generated from this clinical study are exclusive property of Coordinating Investigator and GEDII. Any related publications must be previously approved in written by the Coordinating Investigator and GEDII.
The results of the study will be presented by the Coordinating Investigator in national and international meetings and will published in international papers.
The study results can only be published after the clinical study is terminated, the data analysis is completed and **only** upon the agreement of the study's scientific board. The publication should include the results from all the centers which have participated in the clinical investigation, The publication of results should be agreed by all participating investigators and in strict adherence to the principles originated from the Helsinki Declaration and according to Portuguese Clinical Research law (Law #21/2014, 16th April 2014).[47]

Authorship criteria

For all publications related to this study, the GEDII will comply with recognized ethical standards concerning publications and authorship established by the International Committee of Medical Journal Editors (*Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals - Updated December 2013*).

For all publications related with this clinical study, the order of the authors is as follows:

- The first author position will correspond to the Coordinating Investigator;
- The subsequent author's positions will correspond to the principal investigator from each center, who will be ranked decreasingly according to the number of patients included in the study, as far as the number of co-authors allowed by the journal is not exceeded;
- All the participating investigators not figuring in the authorship (due to journal's limitations in the number of co-authors) will be cited in the acknowledgment section of the publication.
- The last author position will correspond to a GEDII member.

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APPENDIX –