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Séquelles anatomiques et fonctionnelles des maladies inflammatoires chroniques de l'intestin (MICI) de l'enfant.

Claire Dupont

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Normandie Université

THÈSE

Pour obtenir le diplôme de doctorat

Spécialité : Sciences de la vie et de la santé

Préparée au sein de l'Université de Rouen Normandie

Séquelles anatomiques et fonctionnelles des Maladies inflammatoires chroniques de l'intestin (MICI) de l'enfant

**Présentée et soutenue par
Claire LUCAS épouse DUPONT**

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« Failure is not an option »

Gene Kranz, NASA, Apollo 13 flight director

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Titre : Séquelles anatomiques et fonctionnelles des Maladies inflammatoires chroniques de l'intestin (MICI) de l'enfant

Résumé

Les maladies inflammatoires chroniques de l'intestin (maladie de Crohn, rectocolite hémorragique, colite inclassée) de l'enfant peuvent occasionner des séquelles anatomiques telles que la fibrose intestinale responsable de sténoses, ainsi que des troubles fonctionnels intestinaux (TFI) persistants lors de la rémission de la maladie. L'objectif de la thèse était d'estimer l'impact de ces complications et de rechercher une association avec les caractéristiques antérieures de la maladie. Nous avons utilisé pour cela deux séries pédiatriques et deux modèles animaux.

Dans l'étude TFI-MICI, nous avons montré que 20% des enfants et adolescents avec MICI en rémission clinique et biologique avaient des TFI, et 15% des douleurs abdominales fonctionnelles (DAF). Les DAF étaient associées à une fatigue accrue, à des symptômes dépressifs et une diminution de la qualité de vie, mais pas à de l'anxiété. Il n'y avait pas d'association entre la gravité de la MICI et la présence de DAF.

Dans l'étude STENO-PED, nous avons montré que les seuls critères cliniques et radiologiques associés à la nécessité de résection chirurgicale des enfants ayant une maladie de Crohn sténosante du grêle étaient une dilatation sus-sténotique > 30 mm et un PCDAI > 22,5 au diagnostic de sténose. L'administration d'un traitement anti-TNF après le diagnostic de sténose était un facteur protecteur par rapport au risque de chirurgie.

Il manque de modèles expérimentaux permettant de suivre l'histoire naturelle de la fibrose intestinale en lien avec l'inflammation et l'effet des traitements, notamment chez l'animal pré-pubère. Nous avons adapté au rat pré-pubère un modèle de colite aiguë (1 dose de TNBS) et chronique (3 doses de TNBS) et avons montré que les rats développaient une inflammation significative dans les 2 modèles, basé sur un score histologique. L'IRM montrait un épaississement de la paroi colique et des sténoses dans les 2 modèles, et des ulcérations de la muqueuse dans le modèle aigu. En histologie, il y avait une fibrose légère à modérée dans le modèle TNBS chronique et une ébauche non significative de fibrose dans le modèle aigu. Le traitement des rats par MODULEN IBD® exclusif dès le début de l'induction de l'inflammation n'a pas entraîné de diminution de l'inflammation ni de la fibrose histologiques dans les deux modèles.

La prise en charge des séquelles anatomiques et fonctionnelles des MICI pédiatriques devient un enjeu majeur même si la prévalence des douleurs fonctionnelles nous est apparue comme non augmentée par rapport à la population générale. Les modèles animaux pré-pubères pourraient permettre de mieux analyser la cinétique de la fibrogénèse et l'impact d'une suppression précoce de l'inflammation et/ou de nouvelles thérapeutiques anti-fibrosantes.

Mots clés

Inflammation, Fibrose, Développement, Imagerie par Résonance Magnétique, Colite expérimentale, Axe intestin-cerveau.

Title : Functional and structural sequelae of pediatric inflammatory bowel disease

Summary

The course of pediatric-onset inflammatory bowel disease (Crohn's disease, ulcerative colitis and IBD-unclassified) can be complicated by structural or functional sequelae. Structural complications include intestinal fibrosis which can cause bowel strictures. IBD can be associated with functional abdominal pain persisting despite remission of inflammation. The purpose of our work was to determine the burden of these complications and search for association with previous severity of inflammation, based on two clinical studies and two animal models.

In the first study, TFI-MICI, we showed that 20% of children and adolescents with IBD in clinical and biochemical remission had functional gastrointestinal disorders, among which 15% had functional abdominal pain disorders (FAPD). FAPD was associated with increased fatigue, depressive symptoms and reduced quality of life, but not with anxiety. There was no association between the severity of IBD and presence of FAPD.

The second study, STENO-PED, focused on imaging and clinical predictors of response to treatment in children and adolescents with stricturing small bowel Crohn's disease. We showed that the predictors for surgery were a dilation proximal to the stricture of > 30mm, and a PCDAI score at diagnosis of stricture > 22.5. Receiving an anti-TNF α treatment after diagnosis of stricture was a protective factor from surgery.

Experimental models allowing to follow the progression of fibrosis along with inflammation and assess response to treatment are lacking, in particular for pre-pubertal animals. We adapted to Sprague-Dawley pre-pubertal rats a model of acute (1 dose of TNBS) and chronic (3 doses of TNBS) hapten-induced colitis. The rats in both models developed significant inflammation, based on histology and magnetic resonance colonography. Rats in the chronic colitis model developed histologic fibrosis. There was a non-significant trend to fibrosis in the acute model. We treated rats in both models with MODULEN IBD[®] from induction of colitis to collection of colonic samples. This treatment did not reverse inflammation nor fibrosis.

Fibrosis and functional abdominal pain in pediatric-onset IBD are two important problems, although functional pain appeared to be not more frequent than in the general population. Animal models could be of great assistance in order to better decipher the link between inflammation and fibrosis, and see if an effective early suppression of inflammation along with new anti-fibrotic therapies could halt the progression of fibrosis.

Key words

Inflammation, fibrosis, growth and development, magnetic resonance imaging, experimental colitis, Brain-Gut axis.

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Liste des articles préparés dans le cadre de la thèse

Article 1 : **Functional abdominal pain disorders in children and adolescents with IBD in clinical remission: risk factors and association with psychosocial comorbidities, a cross-sectional multicenter French study.** Tran Léa, Martinez Vinson Christine, Bridoux-Henno Laure, Enaud Raphaël, Grandjean Camille, Bertrand Valérie, Chaillou Legault Emilie, Lacotte Édouard, Caron Nicolas, Carré Emilie, Dabadie Alain, Dimitrov Georges, Languépin Jane, Vanrenterghem Audrey, Coopman Stéphanie, Gastineau Swellen, Hugot Jean-Pierre, Lamireau Thierry, Mosca Alexis, Pigneur Bénédicte, Mas Emmanuel, Thomassin Nadège, Jérôme Viala, Dupont-Lucas Claire (liste et position des auteurs à valider par le comité scientifique du GETAID pédiatrique): en attente de soumission à Journal of Crohn's and Colitis

Article 2 : **Risk factors for surgery in stricturing small bowel pediatric Crohn's disease and contribution of magnetic resonance enterography to prediction of response to treatment.** Claire Dupont-Lucas, Edouard Lacotte, Louis Boujonnier, *et al.* (En préparation: analyses complémentaires en cours avec relecture centralisée des IRMs)

Article 3 : **MR-colonography assessment of Acute Trinitrobenzene Sulfonic Acid Colitis in pre-pubertal Rats, and effect of a Polymeric Diet enriched in TGF-beta 2.** Claire Dupont-Lucas, Rachel Marion-Letellier, Mathilde Pala, Charlène Guerin, Christine Bôle-Feysot, Emmeline Salameh, Alexis Goichon, Lionel Nicol, Amelyne David, Moutaz Aziz, Elodie Colasse, Céline Savoye-Collet, Guillaume Savoye: soumis à Journal of Pediatric Gastroenterology and Nutrition le 8/11/2019 (JPGN-S-19-01157)

Article 4 : **Effect of a polymeric diet rich in transforming growth factor beta 2 on chronic 2,4,6-trinitrobenzene sulfonic acid colitis in pre-pubertal rats assessed by magnetic resonance colonography.** Claire Dupont-Lucas, Rachel Marion-Letellier, Mathilde Pala, Charlène Guerin, Asma Amamou, Marine Jarbeau, Christine Bôle-Feysot, Lionel Nicol, Amelyne David, Moutaz Aziz, Elodie Colasse, Céline Savoye-Collet, Guillaume Savoye: soumis à Inflammatory Bowel Diseases le 8/11/2019 (IBD-D-19-00948)

Liste des abréviations

- α -SMA : Alpha- smooth muscle actin
- anti-TNF: traitement anti-tumor necrosis factor α
- COL1A1 : Alpha-1 type 1 collagen
- CTGF : Connective tissue growth factor
- DAF: Douleurs abdominales fonctionnelles
- IBD-DI : Inflammatory Bowel Disease Disability Index
- IRM : Imagerie par résonance magnétique
- MC: Maladie de Crohn
- MEC : Matrice extra-cellulaire
- MICI : Maladies inflammatoires chroniques de l'intestin
- MMP : Matrix metalloproteases
- PCDAI : Pediatric Crohn's Disease Activity Index
- PUCAI : Pediatric Ulcerative Colitis Activity Index
- RCH : Rectocolite hémorragique
- SD (rat) : Rat Sprague-Dawley
- SNA : Système nerveux autonome
- TGF- β : Transforming Growth Factor beta
- TFI : Troubles fonctionnels intestinaux
- TIMP : Tissue Inhibitors of Metallo-Proteases
- TNBS : Acide 2,4,6-trinitrobenzene sulfonique

I) Introduction

1) Les maladies inflammatoires chroniques de l'intestin

a) Définition et diagnostic des MICI chez l'enfant

Les maladies inflammatoires chroniques de l'intestin (MICI) comprennent la maladie de Crohn, la rectocolite hémorragique (RCH) et la colite inclassée (1). Ces maladies évoluent par poussées et périodes de rémission. Cette thèse étant consacrée aux MICI pédiatriques, nous centrerons autant que possible notre présentation sur les particularités de ce groupe d'âge.

Seuls 25% des enfants présentent la triade symptomatique typique comprenant douleur abdominale, diarrhée et amaigrissement. Les MICI de l'enfant peuvent se présenter selon une variété de symptômes et signes biologiques (2). Les symptômes associés plus fréquemment avec la maladie de Crohn sont la diarrhée (73% des patients dans le registre de MICI pédiatrique allemand-autrichien, le GPGE / CEDATA), les douleurs abdominales (74%), l'amaigrissement (61%) et la fatigue (41%). La RCH est associée typiquement à des rectorragies (89%), une diarrhée (86%) et des douleurs abdominales (67%). Un retard de croissance statural est évocateur de maladie de Crohn (16%), de même que l'atteinte péri-anale (17%). Les signes extra-digestifs peuvent toucher tous les patients (1 à 13% selon les atteintes). Une anémie est présente chez 1/3 des patients MICI lors du diagnostic.

En cas de suspicion de MICI chez l'enfant, la confirmation diagnostique repose sur l'endoscopie digestive haute et basse et l'imagerie de l'intestin grêle, selon l'algorithme diagnostique de Porto (3). La maladie de Crohn peut se localiser à tout endroit du tube digestif et cause une inflammation transmurale, tandis que l'inflammation causée par la rectocolite hémorragique est localisée à la muqueuse, avec une atteinte continue, ascendante depuis le rectum, pouvant affecter le colon entier voire l'iléon terminal (backwash ileitis). Une atteinte de l'intestin grêle est recherchée par entéro-IRM ou vidéocapsule du grêle. Au terme du bilan, la maladie est caractérisée selon la classification de Paris (4) comprenant pour la maladie de Crohn : l'âge au diagnostic, la localisation de la maladie, le phénotype (inflammatoire, sténosant ou fistulisant),

et la présence d'un retard de croissance ou d'une atteinte périnéale. La rectocolite hémorragique et la colite inclassée sont caractérisées par leur extension et leur sévérité (définie par un score d'activité (PUCAI) > 65 au cours de la maladie).

b) Epidémiologie et histoire naturelle des MICI : spécificités pédiatriques

Des données récentes américaines montrent une augmentation de prévalence des MICI pédiatriques de 133%, passant de 33 / 100 000 en 2007 à 77 / 100 000 en 2016 (5). L'augmentation touche surtout la tranche d'âge 10-17 ans. La maladie de Crohn reste deux fois plus prévalente que la RCH (45,9 vs 21,6 / 100 000) chez les enfants, et la prévalence des MICI est plus élevée chez les garçons, contrairement aux adultes où la prévalence est plus élevée chez les femmes que chez les hommes. Chez les adultes, l'augmentation de prévalence entre 2007 et 2016 était de 123%. En 2016, 1 enfant américain sur 1299 était atteint de MICI contre 1 adulte sur 209 (5).

Les données françaises issues du registre EPIMAD montrent aussi une augmentation d'incidence des MICI pédiatriques (dans la tranche d'âge 10 - 16 ans) entre 1988 et 2011 de +126% pour la maladie de Crohn (MC) et de +156% pour la RCH (6). Les taux d'incidence au cours de la période 2009-2011 étaient de 9,5 / 100 000 pour la MC et de 4,1 / 100 000 pour la RCH chez les adolescents de 10 à 16 ans, dans la zone géographique couverte par le registre. L'augmentation d'incidence touche aussi les enfants de moins de 10 ans, avec une incidence des MICI étant passée de 0,9 à 1,8 / 100 000 sur la même période ($p < 0,0001$).

De manière intéressante, EPIMAD est le seul registre à avoir montré que le phénotype des MICI pédiatriques au diagnostic a évolué entre les périodes de 1988-1990 et 2009-2011 : la fréquence des formes inflammatoires est passée de 64% à 87% tandis que la fréquence des formes sténosantes est passée de 33% à 11% (6). On pourrait suspecter que le diagnostic de MICI est posé plus tôt, avant le stade des complications, grâce à l'amélioration des techniques diagnostiques et une meilleure sensibilisation des médecins. Cependant il n'y a pas eu de diminution d'incidence des MICI adultes qui suggérerait une simple translation vers le bas de

l'âge au diagnostic, mais au contraire une hausse d'incidence des MICI dans toutes les tranches d'âge (7).

Les MICI pédiatriques sont décrites comme ayant un phénotype plus agressif que les adultes (8, 9). Dans la cohorte européenne Epi-IBD, parmi 488 patients adultes ayant une MC suivis pendant 5 ans (entre 2010 et 2015), 22% ont été opérés et 36% hospitalisés pour la MC (10). Quatorze pourcent des patients ont progressé d'un phénotype B1 vers un phénotype B2 et/ou B3 (10).

En comparaison, une étude américaine sur 6 centres pédiatriques (n=989 Crohn pédiatriques inclus entre 2000 et 2003, diagnostiqués depuis 1979) montrait une incidence de B2 ou B3 de 27% à 5 ans et 38% à 10 ans (11). De plus, 13% des B2 devenaient B3 en 2 ans.

En France, les données EPIMAD sur 535 enfants ayant une MC diagnostiquée entre 1988 et 2004 avec un suivi médian de 11 ans, montraient que 39% avaient un phénotype B2 à la fin du suivi, et que 43% (n=230) avaient eu au moins une résection chirurgicale pendant le suivi pédiatrique (12).

c) Physiopathologie des MICI et rôle de l'axe intestin-cerveau

A ce jour il n'y a pas une cause unique retrouvée aux MICI. La physiopathologie des MICI serait multifactorielle : une perte de tolérance du système immunitaire intestinal vis à vis du microbiote intestinal, chez un individu génétiquement prédisposé, entraînant un processus inflammatoire chronique et auto-entretenu (1). Au delà de la réaction intestinale (barrière épithéliale, microbiote luminal, réponse immune intestinale) l'axe intestin-cerveau en entier aurait un rôle clé dans la pathogénèse des MICI, par une modulation psycho-neuro-endocrino-immune (13).

Le cerveau et l'intestin communiquent par le système nerveux autonome (SNA) et les organes circumventriculaires (Figure 1). L'information viscérale est transmise au système nerveux central par les nerfs vagues, nerfs splanchniques et nerfs pelviens, et est intégrée dans le SNA central. Il s'en suit de nombreuses boucles de rétrocontrôle positives et négatives du système

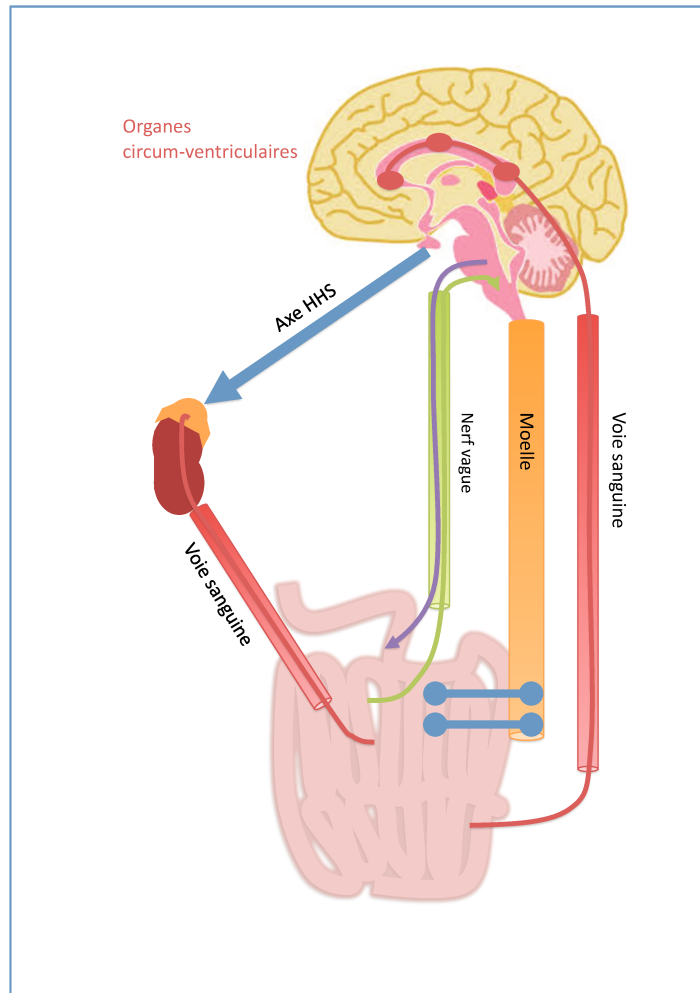


Figure 1. L'axe intestin-cerveau

L'axe intestin-cerveau comprend : 1/ le système nerveux autonome (comprenant les systèmes nerveux sympathique et parasympathique), 2/ le système nerveux central (comprenant cerveau et moelle épinière), 3/ le système de réponse au stress (axe hypothalamo-hypophyso-surrénalien : HHS), 4/ l'axe intestinal du facteur de libération de la corticotrophine (CRF) et 5/ la réaction intestinale (13).

sympathique et parasympathique sur les organes périphériques, y compris sur le système immunitaire. De plus, en situation d'inflammation les interleukines circulantes provenant de la muqueuse intestinale atteignent les organes circumventriculaires par voie sanguine. Ces organes modulent l'activité des neurones de voisinage qui à leur tour stimulent l'axe hypothalamo-hypophyso-surrénalien, inhibant l'inflammation muqueuse via les glucocorticoïdes.

Beaucoup de ces boucles réflexes sont inconscientes ou deviennent conscientes seulement en condition pathologique (13).

Arguments en faveur de l'implication de l'axe intestin-cerveau dans les MICI

Il y a une augmentation de prévalence de l'anxiété et de la dépression chez les patients ayant une MICI par rapport aux sujets sains (14). L'anxiété et la dépression sont corrélées à l'activité de la maladie : des études montrent que les patients ayant une maladie active ont des niveaux d'anxiété et de dépression plus importants que les patients en rémission. Cependant une étude prospective sur 388 adultes ayant une MICI dans le Manitoba a montré que la qualité de vie s'améliorait avec la diminution d'activité de la maladie mais pas le fonctionnement psychologique, faisant suspecter un impact persistant de la maladie même en l'absence de symptômes (15).

De façon plus intrigante, les facteurs pouvant influencer l'axe intestin-cerveau tel une situation stressante, une infection ou une antibiothérapie (par l'effet sur le microbiote) peuvent favoriser les poussées (16). Les patients qui ont un haut niveau d'anxiété et de dépression rechutent plus vite que ceux qui n'en ont pas (17, 18).

Sur modèle animal, les rats qui ont été soumis à un stress dans la petite enfance (séparation précoce de leur mère) avaient une hyperactivation de l'axe hypothalamo-hypophyso-surrénalien. Ils avaient une hyperperméabilité intestinale et développaient des colites plus sévères après instillation de TNBS que des sujets qui n'avaient pas subi le stress (19).

Des arguments en faveur d'une relation inverse sur l'axe intestin-cerveau existent aussi : notre équipe a montré que la colite TNBS causait une hypersensibilité viscérale et des signes d'anxiété persistante chez le rat, malgré la résolution de l'inflammation (20).

En résumé, le modèle biopsychosocial des MICI montre que le devenir (clinical outcome) et les poussées de la maladie influencent et sont influencées par à la fois des facteurs biologiques et des facteurs psychosociaux (13).

d) Traitement des MICI : évolution des paradigmes de traitement

Il n'existe à ce jour pas de traitement curatif des MICI. Les paradigmes du traitement ont évolué depuis le simple contrôle des symptômes vers la recherche d'une rémission endoscopique voire histologique, dans le but de prévenir les complications. L'évidence récente suggère que de maintenir le processus inflammatoire sous contrôle serré pourrait prévenir le handicap induit par la maladie (21). Chez l'adulte, la notion de « treat to target » implique de choisir une cible individualisée pour le patient, et de se donner les moyens de l'atteindre (22). La cible actuellement admise est celle de la rémission muqueuse, qui peut être évaluée par endoscopie ou par des biomarqueurs non invasifs tels que la calprotectine fécale (23-25). La personnalisation des traitements prend en compte les facteurs de risque individuels de progression vers une maladie compliquée, et le dosage des métabolites des médicaments afin d'ajuster les traitements de manière proactive (Proactive Therapeutic Drug Monitoring) (26, 27). Les MICI étant des maladies hétérogènes, il est nécessaire de prendre en compte ces facteurs individuels pour augmenter les chances de succès du traitement. La prise en compte des « Patient Reported Outcomes » est un autre objectif concomitant (21).

Grâce aux stratégies de « step-up accéléré » et au développement de plusieurs classes de biothérapies, le contrôle de l'inflammation devient un objectif réalisable. Il est maintenant nécessaire d'aller plus loin dans la stratégie afin de prévenir les complications anatomiques et fonctionnelles des MICI.

e) Complications des MICI pédiatriques

Les MICI sont associées à des complications anatomiques (sténoses, fistules, lésions ano-périnéales(28)), à un retard de croissance et pubertaire (29). Les manifestations extra-intestinales peuvent évoluer pour leur propre compte avec une morbidité significative (par exemple la cholangite sclérosante). Les médicaments peuvent être associés à des effets secondaires organiques (par exemple abcès cutanés sous anti-TNF α) ou fonctionnels (par exemple l'intolérance au méthotrexate causant des nausées et vomissements anticipatoires nécessitant dans certains cas d'arrêter le traitement (30)) invalidants.

Une revue de 64 articles sur l'histoire naturelle de la maladie de Crohn pédiatrique en population générale a montré les taux de complications suivants : 3-38% avaient une maladie sténosante et/ou fistulisante au diagnostic et 15 à 30% ont évolué vers une forme sténosante ou fistulisante au cours du suivi, 4-45% avaient une atteinte péri-anales, 9-24% un retard de croissance au diagnostic, 29 à 59% des patients déclaraient avoir un retard pubertaire. Enfin, la probabilité de chirurgie dans les 5 ans suivant le diagnostic était entre 13 et 50% (31).

Il y a une mortalité faible mais significative des MICI pédiatriques, en rapport avec des infections opportunistes et des cancers principalement : entre 2006 et 2011 sur 20 pays européens, 31 décès associés aux complications d'une MICI ont été rapportés chez des enfants ayant un âge médian de 10 ans (32).

Sur le plan psychologique et fonctionnel, les MICI peuvent entraîner une diminution de la qualité de vie (33) et une fatigue chronique et être associées à des comorbidités psychologiques (anxiété, dépression) (14). Il a été décrit des douleurs abdominales fonctionnelles chez des enfants ayant une MICI en rémission (34).

Il a été noté un retentissement psychologique de la MICI sur les familles : Jelenova *et al.* ont montré que la qualité de vie des parents d'adolescents ayant une MICI était plus faible, que les mères étaient plus anxieuses et les pères plus déprimés que les parents d'adolescents contrôles (35).

La MICI pédiatrique peut retentir sur la scolarité. Une étude suédoise a montré que les enfants ayant une MICI avaient une note finale moyenne de 4,9 points plus faible que les contrôles, mais que le fait d'accéder à l'enseignement secondaire (qualification for high school) n'était pas affecté par la MICI. Les performances scolaires étaient plus faibles dans un sous-groupe d'enfants ayant des marqueurs de maladie chronique active (par exemple une durée cumulée d'hospitalisation > 30 j) (36).

Comme toute maladie chronique, la MICI peut être source de handicap pour le patient. Selon la version 2011 de la Classification internationale des handicaps : déficiences, incapacités et désavantages (CIH), l'Organisation Mondiale de la Santé (OMS) définit le handicap comme un terme générique pour les déficiences, les limitations de l'activité et les restrictions à la participation. Le handicap est l'interaction entre des sujets présentant une affection médicale et des facteurs personnels et environnementaux (37). Un index de handicap utilisable dans les études de MICI adultes a été créé, le Inflammatory Bowel disease Disability Index (IBD-DI) (38). Le IBD-DI comprend 19 items : 7 sur les fonctions corporelles, 2 sur les structures corporelles, 5 sur la participation aux activités et 5 sur les facteurs environnementaux. Une méta-analyse de 9 études appliquant le IBD-DI a montré que le handicap lié à la MICI était plus élevé en cas de maladie active ou de chômage, et que les patients recevant une biothérapie avaient un niveau de handicap plus faible que ceux recevant une corticothérapie (39). Les études analysées n'ont pas trouvé le lien entre les facteurs suivants et le handicap : chirurgie, tabac, phénotype de la maladie, manifestations extra-intestinales, âge du patient et âge au diagnostic. Il n'existe pas d'index équivalent pédiatrique.

Dans le cadre de la thèse, nous avons choisi de nous concentrer sur une séquelle anatomique : la fibrose intestinale pouvant occasionner des sténoses, et sur une famille de séquelles fonctionnelles : les troubles fonctionnels intestinaux.

2) Fibrose et MICI

La fibrose est une complication fréquente des MICI, survenant dans le lit de l'inflammation. Ainsi, la fibrose compliquant la RCH touche uniquement la muqueuse et sous-muqueuse, causant une perte des haustrations avec un aspect de colon tubulisé ; tandis que la fibrose compliquant la maladie de Crohn est transmurale, affectant muqueuse, sous-muqueuse, musculuse et séreuse et pouvant causer des sténoses à tout endroit du tube digestif (40). Les sténoses de maladie de Crohn peuvent causer des symptômes occlusifs, peuvent persister malgré la résolution de l'inflammation et peuvent nécessiter une dilatation endoscopique, une stricturoplastie ou une résection chirurgicale (41). La chirurgie ne guérit pas le processus fibrosant qui peut récidiver et nécessiter des résections itératives. Les sténoses surviennent dans les sites atteints par l'inflammation, mais évoluent à bas bruit et peuvent survenir des années après l'inflammation initiale. De plus, alors que le phénomène de fibrose est initialement un phénomène physiologique de cicatrisation et de réparation tissulaire, tous les patients ne développent pas une fibrose significative entraînant des sténoses, et il n'y a pas de corrélation entre la sévérité de l'inflammation et le développement de sténose. Les facteurs prédisposant à une évolution sténosante ne sont pas encore connus, même si une prédisposition génétique est suspectée.

a) Un continuum ou une évolution indépendante de l'inflammation ?

L'hypothèse ancienne selon laquelle la fibrose intestinale nécessiterait obligatoirement une inflammation chronique et non contrôlée pour se développer est maintenant remise en question (42). En effet, malgré les progrès des traitements anti-inflammatoires des MICI, l'histoire naturelle des formes compliquées n'a pas changé en 20 ans. Dans une série comparant les données de 1991 et 2014 aux Pays-Bas sur 1162 patients adultes ayant une maladie de Crohn, malgré une augmentation de prescription des immunosuppresseurs (de 30 à 70%), et de biothérapies (de 3 à 41%), et la diminution des taux d'hospitalisation (de 66% à 44%) et de

chirurgie pour maladie réfractaire (de 43 à 17%), la progression vers un phénotype compliqué (B2 ou B3) n'a pas changé dans le temps (21% en 1991 vs 21% en 2014). Si on restreint l'analyse aux seuls utilisateurs d'immunosuppresseurs et de biothérapies, le taux d'hospitalisation, de chirurgie et de progression de phénotype est identique entre les deux périodes (43).

Ceci fait suggérer que le phénotype sténosant n'est pas uniquement secondaire à une inflammation persistante, mais plutôt à des facteurs personnels de prédisposition à un phénotype sténosant. Les mêmes constats sont faits dans la prédisposition à la fibrose d'autres organes : par exemple lien entre hépatite C chronique et évolution cirrhogène (44).

b) Facteurs associés à l'évolution fibrosante dans les MICI

Des études d'histoire naturelle des MICI et de corrélation génotype-phénotype montrent que la localisation de la maladie évolue peu dans le temps, tandis que la progression vers des phénotypes sténosants ou pénétrants survient au cours des 15 premières années de la maladie (45).

Parmi les facteurs génétiques impliqués dans le risque de sténoses au cours de la maladie de Crohn, la mutation NOD2/CARD15 est associée au risque de chirurgie chez les adultes et les enfants seulement en cas de localisation iléale, et une mutation du gène d'autophagie ATG16L1 est associé au risque de sténose chez l'enfant (45-47). Une association entre les polymorphismes T280M et V249I du gène d'un récepteur de chemokine CX3CR1 avec la maladie de Crohn sténosante a été montrée (48, 49). Une association entre une progression plus rapide et plus sévère de la maladie et des signes de réactivité immune accrue a aussi été suspectée : Dubinsky *et al.* ont trouvé que le risque de progression vers une maladie compliquée (fistules, sténoses) et la vitesse de cette progression augmentaient avec le nombre d'anticorps (anti-Cbir1, anti-OMPC, ASCA, ANCA) présents dans le sérum d'enfants ayant une maladie de Crohn (50).

c) Fibrose intestinale : d'un processus de cicatrisation physiologique à une pathologie

Une agression récurrente ou persistante de l'épithélium est nécessaire pour initier et perpétuer l'inflammation intestinale et la fibrogénèse (51). Les lésions épithéliales ou endothéliales entraînent la libération de facteurs chimiotactiques qui permettent le recrutement et l'activation des cellules de l'immunité innée et adaptative, qui à leur tour recrutent et activent les myofibroblastes. Ceux-ci déposent de la matrice extracellulaire (51) (Figure 2). En conditions normales, une fois l'inflammation terminée et l'épithélium réparé, la matrice est dégradée par des enzymes protéolytiques, les MMP (52), et les myofibroblastes sont détruits par apoptose ou reviennent à un état non activé (53). La fibrose intestinale survient lorsque les myofibroblastes activés fabriquent de manière excessive de la MEC, ce qui cause une augmentation de la rigidité du tissu et une dysfonction progressive de l'organe. De plus les systèmes immunitaires inné et adaptatif entretiennent le processus en stimulant la différenciation, le recrutement, la prolifération et l'activation des progéniteurs des myofibroblastes producteurs de MEC (54).

d) Mécanismes cellulaires et médiateurs cytokiniques de la fibrose

Cellules mésenchymateuses : fibroblastes, myofibroblastes, cellules musculaires lisses

L'acteur central de la fibrose intestinale est le myofibroblaste, comme dans les processus de fibrose d'autres organes (foie, poumon, peau...). Une particularité majeure de la fibrose intestinale est que les myofibroblastes ne proviennent pas seulement des cellules mésenchymateuses résidentes (fibroblastes et cellules musculaires lisses), mais aussi de la transdifférenciation de cellules épithéliales intestinales et endothéliales par la transition épithélio-mésenchymateuse ou la transition endothélialo-mésenchymateuse (55, 56). Les myofibroblastes peuvent aussi provenir des cellules stellaires (mais rôle probablement mineur par rapport à leur rôle dans la physiopathologie de la fibrose hépatique et du pancréas), des péricytes et des cellules souches médullaires (56).

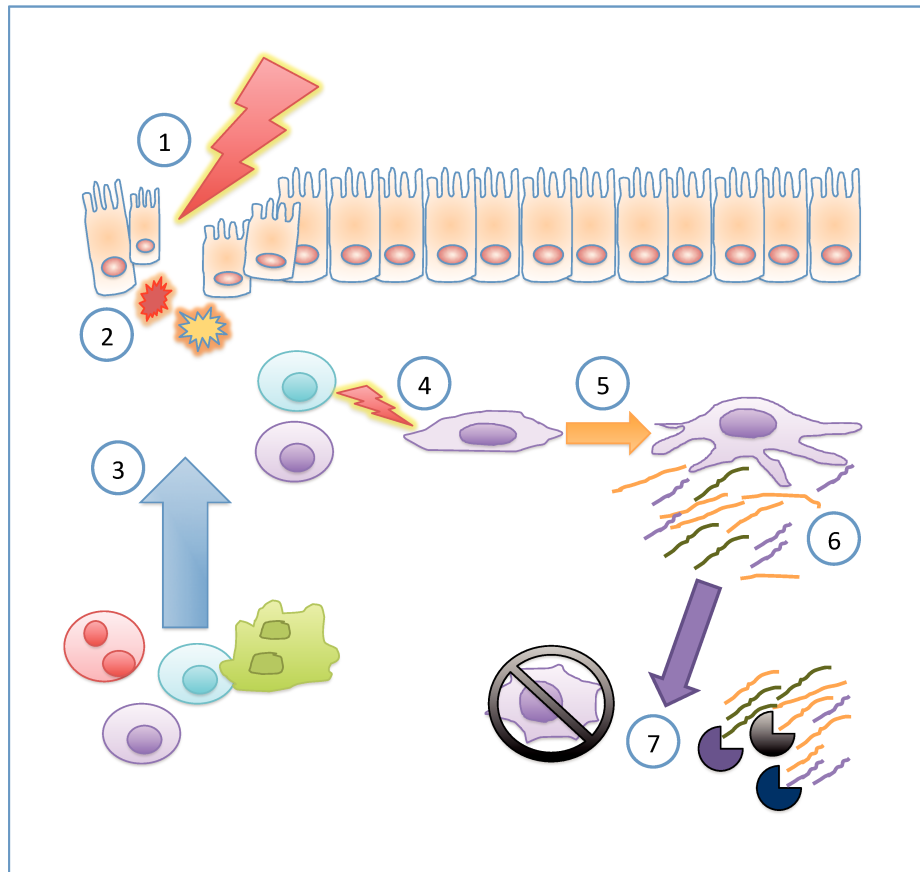


Figure 2. Etapes de la fibrogénèse intestinale

1. Agression de la barrière épithéliale. 2. Libération de chémokines et cytokines. 3. Recrutement de cellules inflammatoires (immunitaires et non immunitaires). 4. Production de cytokines et facteurs de croissance pro-fibrosants. 5. Activation des myofibroblastes. 6. Production de matrice extracellulaire par les myofibroblastes activés. 7. Résolution de la fibrose par apoptose des myofibroblastes et dégradation de la MEC par les enzymes protéolytiques (MMP).

Sous l'effet de différents médiateurs, le myofibroblaste prend un phénotype activé et se met à synthétiser en grande quantité du collagène de type fibrillaire (I, III, V) et non fibrillaire (IV) et d'autres protéines de la matrice extra-cellulaire : glycoprotéines (par exemple fibronectines et laminines), et protéoglycanes (40, 57, 58). Les facteurs qui activent les myofibroblastes sont nombreux : TGF- β 1 (transforming growth factor), activines, CTGF, platelet-derived-growth factor (PDGF), IGF 1 et 2, EGF, endothelines (56).

La voie majoritaire de l'activation des myofibroblastes, commune à de nombreux organes et pathologies, passe par la signalisation TGF- β (59, 60). Sous l'action de l'interleukine 13, le TGF- β 1 se lie à son récepteur membranaire sur les myofibroblastes, entraînant la phosphorylation de

Smad 2 et Smad 3 qui migrent dans le noyau et activent la transcription des gènes codant pour les protéines de la MEC (61).

Parmi les autres cellules mésenchymateuses, les fibroblastes ont un rôle important dans de nombreux organes pour favoriser la cicatrisation tissulaire (54). Une agression ou une inflammation aigue peut déclencher leur activation vers un phénotype cicatrisant ou fibrosant (58).

Les cellules musculaires lisses dans l'intestin se situent principalement dans la couche de musculaire muqueuse. Lors des situations de fibrose, les cellules musculaires lisses se multiplient, causant un épaissement important de la musculaire muqueuse dans la RCH et un épaissement de toutes les tuniques dans la maladie de Crohn participant aux sténoses (51). Même à distance de la zone sténosée, les cellules musculaires lisses isolées dans l'iléon de patients ayant une maladie de Crohn sténosante présentent des anomalies morphologiques et fonctionnelles : diminution de leur longueur et de leur contraction sous l'effet de l'acétylcholine (62).

Cellules de l'immunité innée

On distingue les monocytes, polynucléaires neutrophiles, mastocytes et polynucléaires éosinophiles. Toutes peuvent entretenir la stimulation des myofibroblastes en produisant des cytokines et médiateurs ayant une action pro-inflammatoire et/ou pro-fibrosante, tels que : interleukines, TNF-alpha, TGF beta 1, PDGF (51).

Les macrophages et cellules dendritiques dérivent des monocytes. Parmi les phénotypes de macrophages, les M1 et M2a ont un effet proinflammatoire et profibrosant. Par exemple, en situation d'inflammation les macrophages produisent du TGF-beta 1 comme mécanisme régulateur pour faciliter la résolution d'une réponse inflammatoire (63). Mais le TGF-beta 1 entraîne l'activation des fibroblastes et le développement des myofibroblastes activés qui

facilitent la réparation tissulaire mais aussi la fibrose (63). D'autres macrophages, les M2c auraient un rôle régulateur (51).

Cellules de l'immunité adaptative

Elles comprennent les lymphocytes T, les T régulateurs et les lymphocytes B. Une réponse Th1 entraîne la production d'IFN-gamma qui aurait un effet pro-fibrotique, tandis qu'une réponse Th2 s'accompagne de production d'IL-4, IL-5 et IL-13, ce dernier étant un médiateur majeur de la cascade d'activation des myofibroblastes par la voie de signalisation TGF-beta 1. La réponse Th17 aurait un rôle pro-inflammatoire et pro-fibrotique. Le rôle des T régulateurs dans la fibrose est moins bien connu. Ils auraient un effet inhibant la réponse Th17 et Th2 (51).

Il est probable que les différentes réponses se succèdent au cours du temps dans le contexte d'une inflammation chronique. Fichtner-Feigl *et al.*, sur un modèle de colite chronique au TNBS chez des souris BALB/c, montraient que le profil de la réponse inflammatoire débutait par une réponse Th1 impliquant IL-12p70 et IFN-gamma pendant 3 semaines, à laquelle succédait une production d'IL-23/IL-25 à partir de 4-5 semaines. Apparaissait ensuite une production d'IL-17 et de cytokines associées à une réponse Th2, notamment IL-13 qui atteignait un plateau à 8-9 semaines. Ils ont confirmé que sous l'effet de l'IL-13 sur le récepteur IL-13 R $\alpha 2$ survenait une production de TGF-beta 1 et le début d'une fibrose (64).

e) Vers une résolution de la fibrose intestinale

Malgré l'avènement de traitements anti-inflammatoires de plus en plus efficaces, la proportion de patients ayant recours à la chirurgie ne diminue pas. Ceci suggère que le traitement de l'inflammation, même à la phase précoce où il y aurait le plus de chance que la fibrose soit réversible à l'arrêt de l'agression inflammatoire pro-fibrosante, ne suffit pas à stopper le processus fibrosant. Il pourrait être nécessaire d'adjoindre au traitement anti-inflammatoire un traitement anti-fibrosant (65). Pour le moment, plusieurs cibles sont étudiées dans des modèles *in vitro* ou animaux, mais aucun n'a été testé ou approuvé chez l'homme. De plus, le phénomène

de fibrose ayant une inertie longue, il est probablement nécessaire d'avoir recours à des modèles animaux dans lesquels l'échelle du temps est raccourcie, permettant d'observer l'effet des interventions testées dans un délai raisonnable (66). Les différentes pistes testées sont l'induction de l'apoptose des myofibroblastes activés (67) ou l'inhibition de leur activation (68), l'inhibition de la transition épithélio-mésenchymateuse (69), l'activation des MMP qui dégradent la MEC ou l'inactivation des TIMP. Les cibles ont fait l'objet de revues de la littérature (53, 56, 70).

3) TFI et MICI

a) Définitions

Les Troubles fonctionnels intestinaux sont définis actuellement selon les critères de Rome IV comme une combinaison variable de symptômes digestifs et extra-digestifs, sans cause organique retrouvée au terme d'un bilan orienté sur les signes d'alerte. Il existe trois classifications par groupe d'âge : nourrisson, enfants et adolescents, adultes. Les critères de Rome IV, qui diffèrent peu des critères de Rome III, distinguent 12 TFI chez l'enfant et l'adolescent répartis en 3 familles : les douleurs abdominales, les nausées et vomissements, les troubles de défécation (Tableau 1) (71). Les critères diagnostiques sont indiqués en Annexe 1.

b) TFI : épidémiologie chez l'enfant « sain »

Les troubles fonctionnels intestinaux sont des phénomènes fréquents chez l'enfant d'âge scolaire. Une méta-analyse mondiale (58 articles, 196 472 enfants), a montré une prévalence poolée de 16,4% des douleurs abdominales fonctionnelles selon les critères de Rome III (72). La prévalence était plus importante chez les filles que chez les garçons : 15,9 vs 11,5%. Les douleurs abdominales fonctionnelles étaient associées à des troubles anxieux et dépressifs, au stress et aux évènements de vie traumatiques.

Tableau 1. Comparaison des classifications de Rome III et Rome IV des troubles fonctionnels intestinaux de l'enfant et de l'adolescent

Rome III (Rasquin 2006(73))	Rome IV (Hyams 2016(71))
H1. Vomissements et aérophagie	H1. Troubles fonctionnels avec nausées et vomissements
H1a. Syndrome de rumination de l'adolescent	H1a. Vomissements cycliques
H1b. Syndrome des vomissements cycliques	H1b. Nausées et vomissements fonctionnels
H1c. Aérophagie	H1c. Rumination
	H1d. Aérophagie
H2. TFI avec douleur abdominale	H2. Troubles fonctionnels douloureux abdominaux
H2a. Dyspepsie fonctionnelle	H2a. Dyspepsie fonctionnelle
H2b. Syndrome de l'intestin irritable	H2b. Syndrome de l'intestin irritable
H2c. Migraine abdominale	H2c. Migraine abdominale
H2d. Douleur abdominale fonctionnelle de l'enfant	H2d. Douleurs abdominales fonctionnelles non classées ailleurs
H2d1. Syndrome de douleur abdominale fonctionnelle de l'enfant	
H3. Constipation et incontinence	H3. Troubles fonctionnels de la défécation
H3a. Constipation fonctionnelle	H3a. Constipation fonctionnelle
H3b. Incontinence fécale non rétentionnelle	H3b. Incontinence fécale

c) Le concept de IBD-IBS

La présence de symptômes évocateurs de Syndrome de l'intestin irritable ou de TFI a été souvent décrite chez les patients adultes ayant une MICI. Les premières études, utilisant les critères de Rome II, trouvaient une prévalence des TFI entre 33 et 82% chez des patients ayant une MICI en rémission (74, 75). L'entité a été appelée IBD-IBS (inflammatory bowel disease - irritable bowel syndrome) (76, 77). Les symptômes étaient associés à de l'anxiété et une diminution de qualité de vie.

Plusieurs auteurs ont par la suite rapporté une association des symptômes fonctionnels à de l'inflammation de bas grade, définie par une calprotectine fécale augmentée. Progressivement il a été admis que cette inflammation de bas grade témoignait bien d'une MICI active et que les symptômes étaient liés à l'activité de la maladie et non seulement à des troubles fonctionnels (78-80). Actuellement il est recommandé de s'assurer de l'absence d'activité résiduelle de la maladie (calprotectine fécale) ou de diagnostic différentiel (par exemple : malabsorption des sels biliaires, pullulation microbienne du grêle), avant de conclure au diagnostic de TFI associée à la MICI (81, 82).

d) Genèse des TFI : hypothèse multifactorielle et modèle biopsychosocial

La physiopathologie des MICI et des TFI comprend plusieurs points communs (82, 83).

L'événement initiateur semble être l'augmentation de perméabilité intestinale permettant à des composants microbiens du microbiote intestinal de pénétrer dans la muqueuse et d'y être en contact avec le système nerveux entérique. Ceci déclencherait une augmentation des récepteurs à la douleur, par exemple TRPV1, qui peut persister dans le temps. L'inflammation en elle-même pourrait avoir un effet sur l'axe intestin-cerveau, par le relargage de cytokines pro-inflammatoires par voie sanguine, intégrée au niveau des organes circumventriculaires.

Faure et Giguere ont montré une hypersensibilité viscérale chez les enfants ayant une MICI en rémission et des douleurs abdominales fonctionnelles (MICI-DAF), en utilisant un barostat rectal

(84). Le seuil de sensibilité rectal à la douleur était abaissé chez les patients MICI-DAF par rapport aux témoins sains, mais n'était pas différent de celui des patients ayant des douleurs fonctionnelles. Sur modèle animal, la colite chronique est associée à une surreprésentation de filets nerveux exprimant le récepteur de la douleur TRPV1 dans la muqueuse colique (85). Une augmentation de l'expression des récepteurs de TRPV1 a aussi été trouvée sur des biopsies de muqueuse colique de patients ayant une MICI en rémission et des douleurs abdominales fonctionnelles (86).

La transmission du signal douloureux vers le cerveau a pu être mesurée par IRM fonctionnelle par Berstein *et al.* Ils ont montré une activation similaire du gyrus cingulaire antérieur (partie frontale du cortex cingulaire) chez des patients MICI et patients SII par rapport à des contrôles sains (87).

Le rôle du terrain anxieux et/ou dépressif associé pourrait être médié par l'axe hypothalamo-hypophyso-surrénalien. Les modèles de stress néonatal chez l'animal (séparation de la mère) causent des altérations à long terme de la barrière épithéliale intestinale et de l'immunité muqueuse (19).

Toutes ces hypothèses physiopathologiques mettent en avant le rôle central de l'axe cerveau-intestin dans les douleurs abdominales fonctionnelles des MICI. Un modèle biopsychosocial des MICI et des TFI permet d'appréhender la complexité de l'interaction entre les événements médicaux sensibilisateurs, les événements psychosociaux sensibilisateurs, la prédisposition génétique, les événements de vie précoce, le microbiote intestinal et la perméabilité intestinale (Figure 3).

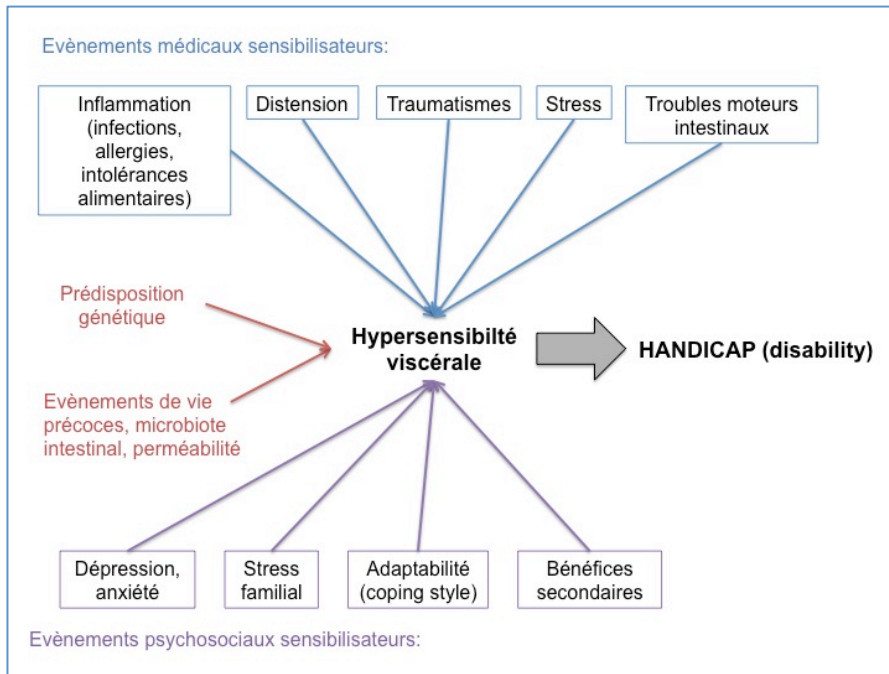


Figure 3. Modèle biopsychosocial des douleurs abdominales fonctionnelles de l'enfant

(D'après Carlo di Lorenzo, ESPGHAN 2018.)

4) Les questions à éclaircir, justifiant mon travail de thèse

Nous avons vu que les TFI étaient fréquents dans la population générale pédiatrique, touchent environ 1/3 des patients adultes ayant une MICI en rémission, mais que leur fréquence n'est pas connue chez les enfants ayant une MICI. Parmi les TFI, certains symptômes (diarrhée, douleur abdominale) sont communs avec une poussée de MICI et pourraient être attribués à tort à une poussée, entraînant des explorations voire des traitements non nécessaires. De plus, l'augmentation de prévalence de l'anxiété et de la dépression chez les enfants ayant une MICI, ainsi que la perméabilité intestinale induite par l'inflammation les placeraient à risque de développer des douleurs abdominales fonctionnelles. Il n'a pas été étudié à notre connaissance de lien entre la gravité de l'inflammation antérieure et le développement de douleurs fonctionnelles. Nous avons voulu évaluer la prévalence des TFI chez les enfants ayant une MICI en rémission et le lien avec la gravité antérieure de la maladie.

Notre première étude (TFI-MICI) nous a montré que les douleurs fonctionnelles n'étaient pas aussi fréquentes qu'attendu, et n'étaient pas liées à la gravité antérieure de la maladie. Ceci nous a conforté dans la nécessité de nous concentrer sur les séquelles anatomiques des MICI, en premier lieu la fibrose pouvant être responsable de sténoses.

La fibrose intestinale occasionnant des sténoses intestinales est la première cause de chirurgie chez les enfants ayant une MICI. Un des enjeux importants en cas de sténose intestinale est de pouvoir déterminer de manière individuelle quels enfants ont une chance de réussite d'un traitement médical pour sténose du grêle et ceux qui nécessiteront une chirurgie de résection. En effet une chirurgie précoce peut être bénéfique dans certains cas, mais la chirurgie est associée à un risque de récurrence et de complications post opératoires (88). Il manque de données sur les facteurs prédictifs de chirurgie dans les sténoses de l'intestin grêle (89). Nous avons voulu évaluer en rétrospectif l'apport de l'entéro-IRM pour prédire la réponse au traitement des enfants ayant une maladie de Crohn avec sténoses du grêle, et rechercher les facteurs cliniques associés à la réponse.

Notre seconde étude (STENO-PED) nous a montré que l'entéro-IRM de routine était insuffisante à elle-seule pour prédire la réponse au traitement des sténoses. De plus, nous n'avons pas pu identifier de lien entre la sévérité de la maladie antérieure et l'évolution des sténoses sous traitement. Il nous est alors apparu nécessaire d'avoir recours à un modèle animal pour améliorer la connaissance de l'histoire naturelle de la fibrose, son lien avec l'inflammation et sa réversibilité par un traitement anti-inflammatoire.

Des modèles animaux de colite expérimentale sont utilisés depuis plus de 40 ans pour analyser les mécanismes physiopathologiques immunologiques et inflammatoires sous-tendant les MICI et étudier la réponse aux traitements. Ces modèles ne sont utilisés que depuis peu de temps pour l'analyse de la fibrose intestinale. Parmi ces modèles, le modèle de colite induite par TNBS cause une inflammation transmurale et granulomateuse, avec une inflammation médiée par les lymphocytes T. Malgré de nombreuses études menées sur le modèle de colite TNBS, il n'y en a eu que peu sur des rats en croissance (90, 91). Il est pourtant nécessaire, étant donné les spécificités pédiatriques des MICI, de disposer d'un modèle d'animal en croissance pour la colite aigue et chronique. Les difficultés à prévoir sont une mortalité plus importante des animaux, puisqu'étant plus fragiles, ainsi qu'une possible susceptibilité accrue au TNBS.

Nous avons adapté au rat pré-pubère successivement un modèle de colite aigue et un modèle de colite chronique au TNBS, que nous avons validés par histologie. Ceci nous a permis de montrer l'intérêt d'un suivi non invasif de la colite par IRM. Nous avons testé le modèle en administrant un traitement anti-inflammatoire, le MODULEN IBD®, dans le but de réverser l'inflammation et si possible, de prévenir la fibrose intestinale.

II) Articles

Article 1 : Etude TFI-MCI

Functional abdominal pain disorders in children and adolescents with IBD in clinical remission: risk factors and association with psychosocial comorbidities, a cross-sectional multicenter French study

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ABSTRACT

Background: Chronic abdominal pain occurs frequently in pediatric patients with inflammatory bowel disease (IBD) despite being in remission, and can lead to unnecessary treatment escalation. This phenomenon could be a functional scar of past inflammation. We aimed to characterize IBD patients in remission suffering from Functional Abdominal Pain Disorders (IBD-FAPD, including functional dyspepsia, abdominal migraine, irritable bowel syndrome, functional abdominal pain and functional abdominal pain syndrome) and look for an association with past severity of IBD as well as psychosocial comorbidities.

Methods: Patients with IBD for > 1 year, in clinical remission for at least 3 months, answered Rome III questionnaire and self-reported questionnaires of anxiety, depression, health-related quality of life and fatigue. Parental anxiety and depression were assessed.

Results: Among 80 included patients, there were 40 boys, mean age was 15.0 ± 2.0 years and 76% had Crohn's disease. Sixteen patients (20%) had at least one Functional Gastrointestinal Disorder (FGID) and 12 (15%) had at least one IBD-FAPD. IBD-FAPD was not associated with past severity of disease or treatments received. Level of remission assessed by ESR, CRP, fecal calprotectin, and most recent imaging and/or endoscopy, was not associated with having IBD-FAPD. Patients with IBD-FAPD had more fatigue (Peds FACIT-F score: 37 (IQR 25-75: 20 – 44) vs 44.5 (IQR 25-75: 38.5 – 48.0), $p=0.007$), depression ($p=0.03$), and a lower health-related quality of life (IMPACT III: 73.2 (IQR 25–75: 63.4 – 84.8) vs 81.9 (IQR 25-75: 75.0 – 87.3), $p=0.04$) than patients with no FAPD. There was no difference in anxiety between groups (SCARED-R51). Parents of patients from the IBD-FAPD Group had higher Trait anxiety (STAI-Y-B), but not State anxiety or Depression (BDI) than parents from the “No FAPD” group.

Conclusions: Prevalence of IBD-FAPD was low in this cohort of IBD patients in clinical remission. IBD-FAPD was not associated with past severity of disease, but with fatigue, depression and lower quality of life.

Keywords: Anxiety, Depression, Quality of Life, Pediatric, Patient-reported outcomes.

INTRODUCTION

The paradigms of pediatric inflammatory bowel disease (IBD) care have evolved over the last thirty years. Compared to adults, IBD in children has been described as having a more aggressive phenotype from diagnosis (1). Progressively we have moved from treating inflammation with step-up immunomodulators and then biologics, to trying to reverse natural history of disease in order, for example, to prevent intestinal damage. The risk factors for complicated disease in pediatric IBD have been described, and pediatric IBD patients can now benefit from highly effective biologics and treatment strategies early on in the course of disease (2, 3). Besides the better understanding of mechanisms of tissue damage caused by recurrent or unresolved inflammation, pediatric gastroenterologists have raised awareness on psychosocial consequences of a life-long disease in young individuals. It has been shown that pediatric IBD patients suffer from increased anxiety, depression, and lower quality of life than healthy controls (4, 5). These symptoms are increased in the setting of active disease (6).

In addition, a significant number of patients have persistent abdominal pain although they are in remission from IBD. In adults, the prevalence of these symptoms, termed IBD-IBS, reaches up to 35% of patients (7). The cause of this functional pain is not fully understood, but seems to result from disorders in the Brain-Gut axis and in pain perception (8). Animal models have shown evidence of visceral hypersensitivity and anxiety persisting several weeks after chronic TNBS colitis (9).

We hypothesized that functional abdominal pain in children with IBD in remission might be a consequence of the severity of previous inflammation. The first objective of our study was to test for an association between having a Functional Abdominal Pain Disorder (FAPD) although in remission from IBD, and past characteristics of IBD. Since disorders of the Brain-Gut axis have been shown to be associated with psychosocial difficulties such as depression and anxiety, our second objective was to evaluate correlation between psychosocial

comorbidities and IBD-FAPD. Finally, to understand the patient's family context and how it may impact IBD-FAPD, we studied the association between parental anxiety and depression and IBD-FAPD.

MATERIALS AND METHODS

This was a multicenter cross-sectional study conducted in pediatric gastroenterology units participating in the GETAID pédiatrique (Groupe d'Etude des Affections Inflammatoires Digestives pédiatriques), a French national collaborative research network on pediatric IBD.

Inclusion criteria:

All pediatric IBD patients seen in the outpatient clinic, inpatient ward or infusion clinic between April 2018 and August 2019 and who were in clinical and biological remission were invited to participate. The inclusion criteria were: age between 9 and 18 years at the time of inclusion, a diagnosis of IBD confirmed according to the ESPGHAN Porto criteria (10), duration of IBD > 1 year, clinical remission defined as (all criteria mandatory): Physician's global assessment = "Remission", no nocturnal stools, no blood in stools, ≤ 3 stools per day, CRP < 10 mg/L, ESR < 20 mm/h, no ongoing steroid therapy, no recurrence of disease or optimization of treatment in the last 3 months.

Exclusion criteria were: presence of an ileostomy or colostomy, inability to understand French or refusal to participate.

Data collection:

Patients were asked to complete 5 questionnaires, either on a web-based Limesurvey module, or on paper forms. Parents were asked to complete 2 questionnaires and give socio-demographic information. Treating pediatric gastroenterologists were asked to fill in a web-based CRF.

Patient questionnaires were the following:

- Fr-qPGS (Rome III Diagnostic Questionnaire for the Pediatric Functional GI Disorders) (11): a French-language validated version of the qPGS questionnaire using Rome III criteria for Functional gastrointestinal disorders. The Fr-qPGS comprises 49 questions divided into 4

sections. Interpretation of questionnaire classifies functional syndromes in 3 categories: pain disorders (Further referred to as Functional Abdominal Pain Disorders, comprising Functional Dyspepsia, Abdominal Migraine, Irritable Bowel Syndrome, Functional Abdominal Pain, Functional Abdominal Pain Syndrome), nausea and vomiting (Aerophagia, Cyclic Vomiting Syndrome, Adolescent Rumination Syndrome) and defecation disorders (Functional Constipation, Nonretentive Fecal Incontinence).

- peds FACIT-F (Pediatric Functional Assessment of Chronic Illness Therapy - Fatigue) (12): a pediatric validated questionnaire on fatigue in chronic diseases, originally developed for adults with anemia in oncology (13) and validated for children with cancer by Lai *et al.* (12). We used the copyrighted French version of the pediatric questionnaire, upon authorization from Dr Cella. The questionnaire comprises 13 questions, with answers on a 5-point Likert scale (0: never, 1: rarely, 2: sometimes, 3: most of the time, 4: all the time). The questionnaires were interpreted according to the scoring guidelines (version 4). Eleven items were reversed (4-item) and summed to compose the tiredness score, ranging from 0 to 44. Two items composed the energy score, ranging from 0 to 8. The sum of the tiredness score and the energy score composed the total score, ranging from 0 to 52, with lowest scores representing the most fatigue.

- CDI (Children's Depression Inventory) (14): the French version of the questionnaire comprises 27 items with 3 statements of increasing severity (0 to 2). The total score ranges from 0 to 54, with highest scores representing most severe depression. In order to define mild, moderate and severe depression, we used the cutoffs proposed by a Korean team (15): 15 for mild, 20 for moderate and 25 for severe depression.

- SCARED-R-51 (Screen for Child Anxiety Related Disorders). The original questionnaire comprising 41 items was developed by Birmaher *et al.* (16) We used a French-language version adapted by Martin and Gosselin (17). The French version comprised 51 items, with 3 statements of increasing severity (0 to 2). The total score ranges from 0 to 102, with highest scores representing highest levels of anxiety. The items can be subdivided into different

aspects of anxiety disorders: panic disorder (items 1, 9, 14, 27, 35, 42, 45), generalized anxiety disorder (items 8, 17, 29, 30, 36, 41, 43), separation anxiety disorder (items 7, 10, 15, 24, 32, 37, 38), social phobia (social anxiety disorder: items 4, 11, 34, 39), obsessive compulsive disorder (items 6, 20, 22, 40, 47), post traumatic stress disorder (items 21, 31, 33, 49), specific phobia: school phobia (items 3, 13, 25, 44), animal phobia (items 18, 28, 50), situational phobia (items 2, 19, 23, 46, 48) and phobia of medical procedures (items 5, 12, 16, 26, 51).

- IMPACT III: this Health-related quality of life (HRQoL) questionnaire was originally developed by Griffiths *et al.* in 1999 for children and adolescents with IBD (18), and further adapted by Loonen *et al.* (IMPACT II) (19) and by Otley *et al.* (IMPACT III) (20). We used the validated French translation and the Six Domain Scoring instructions provided by Dr Otley (2013 version). The questionnaire comprises 35 items, with five Likert response options per question. Total score is calculated by summing the 35 questions, and dividing by the number of questions answered. The total score ranges from 0 (poor HRQoL) to 100 (best HRQoL). The 35 items can be subdivided into 6 domains: emotional functioning (items 4, 5, 11, 12, 13, 16, 29), treatment / intervention (items 2, 22, 24), social functioning (items 8, 9, 14, 17, 18, 20, 23, 26, 27, 30, 34, 35), bowel symptoms (items 1, 3, 10, 19, 21, 25, 31), systemic symptoms (items 6, 28, 32), and body image (items 7, 15, 33). To score each domain, the questions are summed and divided by the number of questions answered, each domain score ranging from 0 (poor) to 100 (best).

Parent questionnaires:

Parents were asked for their age group, socio-professional category, child's school level, number of school absences, child's medications and if he/she had received complementary medicines. The psychological self-reported questionnaires for parents were:

- STAI-Y: State Trait Anxiety Inventory / Form Y-A and Y-B. The Spielberger STAI questionnaire is one of the most frequently used questionnaires on anxiety (21). The form Y,

produced in 1983, consists of two questionnaires, one that measures State anxiety (STAI-Y-A, how one feels at the moment), the other measuring Trait anxiety (STAI-Y-B, how one feels generally). Each questionnaire comprises 20 questions, scored from 1 to 4. We used a French version of the questionnaire adapted by Gauthier and Bouchard (22). For each questionnaire the scores range from 20 (least anxiety) to 80 (most anxiety).

- BDI: Beck's Depression Inventory (23). Comprises 13 items scored from 0 (no depression) to 3 (most depression), with a total score ranging from 0 to 39. We used the French version adapted by P. Pichot from the revised BDI-II version of the inventory (24) the following cutoffs for the CDI: mild depression > 4, moderate > 7, severe > 15.

Clinical data retrieved from the chart were disease characteristics at diagnosis of IBD (Paris classification), treatments received since diagnosis, history of steroid dependency or being refractory to steroids, comorbidities. Steroid dependency was defined as partial or complete clinical response to treatment with prednisone 1 mg/kg/day (up to 40-60 mg) and relapse within 30 days after prednisone treatment was completed or relapse with a dose reduction of prednisone resulting in the use of prednisone at doses less than or equal to 15–25 mg/day for at least 6 months (25). Steroid-refractory disease was defined as IBD patients who still have active disease, despite prednisolone or an equivalent of up to 0.75 mg/kg/day over a period of 4 weeks (26).

We collected data on most recent imaging, most recent endoscopy, laboratory results from the last 3 months including Fecal calprotectin, and clinical activity indices (PCDAI, PUCAI), in order to evaluate depth of remission: clinical vs biological vs endoscopic vs histologic. Clinical remission was defined as PCDAI or PUCAI < 10. Biological remission was defined as CRP < 5 mg/L and ESR < 10 mm/h (27). Endoscopic remission was defined as no ulcerations and/or CDEIS < 6 for CD and no bleeding, ulceration or erosion and/or Mayo score ≤ 1 for UC and IBD-U (28). Histologic remission was defined as absence of neutrophils, and normal levels of plasmacytes and eosinophils in lamina propria (29).

Statistics:

Characteristics of patients having at least one pain-Functional Abdominal Pain Disorder (“IBD-FAPD group”) according to Rome III criteria based on Fr-qPGS questionnaire were compared to those of patients with no pain (further referred to as “No FAPD group”). Categorical variables were compared by Chi-square test or Fisher’s exact test if $n < 5$. Gaussian distribution of continuous variables was tested by Shapiro-Wilk test. Continuous variables were compared by T-Test if normally distributed and Wilcoxon non-parametric tests if not. Variables associated with IBD-FAPD with a $p < 0.10$ were included in a multivariable model where the explanatory variables were baseline characteristics of IBD, treatments received, and phenotype of disease. Correlation between psychological scores was tested by Spearman’s correlation coefficient, and r values $> 0,7$ with $p < 0.05$ were considered relevant. SAS version 9.2 (Cary, N.C., USA) was used for the analyses. A two-tailed p value < 0.05 was considered significant for all analyses.

ETHICAL CONSIDERATIONS

The study protocol was accepted by a French ethics committee (Comité de Protection des Personnes Nord-Ouest I, N°RCB: 2017-A02420-53). Patients received a non-financial compensation for their participation in study (a gift card).

RESULTS

Description of patients

From April 2018 to August 2019, 129 patients were included in the study, 108 patients filled in the questionnaires, 83 had both complete questionnaires and Case Report Form and 3 were excluded (1 for IBD duration < 1 year, 1 for ESR > 20 mm/h and 1 for CRP > 10 mg/L). In all, 80 patients were analyzed.

Among the 80 patients there were 40 boys, and the mean age was 15.0 ± 2.0 years (min-max 9.6 – 17.9). Seventy-six percent had Crohn's disease (CD), 19 % had Ulcerative colitis (UC) and 5% had Unclassified-IBD (IBD-U). Mean duration of IBD was 3.7 ± 2.2 years (min-max: 1.1– 12.7). Treatments received since diagnosis (including current treatment) were: aminosalicylates (n=33, 41%), corticosteroids (n=43, 54%, none currently taking corticosteroids at inclusion in study), immunomodulators (n=56, 70%), anti-TNF alpha therapy (n=55, 69%), enteral nutrition (n=28, 35%, only 2 patients currently receiving enteral nutrition), parenteral nutrition (n=3, 4%, all were past treatments). Previous IBD-related surgeries were: perianal surgery (n=5, 6%) and ileocecal resection (n=1, 17%) (Table 1). Features of complicated IBD course since diagnosis are indicated in Table 2.

At the time of inclusion, IBD was in clinical remission for all (PCDAI: 1.6 ± 4.1 , PUCAI: 1.1 ± 2.7) (Table 3). Fifteen patients had had an endoscopy within the last 6 months, among which 12 were in endoscopic remission, and 11 in histologic remission. Twenty-five patients had had an abdominal imaging within the last 6 months, among which 23 had no signs of active disease. Sixty-three patients had laboratory results from < 3 months: mean ESR was 7.7 ± 5.0 mm/h, mean CRP was 1.1 ± 0.8 mg/L, mean Albumin was 4.4 ± 0.3 g/dL.

Comparison of patients with functional pain and those without

Among the 80 patients, 16 (20%) met diagnostic criteria for at least one Functional gastrointestinal disorder (FGID) among which 12 (15%) had diagnostic criteria for at least

one Functional abdominal pain disorder (IBD-FAPD): 4 had IBS-lower location, 3 IBS-upper location, 2 abdominal migraines, 3 FAP-lower location, 1 FAP-upper location, 1 FAPS-lower location (Figure 1). According to Rome III criteria, some FAPDs and FGIDs can coexist, as shown in Figure 1.

The baseline characteristics of patients with and without FAPD did not differ significantly (Table 1), except for Crohn's disease Behavior: 20% stricturing disease in IBD-FAPD group, 8% penetrating disease in No FAPD group ($p=0.04$). There was no difference in number of hospitalizations, flares requiring steroids, treatments received or further IBD complications since diagnosis between groups (Table 2 and 3).

Among the patients who had recent (< 3 months) laboratory results, or an abdominal imaging or endoscopy within the last 6 months, there was no difference between the proportion of patients in biological, endoscopic, histologic or imaging remission between the 2 groups (Table 3). Median Fecal calprotectin was 30 $\mu\text{g/g}$ (IQR 25-75: 26-78) in the IBD-FAPD group vs 40 $\mu\text{g/g}$ (IQR 25-75: 21 – 125) in the No-FAPD group ($p=NS$).

In the multivariable logistic regression model including all clinical and demographic variables associated with IBD-FAPD with a $p<0.10$, and taking into account the interaction between number of hospitalizations and number of flares treated with corticosteroids, no variable was significantly associated with IBD-FAPD.

Psychosocial comorbidities of IBD-FAPD

Patients in the IBD-FAPD group reported increased levels of fatigue, as shown by lower total Peds FACIT-F scores than the No-FAPD group ($p=0.007$), with a lower Tiredness subscale ($p=0.006$) but no difference in Energy subscale ($p=0.11$) (Suppl Table 1, Figures 2 and 3).

Considering the CDI depression score and using the cutoffs proposed by Bang *et al.*, there were significantly more patients with mild to severe depression in the "Pain" group than in the "No FAPD" group ($p=0.03$) although the total increase in the CDI score did not reach significance ($p=0.11$) (Suppl Table 1, Figures 2 and 3).

The anxiety SCARED R-51 score did not differ between groups (Suppl Table 1, Figure 2), nor did the subscores of anxiety (Figure 3).

Health-related quality of life was significantly lower in the “IBD-FAPD” group, as reflected by a lower IMPACT-III score compared to the “No FAPD” group ($p=0.04$) (Suppl Table 1, Figure 2). The domains of IMPACT-III that were significantly lower in the “IBD-FAPD” group were: “Bowel symptoms” ($p=0.02$) and “Body image” ($p=0.04$) (Suppl Table 1, Figure 3).

Fifty percent of patients in the “IBD-FAPD” group and 46% of patients in the “No FAPD” group had been absent from school for 1 to 3 half-days per month on average over the school year. Rate of school absences was not different between groups (Suppl Table 1).

There was a significant correlation between psychological questionnaires scores in the IBD-FAPD group but not in the No FAPD group (Suppl Table 2). IMPACT III score was correlated with CDI score ($r=-0.88$, $p=0.0002$), Peds FACIT-F score ($r=0.77$, $p=0.003$) and SCARED-R-51 score ($r=-0.73$, $p=0.008$). In addition, Peds FACIT-F score was correlated with SCARED-R-51 score ($r=-0.83$, $p=0.0009$).

Parents of children with IBD-FAPD have higher levels of anxiety

In order to test for correlations between patient’s and parent’s answers, we restricted the analyses to one parent per child: if both parents had answered (only 5 patients) the mother’s questionnaire was retained for analysis since anxiety and depression have been shown to be more frequent in adult women in the general population. Age and socio-professional group were not different whether their child was in the “IBD-FAPD” group or not (Table 4). Parents of children in the “IBD-FAPD” group had a higher level of Trait anxiety (STAI-Y-B 42.6 ± 9.5 vs 36.4 ± 8.8 , $p=0.03$) but not State anxiety, than the parents of children in the “No FAPD” group. There was no correlation between parental and child anxiety based on STAI-Y A or B and SCARED-R51 scores (Pearson $r=0.18$).

Using the cutoffs proposed by Pichot *et al.*, there was a trend to a higher proportion of parents with Moderate depression in the “IBD-FAPD” group than in the “No FAPD” group

($p=0.04$), although BDI score was not statistically higher ($p=0.26$). There was no correlation between parental and child depression based on CDI and BDI scores (Pearson $r=0.09$).

Use of complementary medicines was marginal in the cohort. Parents reported the use of the following for their child: manual therapies ($n=6$), osteopathy ($n=5$), shiatsu ($n=1$), administrated surface therapies such as balms and creams ($n=1$), mind-body therapies ($n=6$), thermal water cure ($n=1$), relaxation therapy and Eye Movement Desensitization and Reprocessing (EMDR) therapy ($n=1$).

DISCUSSION

The overlap of IBS-like symptoms in adults with IBD in clinical and biological remission is now well known (8, 30). However there have been only few reports in pediatrics, and none that has examined simultaneously multiple aspects of psychosocial repercussions (31-33). In addition, most studies have focused on IBS or on FAP, but not on all FGIDs according to the Rome III classification.

In this study we have shown that the prevalence of FGIDs among a group of children and adolescents with IBD in remission was 20% (16 / 80) among which IBD-FAPD represented 15% (12 / 80) and IBS 8% (6 / 80) of patients. These rates are similar to those observed in the general population: a meta-analysis showed a worldwide pooled prevalence of FAPD in children of 13.5%, with IBS representing 8.8% (34). It was noted a discrepancy between prevalences depending on areas in the world which was different from that of IBD; the prevalence rates were higher in South America (16.8%) and Asia (16.5%) compared to Europe (10.5%). FAPD was more prevalent in girls than in boys (15.9% vs 11.5%), a feature of IBS in adults that we did not observe in our study. FAPD was associated with anxiety and depressive disorders, stress and traumatic life events (34).

As for the prevalence of other FGIDs, a study by Lewis *et al.*, on a sample of 949 mothers living in the USA who filled out QPGS and PedsQL 4.0 questionnaires on behalf of their healthy children showed that 23.1% of children had at least one FGID: constipation was the most prevalent (12.9%), followed by abdominal migraine (9.2%), aerophagia (4.3%), IBS (2.8%), fecal incontinence (1.8%), cyclic vomiting syndrome (1.1%), FAPS (0.8%), FAP (0.3%) and functional dyspepsia (0.2%) (35). These prevalence rates of FAPD are lower than those of the meta-analysis and might have been biased by the fact that the questionnaires were not self-administered by the children themselves, which we have done in our study.

Moving on to prevalence of FAPD in pediatric IBD patients in remission, Zimmerman *et al.* in a multicenter cross-sectional study in the USA showed a prevalence of functional abdominal pain of 10.3% in inactive pediatric IBD (31). Prevalence of pediatric IBD-FAPD according to

Rome III criteria was 26% in a study by Watson *et al.* (33), while a Dutch multi-center study reported IBS-like symptoms in 16% and IBS or FAP symptoms in 22.6% of pediatric IBD patients in biochemical remission (32). Our prevalence rates are thus in the lower range of those reported in pediatric IBD patients, tending to be closer to general population rates, and in all cases much lower than the rates of 35% observed in adult IBD patients thought to be in remission (7).

The first reports on overlapping IBS symptoms in adults with IBD identified an association with subclinical inflammation, suggesting that in case of residual symptoms it was first necessary to ascertain the absence of inflammation that could per se cause abdominal symptoms (30). For this reason we chose strict inclusion criteria in order to avoid biasing the data with patients with active subclinical disease. All patients had a normal CRP and ESR and low levels of Fecal calprotectin, although some outliers had a calprotectin level higher than the cutoff of 250 µg/g proposed in adult studies (8). In pediatric IBD, a cutoff of 300 µg/g has been associated with mucosal healing, although the range of 100 – 599 µg/g is considered a gray range with low discriminatory accuracy (27). In order to increase diagnostic accuracy, the MINI index (mucosal inflammation noninvasive index for pediatric Crohn's disease) was recently developed by the researchers from ImageKids study (27). The index comprises the stooling item of the PCDAI, ESR, CRP and fecal calprotectin. A MINI score < 8 identifies children with mucosal healing with higher accuracy than fecal calprotectin alone.

Since current inflammation was not associated with IBD-FAPD in our study, we sought for an association with previous inflammation, with the hypothesis that a severe past disease might lead to post inflammatory alterations in pain perception or visceral hypersensitivity, as it has been shown in animal models (9). We used as a proxy for severe disease the presence of either steroid dependency, steroid refractory disease, requiring anti-TNF therapy, complicated disease (B2, B3), perianal disease or growth retardation. Among these, only behavior of disease for Crohn's patients was associated with IBD-FAPD. In particular, there

were 20% of patients with stricturing disease at diagnosis in the IBD-FAPD group. Obstructive pain is among the most intense pains described in Medicine. We hypothesize that a pain sensitization might occur at the moment of obstructive pain and that a “memory of the pain” might persist even several years after. However, given that this represented only 2 patients, the significance of this hypothesis would need to be verified on a larger sample.

In our study there was a lower health-related quality of life in the IBD-FAPD group. This had also been reported by Watson *et al.* (33). Two domains differentiated the groups, the “bowel symptoms” and the “body image” domains. Since the “bowel symptoms” domain comprises questions on eating, abdominal pain, diarrhea and flatulencies and the “body image” domain comprises questions on satisfaction with ones’ weight, height and physical appearance, it was expected that the patients from the IBD-FAPD group would report more impact of these symptoms on their quality of life. We did not have a control group for comparison, but a study by Varni *et al.* showed lowered quality of life (using PedsQL questionnaire) in children with Functional gastrointestinal disorders and in IBD patients, compared to controls (5). Interestingly they observed that quality of life was lower in healthy children with FGID than in children with organic digestive diseases (IBD and gastro-oesophageal reflux). They had not included children who combined the two diagnoses, but based on our data we show that combination of functional abdominal pain and IBD reduces quality of life compared to IBD alone.

Depression and anxiety are frequently reported in children and adolescents with IBD (4). A recent meta-analysis showed pooled prevalence of anxiety symptoms of 16.4% and depressive symptoms of 15% in pediatric IBD (4). Rates of depression remained lower than those observed in adult IBD. The authors mandated caution when extrapolating this data, since many different questionnaires and many different cutoffs for the same questionnaires were used (4). We used the Children’s Depression Inventory and found a mean score of 9.0 ± 5.5 with no statistical difference between groups. Using the cutoffs of 15, 20 and 25 validated in a school-based study on Korean adolescents (15), there was a higher rate of

moderate depression in the IBD-FAPD group than in the No FAPD group. Zimmerman *et al.*, with a CDI cutoff of > 9 showed that 55% of children with functional abdominal pain and IBD in remission had depression, compared to 30% in children with no pain and a PCDAI ≤ 10 ($p=0.03$) (31). Had we used the same cutoff as Zimmerman *et al.* we would have had a rate of depression of 58% in the IBD-FAPD group vs 35% in the No FAPD group ($p=0.20$). Regardless of the cutoff chosen, chronic pain in children in general has been shown to be associated with depression (36). The association also stands for depression and FAPD in children with IBD in remission. We believe children with IBD in remission who suffer from abdominal pain should be evaluated for possible depressive symptoms and referred accordingly.

We were expecting to find an association between anxiety and FAPD. There was no difference between IBD-FAPD and no FAPD groups regarding the overall SCARED-R-51 score. However, the parents of children from the IBD-FAPD group had significantly more Trait anxiety than the other parents. We can speculate that although the children were not exceedingly anxious, the fact that their parents had anxiety might have amplified their perception of pain. One of the hallmarks of care of pediatric patients with functional abdominal pain is reassurance by the caregivers (37). The alternative explanation would be that their child's disease caused anxiety in the parent, and that anxiety persisted because of persisting symptoms.

Finally, we noted that children in the IBD-FAPD group had significantly more fatigue than the "No FAPD" group, although the overall scores using the FACIT-F 13-item questionnaire were in the same range as reported in adult IBD patients (38). In the adult study, patients with inactive disease had a mean FACIT-F score of 40.2 in CD and 42.2 in UC(38). Fatigue is a symptom frequently reported in adults with IBD, but data is lacking in pediatric IBD (39). The fatigue symptom can be associated with organic diseases such as hypothyroidism or iron deficiency. We had planned to collect the most recent iron levels, Thyroid Stimulating

Hormone and vitamin D levels, but these tests were not done in routine and were available for only a small number of patients.

All of the psychological aspects were correlated (quality of life, fatigue, depression and anxiety), which shows a complex interplay of these factors and the necessity to take each of them into account. Several parents reported that their child had received complementary medicines. To date there are no recommendations concerning optimal management of IBD-FAPD in children, although there have been reports of successful implementation of mind-body interventions (40).

Considering reproducibility of our results, we recognize that the patients who accepted to participate in the study might reflect a milder course of disease, although their baseline characteristics (Paris classification and age) are comparable to data from population-based registries (1). Indeed, although mean duration of IBD was 3 years, the majority had not been hospitalized (apart from initial hospitalization for diagnostic work-up), and had less than one flare requiring steroids since diagnosis. Another clue is that almost 70% of patients participating in the study were currently on anti-TNF alpha treatment, which is higher than population-based pediatric studies. There might have been an inclusion bias based on mode of recruitment of patients (in the infusion clinic rather than outpatient clinic?). Nonetheless, we seem to have captured an interesting group of patients who as a whole are doing well despite their IBD, and have lower depression scores, anxiety, and fatigue than comparable studies. In the absence of IBD complications, it seems possible that the anti-TNF therapy was initiated early in the course of disease, due to predictive factors of poor outcome according to ECCO/ESPGHAN pediatric recommendations and not after complications had occurred (2). Whether this therapeutic strategy could also have beneficial effects on Brain-Gut axis and psychological outcomes should be further evaluated.

CONCLUSION

In conclusion, we have shown that the prevalence of IBD-FAPD in pediatric patients in clinical and biochemical remission is similar to that of the general population. IBD-FAPD was associated with fatigue, lower quality of life, depression and parental anxiety, but not with child anxiety. Although past severity of disease did not differ between groups, the patients in the study had a high rate of current biologic medications and had had few flares since the diagnosis of IBD. We speculate that beginning early in the course of disease highly efficacious medications and obtaining rapid remission might have prevented disturbances of the brain-gut axis.

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TABLES

Table 1. Baseline characteristics of patients since diagnosis of IBD, comparing patients with at least one IBD-FAPD to those without.

Functional Abdominal Pain Disorders (FAPD) include functional dyspepsia, irritable bowel syndrome (IBS), abdominal migraine, functional abdominal pain and functional abdominal pain syndrome. Data expressed as n (%), mean \pm SD for normally distributed variables and median (IQR 25-75) for non-normally distributed variables.

	At least one IBD-FAPD (n=12)	No FAPD (n=68)	p
Male (n,%)	5 (42%)	35 (51%)	NS
Duration of IBD (yrs, median [IQR 25-75])	2.4 (2.1-3.6)	3.4 (2.4-4.7)	0.10
Diagnosis			NS
Crohn's disease (n,%)	10 (83%)	51 (75%)	
Ulcerative colitis (n,%)	1 (8%)	14 (21%)	
IBD-unclassified (n,%)	1 (8%)	3 (4%)	
Crohn's disease: Age at diagnosis (Paris classification)			NS
A1a: 0 - <10 years (n,%)	0	10 (20%)	
A1b: 10 - <17 years (n,%)	10 (100%)	41 (80%)	
Crohn's disease: Location (Paris classification)			NS
L4a (n,%)	3 (30%)	9 (18%)	
L4b (n,%)	0	4 (8%)	
No upper GI disease (n,%)	7 (70%)	36 (73%)	
L1 (n,%)	2 (20%)	L1: 6 (12%)	
L2 (n,%)	0	14 (27%)	
L3 (n,%)	8 (80%)	27 (53%)	
No lower GI disease (n,%)	0	4 (8%)	
Crohn's disease: Behavior (Paris classification)			0.04
B1: non stricturing, non penetrating (n,%)	8 (80%)	45 (92%)	
B2: stricturing (n,%)	2 (20%)	0	
B3: penetrating (n,%)	0	4 (8%)	
p (perianal disease)	3 (30%)	23 (45%)	NS
G (growth failure)	3 (30%)	16 (31%)	NS
Ulcerative colitis and IBDU: Extension (Paris classification)			NS
E1 (n,%)	0	0	
E2 (n,%)	1 (50%)	5 (31%)	
E3 (n,%)	0	2 (13%)	
E4 (n,%)	1 (50%)	9 (56%)	
Ulcerative colitis and IBDU: Severity (Paris classification)			NS
S0: Never severe (n,%)	2 (100%)	14 (82%)	
S1: Ever severe (n,%)	0	3 (18%)	
Previous medications for IBD (n,%)			
Aminosalicylates	0	17 (25%)	NS

Corticosteroids	5 (42%)	38 (56%)	NS
Immunosuppressant drugs	4 (33%)	29 (43%)	NS
anti-TNF α agents	0	3 (4%)	NS
2 nd line anti-TNF α agents	0	5 (8%)	NS
Enteral nutrition	6 (50%)	20 (29%)	NS
Parenteral nutrition	0	3 (4%)	NS
Previous IBD-related surgery (n,%)			
Perianal surgery	1 (8%)	4 (6%)	NS
Ileocecal resection	1 (8%)	0	NS

Table 2. Features of complicated IBD, in patients with and without Functional abdominal pain disorders

Data expressed as n (%), mean \pm SD for normally distributed variables and median (IQR 25-75) for non-normally distributed variables.

	IBD-FAPD (n=12)	No FAPD (n=68)	p
Number of hospitalizations since diagnosis of IBD*	0 (0-0)	0 (0-1)	0.10
Number of flares requiring steroids	0.5 (0-1)	0 (0-1)	NS
Steroid-dependency (any)	1 (8%)	12 (18%)	NS
Steroid-resistance (any)	0	4 (6%)	NS
Complications since diagnosis of IBD			
Bowel stricture	1 (8%)	0	NS
Intestinal fistula	0	2 (3%)	NS
Perianal disease	2 (17%)	17 (25%)	NS
Comorbidities			
Liver disease	0	3 (4%)	NS
Psoriasis	2 (17%)	2 (3%)	0.10
Arthritis	1 (8%)	4 (6%)	NS

* Not counting the first hospitalization for diagnostic work-up

Table 3. Characteristics of patients with and without FAPD at inclusion in the study

Data expressed as n (%), mean \pm SD for normally distributed variables and median (IQR 25-75) for non-normally distributed variables.

	IBD-FAPD (n=12)	No FAPD (n=68)	p
Age at inclusion (yrs)	15.8 \pm 1.3	14.9 \pm 2.1	NS
Weight (kg)	52.4 \pm 12.2	52.5 \pm 14.3	NS
Height (cm)	163.3 \pm 9.4	163.1 \pm 11.1	NS
PCDAI	0 (0-5)	0(0-0)	NS
PUCAI	2.5 (0-5)	0 (0-0)	NS
Laboratory results (< 3 months)	n=10	n=53	
Hemoglobin (g/dL)	13.7 \pm 1.3	14.0 \pm 1.3	NS
Hematocrite (%)	41.6 \pm 4.1	40.4 \pm 3.8	NS
Platelets (G/L)	270 \pm 55	282 \pm 71	NS
Leucocytes (G/L)	7.6 \pm 1.8	6.9 \pm 2.4	NS
ESR (mm/h)	8.8 \pm 5.5	7.5 \pm 5.0	NS
CRP (mg/L)	1.4 \pm 1.3	1.1 \pm 0.6	NS
Albumin (g/dL)	4.6 \pm 0.3	4.3 \pm 0.3	0.06
Fecal calprotectin (μ g/g) (< 3 months)	30 (26-78) (n=5)	40 (21-125) (n=25)	NS
Endoscopic remission*	2 / 3	10 / 12	NS
Histologic remission*	2 / 3	9 / 12	NS
Imaging remission*	3/3	20/22	NS
Current medication for IBD (n,%)			
Aminosalicylates	2 (17%)	14 (21%)	NS
Immunomodulators	3 (25%)	20 (29%)	NS
anti-TNF α agents	8 (67%)	47 (69%)	NS
Enteral nutrition	0	2 (3%)	NS
Psychotropic treatments			
Past psychotherapy	1 (8%)	8 (12%)	NS
Ongoing treatment	1 (8%)	0	NS
Ongoing therapy	1 (8%)	3 (4%)	NS

* Based on results of most recent endoscopy or abdominal imaging, if performed less than 6 months before inclusion

Table 4. Parents' characteristics (one parent per child)

Data expressed as n (%), mean \pm SD for normally distributed variables and median (IQR 25-75) for non-normally distributed variables.

	At least one IBD-FAPD (n=12)	No FAPD (n=68)	p
Gender of the parent answering the questionnaire (female, n,%)	10 (83%)	50 (86%)	NS
Parent age group			NS
30 – 39 years (n,%)	1 (8%)	9 (16%)	
40 – 49 years (n,%)	6 (50%)	37 (66%)	
50 – 59 years (n,%)	4 (33%)	8 (14%)	
60 – 69 years (n,%)	1 (8%)	2 (4%)	
Parent Socio-professional group			NS
Farmers (n,%)	1 (8%)	0	
Craftsmen, Tradesmen, Entrepreneurs (n,%)	0	3 (5%)	
Person without professional activity (n,%)	1 (8%)	5 (9%)	
Senior managers and higher intellectual occupations (n,%)	4 (33%)	16 (28%)	
Employees (n,%)	5 (42%)	15 (26%)	
Workers (n,%)	0	2 (3%)	
Intermediate professions (n,%)	1 (8%)	15 (26%)	
Retirees (n,%)	0	2 (3%)	
Parent anxiety and depression			
STAI-Y-A	40.1 \pm 12.2	34.7 \pm 11.0	NS
STAI-Y-B	42.6 \pm 9.5	36.4 \pm 8.8	0.03
BDI score	2 (1-8)	2 (0-4)	0.26
No depression (BDI \leq 4)	6 (60%)	51 (82%)	<i>0.05 for the table</i>
Mild depression (BDI > 4 - \leq 7)	0	5 (8%)	
Moderate depression (BDI > 7 - \leq 15)	4 (40%)	6 (10%)	
Severe depression (BDI > 15)	0	0	

Suppl Table 1. Psychosocial comorbidities of Functional abdominal pain disorders among pediatric IBD patients in clinical and biochemical remission.

IBS: irritable bowel syndrome, FAP: functional abdominal pain, FAPS: functional abdominal pain syndrome. Data expressed as n (%), mean \pm SD for normally distributed variables and median (IQR 25-75) for non-normally distributed variables.

	IBD-FAPD (n=12)	No FAPD (n=68)	p
Rome III IBD-FAPD disorders			
Functional dyspepsia	0	0	
IBS-lower location	4 (33%)	0	
IBS-upper location	3 (25%)	0	
Abdominal migraine	2 (17%)	0	
FAP-lower location	3 (25%)	0	
FAP-upper location	1 (8%)	0	
FAPS-lower location	1 (8%)	0	
FAPS-upper location	0	0	
Rome III non-pain FGID syndromes			
Functional constipation	0	4 (6%)	
Fecal incontinence	1 (8%) #	0	
Aerophagia	0	0	
Cyclic vomiting syndrome	0	0	
Rumination syndrome	1 (8%) #	0	
Chronic fatigue			
Tiredness subscore	30 (16.5 – 36.5)	37.5 (32.5 – 41.0)	0.006
Energy subscore	5.5 (3.5 – 7.5)	7 (6 – 7)	0.11
Total Peds FACIT-F score	37 (20 – 44)	44.5 (38.5 – 48.0)	0.007
Depression			
CDI score	12.7 \pm 8.5	8.3 \pm 4.5	0.11
No depression (CDI<15)	9 (75%)	60 (88%)	0.03
Mild depression (CDI 15- <20)	1 (8%)	7 (10%)	
Moderate depression (CDI 20 - <25)	0	1 (1%)	
Severe depression (CDI \geq 25)	2 (17%)	0	
Anxiety			
SCARED-R-51 score*	27.1 \pm 13.1	24.1 \pm 11.8	NS
HRQoL			
IMPACT-III score	73.2 (63.4 – 84.8)	81.9 (75.0 – 87.3)	0.04
Emotional functioning	64.3 (48.2 – 89.3)	82.1 (71.4 – 89.3)	0.17
Treatment/intervention	75.0 (41.7 – 83.3)	66.7 (50.0 – 83.3)	0.72
Social functioning	86.5 (82.3 – 88.5)	89.6 (81.3 – 95.8)	0.11
Bowel symptoms	76.8 (67.3 – 85.4)	85.7 (78.9 – 92.9)	0.02
Systemic symptoms	66.7 (45.8 – 83.3)	79.2 (66.7 – 87.5)	0.07
Body image	50.0 (33.3 – 58.3)	66.7 (50.0 – 75.0)	0.04
Academic impact			
Repeated a school year	0	6 (10%)	
School absences			NS
None	4 (33%)	24 (39%)	
1-3 ½ days per month	6 (50%)	28 (46%)	
4-10 ½ days per month	2 (17%)	6 (10%)	
> 10 ½ days a month	0	3 (5%)	

These 2 patients who had a FGID-non FAPD, also had criteria for a FAPD and thus were analyzed with the FAPD group

* No statistically significant difference between groups for all sub-items of the SCARED-R-51

Suppl Table 2. Correlation between psychological Patient Reported Outcomes, among patients with and without IBD-FAPD.

r= Spearman's correlation coefficient.

A. Patients with IBD-FAPD (n=12)

r p	IMPACT III	CDI	Peds FACIT-F	SCARED R51
IMPACT III	1	-0.88 0.0002	0.77 0.003	-0.73 0.008
CDI	-0.88 0.0002	1	-0.63 0.03	0.58 0.05
Peds FACIT-F	0.77 0.003	-0.63 0.03	1	-0.83 0.0009
SCARED R51	-0.73 0.008	0.58 0.05	-0.83 0.0009	1

B. Patients without FAPD (n=68)

r p	IMPACT III	CDI	Peds FACIT-F	SCARED R51
IMPACT III	1	-0.58 <0.0001	0.50 <0.0001	-0.38 <0.0001
CDI	-0.58 <0.0001	1	-0.38 0.007	0.53 <0.0001
Peds FACIT-F	0.50 <0.0001	-0.32 0.007	1	-0.23 0.06
SCARED R51	-0.38 <0.0001	0.53 <0.0001	-0.23 0.06	1

FIGURE LEGENDS

Figure 1. Graphical description of answers to the Fr-qPGS questionnaire based on Rome III criteria among the 80 patients included.

The “n=” represent number of patients by functional gastrointestinal disorder (FGID). This figure shows the overlap between IBD-FAPD (IBS, abdominal migraine, FAP and FAPS) and the other FGIDs (constipation, rumination, fecal incontinence). Functional dyspepsia, aerophagia and cyclic vomiting syndrome are not shown since no patient had criteria for these FGIDs.

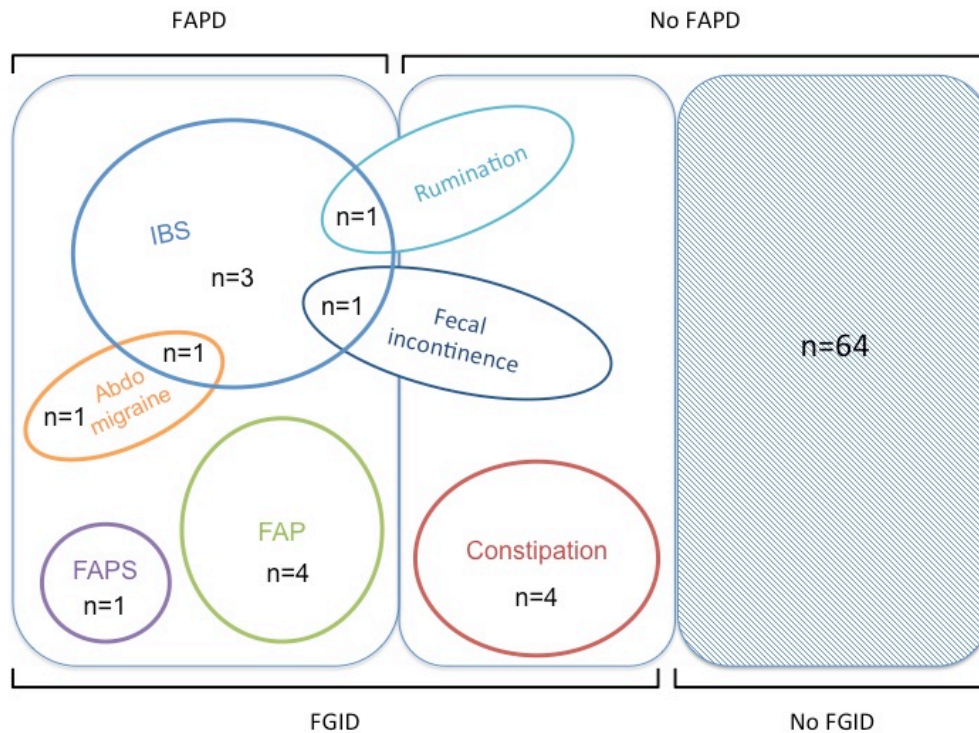


Figure 2. Psychological comorbidities of Functional abdominal pain disorders based on self-administered questionnaires.

Panel A: Total Peds FACIT-F score by group (No FAPD: n=68, IBD-FAPD: n=12), lower scores represent higher levels of fatigue. Panel B: Total CDI score by group, higher CDI scores indicate higher levels of depression. Panel C: Total SCARED-R-51 score by group, higher SCARED-R-51 scores indicate higher levels of anxiety. Panel D: Total IMPACT III score by group, lower scores indicate lower health-related quality of life. Scores are represented as median \pm IQR and whiskers indicate min and max values. * p-value < 0.05, ** p-value < 0.01.

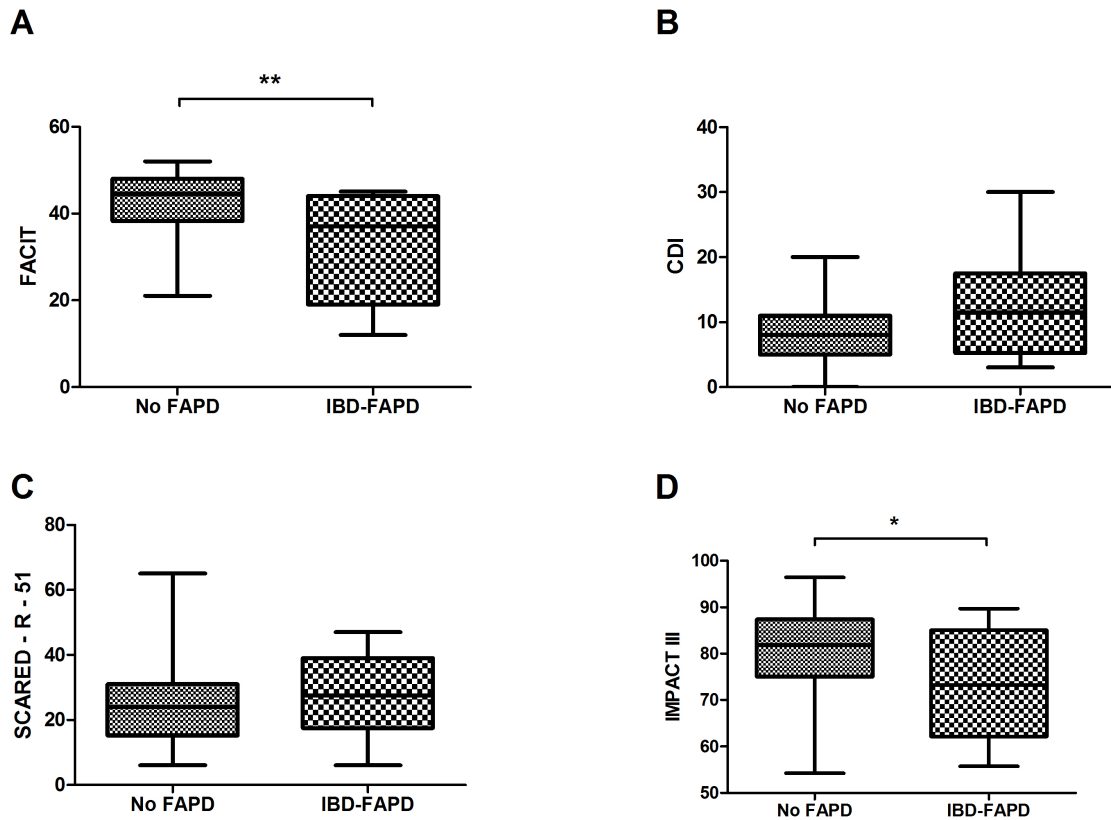
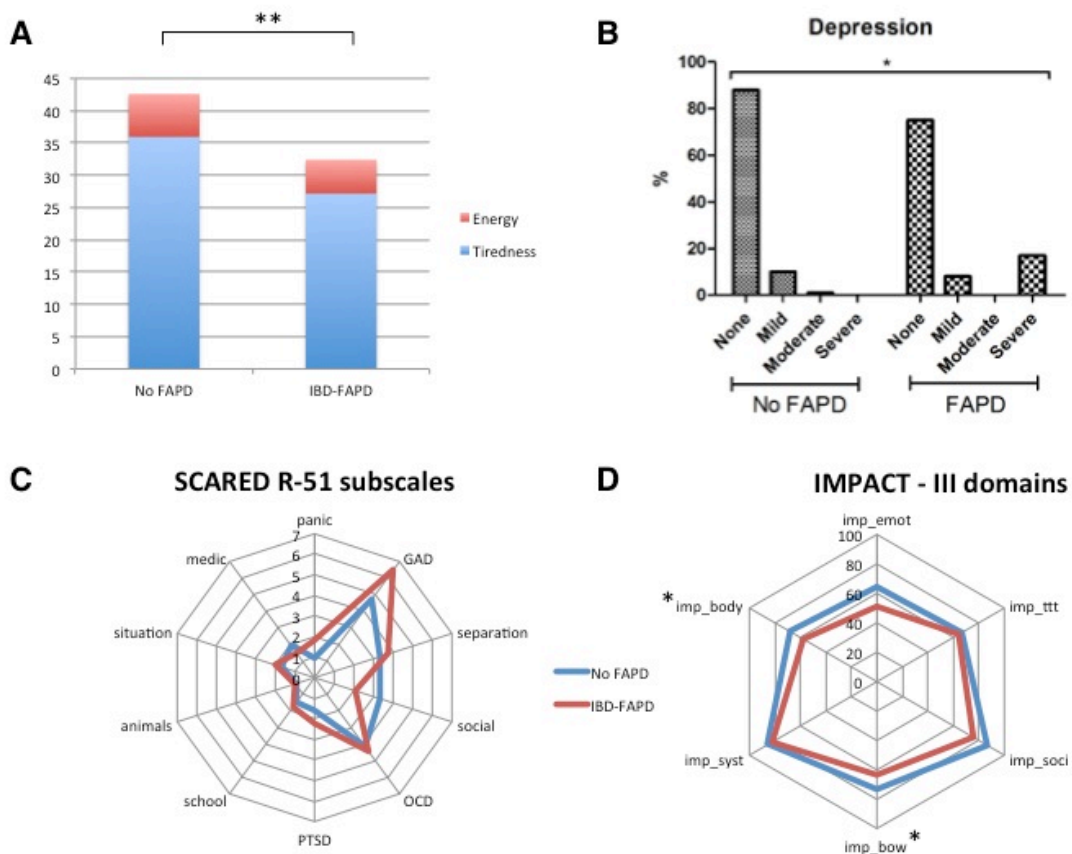


Figure 3. Detailed answers to the psychological questionnaires, by subscore, among pediatric IBD patients in remission, comparing patients with IBD-FAPD (n=12) and patients without (n=64)

Panel A: FACIT subscores: A higher Tiredness subscore indicates less tiredness, a Lower energy subscore indicates lower energy. Panel B: Intensity of depression based on Bang et al. cutoffs for CDI: mild (CDI 15-19), moderate (CDI 20-24), severe (CDI ≥ 25). Panel C: SCARED-R-51 subscales of Anxiety Disorder: “panic”: Panic Disorder, “GAD” : Generalized Anxiety Disorder , “separation”: Separation Anxiety, “social” : Social Anxiety Disorder, “OCD”: obsessive-compulsive disorder, “PTSD” : Post Traumatic stress disorder, “school”: Significant School Avoidance, “animals” : animal phobias, “situation” : situational phobias, “medic” : phobia of medical procedures. Panel D : IMPACT III domains of health-related quality of life : “imp_emot” : emotional functioning, “imp_ttt” : treatment/intervention, “imp_soci” : social functioning, “imp_bow” : bowel symptoms, “imp_syst” : systemic symptoms, “imp_body” : body image. * p-value < 0.05, ** p-value < 0.01.



Article 2 : Etude STENO-PED

Risk factors for surgery in stricturing small bowel pediatric Crohn's disease and contribution of magnetic resonance enterography to prediction of response to treatment

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Abstract:

Background and aims: The optimal management of small bowel strictures in pediatric Crohn's disease and decision of whom and when to refer to surgery are not well identified. A clinical and radiological prognostic score (the CREOLE score) was developed to predict response to adalimumab in symptomatic small bowel strictures in adults with Crohn's disease. Our aims were: 1/ to identify risk factors associated with surgery in pediatric stricturing small bowel Crohn's disease, 2/ to test if routinely collected MRI signs can predict response to treatment, 3/ to evaluate if the CREOLE score could be used in a pediatric population based on imaging data collected routinely.

Methods: Pediatric patients with stricturing small bowel Crohn's disease diagnosed between 2009 and 2018 were recruited from 9 French tertiary hospitals. CREOLE score was calculated retrospectively from MRE report and medical charts. Demographic and clinical data were collected from chart review.

Results: Fifty patients (62% boys) with a median follow-up of 3.9 years (IQR 25-75: 2.5 – 6.3) were included. Twenty-nine (58%) required small bowel surgery for the stricture, within a delay of 2.6 months (IQR 25-75: 1.1 – 6.2) after diagnosis of stricture. Using a Cox multivariable model, PCDAI > 22.5 at diagnosis of stricture was associated with risk of surgery (OR 6.73 (95% CI: 2.27-19.91), $p=0.0006$) whereas anti-TNF treatment after diagnosis of stricture was a protective factor (OR 0.21 (95%CI: 0.08-0.59), $p= 0.003$). Among imaging features, diameter proximal to dilation of more than 30 mm was the only criteria associated with surgery, but complete CREOLE score could not be calculated for all due to missing data.

Conclusion: Routine MRE reports frequently lack data that could potentially serve as prognostic factors. Implementing standard MRE reports in pediatric CD should be a priority. Clinical data alone might not be sufficient to predict response to treatment, although our study suggests that patients with higher activity scores and who did not receive an anti-TNF after diagnosis of stricture were at increased odds for surgery.

Keywords:

Magnetic resonance imaging, child, surgery, risk factors.

Introduction

The prevalence of stricturing phenotype in pediatric Crohn's disease (CD) has been reported from 20% at diagnosis to 40% at ten years since diagnosis, and is the leading cause of surgery in pediatric CD (1).

Chronic inflammation is a major determinant of intestinal fibrosis. Following a cascade of proinflammatory mediators implicating principally the IL-13 – TGF-Beta 1 – Smad 2/3 pathway, fibroblasts are stimulated to become activated myofibroblasts and deposit collagen and other proteins in the extracellular matrix. Fibrosis results from an imbalance between pro-fibrotic and anti-fibrotic factors (2). Intestinal strictures may also result from a localized inflammation of the intestinal wall causing a narrowing of the lumen and obstructive symptoms. It may be difficult to distinguish the nature of the stricture, inflammatory *vs* fibrotic. Mounting evidence based on surgical resection specimens shows that strictures are composed of both inflammatory and fibrotic tissue (3, 4).

To date no medical treatment can reverse intestinal fibrosis and the natural history of intestinal fibrosis is not well known. It has been suggested that optimizing anti-inflammatory treatments before the fibrosis becomes fixed may reduce need for surgery (5). Surgical resections can add to intestinal tissue damage burden and be associated with post-operative complications and recurrences (6, 7). The optimal timing of surgery in children must take into account several factors specific to that age group, among which correction of growth delay, and minimization of body-image related psychological outcomes. Early surgery in children can be beneficial. A pediatric series in the general population, based on EPIMAD registry, showed better recaptured growth and less necessity for immunomodulators if surgery was performed within 3 years from diagnosis *vs* after (7).

It is thus of paramount importance to be able to identify patients who could probably benefit from medical treatment alone compared to those for whom the probability of failure is high, leading to discuss early surgery. In this aim, several new imaging techniques have ambioned to better discriminate the inflammatory or fibrotic nature of strictures. Over the last 10 years, MR-enterography (MRE) has become the reference technique for mapping Crohn's disease extent to the small bowel, identifying jejunal and ileal locations of CD, as well as stricturing and fistulizing

complications (8, 9). Although MRE has a detection rate of 81% for small bowel strictures, its discriminatory rate between inflammatory strictures and fibrotic strictures is low, around 57% according to a series by Lenze *et al* (10). In addition, duration of disease, burden of past inflammation and location of inflammatory sites could have an impact on the fibrotic load. It seems necessary to add clinical data to the imaging in order to better characterize the strictures with the aim of being able to predict those that will require surgical intervention. A recent prospective study by the French collaborative research group on IBD in adults (GETAID) established a clinical and radiological prognostic score, the CREOLE score, in order to predict response to adalimumab of adult patients with symptomatic small bowel strictures (11). Based on MRE and clinical data, the probability of response to adalimumab was classified as: good if CREOLE score ≥ 4 (89% treatment success defined as absence of surgery or endoscopic dilatation during the first 24 weeks of treatment), moderate if score = 3 (61% success) and poor if score ≤ 2 (6% success). The score has never been validated outside of the reference population.

The primary objective of our study was to identify risk factors for surgery in pediatric patients with stricturing small bowel Crohn's disease. The secondary objective was to evaluate if MRE imaging characteristics of the stricture could be associated with response to treatment, based on criteria collected routinely and present on MRE report. Our final objective was to determine the feasibility of implementing the clinical-radiological CREOLE score in pediatric stricturing small bowel disease and whether it would be associated with response to treatment.

Patients and methods

This was a multicenter retrospective observational study elaborated by investigators of the GETAID pédiatrique (Groupe d'Etudes Thérapeutiques des Affections Inflammatoires Digestives pédiatrique), a French collaborative research group on pediatric IBD.

Patient selection

Inclusion criteria were: all patients with Crohn's disease based on Porto criteria (12), who had at least one small bowel stricture diagnosed between January 2009 and December 2018, aged less than 18 years at diagnosis of stricture, and with a follow-up of at least 6 months after diagnosis of stricture. Stricture was defined as a constant luminal narrowing, associated with a pre-stenotic dilation on imaging and/or obstructive symptoms (13).

Exclusion criteria were: patients who had only colonic strictures and patients who had an intra-abdominal abscess at the time of diagnosis of the stricture, since this could have modified the surgical indication.

The patients were identified by the hospitals' electronic health records database (K50 = "Crohn's disease", K56 = "ileus or occlusion without hernia", and/or R11 = "nausea/vomiting"), by systematic screening of all MR-enterography performed in patients < 18 years between 2009 and 2019, and by questioning the physicians on their cohort of patients.

Data collection

The data collected retrospectively from the charts were: age and gender, CD phenotype at diagnosis according to Paris classification (13), treatments received before the diagnosis of small bowel stricture. The date of diagnosis of stricture, disease activity based on Pediatric Crohn's Disease Activity Index (PCDAI), laboratory results at the time of stricture diagnosis were collected. Obstructive symptoms were quantified according to the Crohn's Disease Obstructive Score (CDOS) (11). Small bowel imaging reports (MR-enterography, CT-scan enterography, small bowel ultrasound) were analyzed for the following: number of strictures per segment (duodenum, jejunum, ileum, ileocecal valve, ileocolonic anastomosis, colon), length of the longest stricture (cm), pre-stenotic

dilatation diameter (mm), inflammatory characteristics based on MRE: spontaneous hypersignal on T2-weighted images, delayed T1-w enhancement after gadolinium injection, presence of a small bowel fistula. The same criteria were analyzed on CT-scan enterography and ultrasound reports if available, inflammatory characteristics being defined by enhancement of the stricture after contrast injection in CT-scan, or spontaneous hyperemia on Doppler signal in ultrasound.

We noted treatment modification following diagnosis of small bowel stricture, date of surgery and indication for surgery if applicable (refractory stricture *vs* acute indication for surgery), date of endoscopic dilation if applicable, date of last news and if the stricture was still present on the most recent imaging.

CREOLE score was calculated retrospectively, based on characteristics at diagnosis of small bowel stricture. The CREOLE score comprises 7 factors associated with a high rate of success of adalimumab: immunosuppressive treatment / yes, Crohn's disease obstructive score / > 4, duration of obstructive symptoms (weeks)/<5, length of stricture <12 cm, maximal small bowel diameter proximal to stricture(s) (mm) / between 18 and 29 mm, enhancement on delayed T1-weighted sequence / marked, fistula/no. Each factor is attributed 1 point when present, the total CREOLE score ranging from 0 to 7 (highest chance of success).

Statistics

Patient characteristics were described by mean \pm SD or median [IQR 25-75] for non-normally distributed continuous variables, and n (%) for categorical variables.

Patients having required surgery for small bowel stricture during their pediatric follow-up were compared to those who did not. Imaging characteristics were compared using Fisher's exact test for categorical variables, and Wilcoxon's exact test for continuous variables. Time until surgery or transition to adult care (whichever came first), or last news was modeled using survival curves, and groups were compared by Kaplan-Meier tests. The event studied was "surgical resection of small bowel stricture". Variables associated with the event with $p < 0.10$ were included in a multivariate Cox regression analysis, after verification of Hazards proportionality hypothesis. The cutoffs used for hematocrit, ESR and albumin were those from PCDAI score. Age was divided into 3 groups (0-5, 6-12

and 13-17 years) according to methodology from previous studies (14, 15). Weight and height were categorized into $<-2SD$, -2 to $+2SD$ and $> 2 SD$ for age and sex, according to French growth charts. The other continuous variables were dichotomized according to their median value in the whole study group. Imaging characteristics were not included in multivariable model since none reached the significance level for entry in the model. CREOLE score could be calculated only for a small sample of patients and was not included in the multivariable model. Effect of study center was taken into account by studying study center as a covariate in the multivariate model. Identical results were obtained by an analysis of clustered data. SAS 9.2 (Cary, N.C., USA) was used for the analyses. A two-tailed p-value < 0.05 was considered significant for all analyses.

Ethics

The study was approved by the French Pediatric Society Ethics Committee (“Comité d’éthique de la recherche de la Société Française de Pédiatrie”), reference CERSFP_2017_061-2.

Results

Patient baseline characteristics

Fifty patients (62% boys) with Crohn's disease diagnosed between August 2006 and August 2018 and a median follow-up of 3.9 years (IQR 25-75: 2.5 – 6.3) were included from 9 French Tertiary hospitals. Small bowel strictures had been diagnosed between April 2010 and November 2018. At the time of diagnosis of CD, mean age was 12.3 ± 2.8 years (range 5.6 – 16.7), 34% had disease limited to terminal ileum and/or caecum (L1), 54% had ileocolonic disease (L3), and 22% had a stricturing phenotype (B2). Among treatment regimens received for Crohn's disease before diagnosis of small bowel stricture, 62% of patients had received MODULEN IBD®, 60% steroids, 52% immunomodulators, 48% had received an anti-TNF α and 24% had received both an immunomodulator and an anti-TNF α (Detailed baseline characteristics are in Table 1).

Diagnosis of small bowel stricture

Diagnosis of stricture was made a median 12.4 months [IQR 25-75: 2.0 – 34.7] after diagnosis of CD, based on obstructive signs (CDOS ≥ 2) in 39 patients and/or on small bowel dilation on imaging in 39 patients. Of note, 22% patients (n=11) had only small bowel dilation on imaging without clinical obstructive symptoms. Duration of obstructive symptoms was < 5 weeks in 44% of patients (Table 2). Sixteen percent of patients were in clinical remission (PGA) at the diagnosis of stricture. Among the other patients, the signs of active disease were: PCDAI > 10 in 34 patients (83%), CRP > 10 mg/L in 29 patients (66% of patients who had recent laboratory results), ESR > 20 mm in 15 patients (44%).

Comparison of imaging techniques used to diagnose stricture

The imaging reports available were: MRI-enterography (n=41), TDM-enterography (n=5), abdominal ultrasound (n=18) (Table 3). Fourteen patients had more than one type of imaging at diagnosis of stricture: 3 had MRE and TDM, 11 had MRE and US, none had TDM and US. Based on MRE, 33 patients had a single ileal stricture, 7 had 2 ileal strictures, 1 had no ileal strictures. Two patients had a

jejunal stricture. There were no duodenal strictures in the cohort. There was imaging evidence of ileocecal valve stricture in 14 patients. None of the patients had an associated colonic stricture based on MRE. Median length of longest stricture as measured by MRE was 9.5 cm [IQR 25-75 : 5.0 – 15.0] and stricture wall thickness was 8.0 mm [IQR 25-75: 5.65-8.50]. Luminal diameter in the most narrowed segment was indicated in none of the reports and could not be measured since dynamic sequences are necessary. Comparing MRE and abdominal ultrasound reports in the 11 patients who had had the 2 exams at an interval of less than 3 months, length of stricture was 5.5 cm (4.0-7.0) by MRE vs 5.0 cm (3.50 – 10.0) by ultrasound (Pearson $r=0.25$, NS) and stricture wall thickness was 7.35 mm (5.0 – 8.0) by MRE vs 7.0 mm (5.0 – 8.0) by ultrasound (Pearson $r=-0.98$, $p=0.02$).

Treatment modifications after diagnosis of small bowel stricture

Ten percent of patients ($n=5$) continued the same treatment as before diagnosis of stricture.

Twenty-two patients started steroids (5 of which were steroid-naïve), 14 patients started MODULEN IBD[®] (5 of which were naïve to it). Twelve patients who were naïve to immunomodulators started: methotrexate ($n=4$) or azathioprine ($n=8$), while 18 continued a previous immunomodulator and 8 stopped. Thirty-two patients started or switched anti-TNFs, among which 19 infliximab-naïve patients started infliximab and 12 adalimumab-naïve patients started adalimumab. Twenty patients (40%) received both an anti-TNF α and an immunomodulator. In addition, 2 patients started vedolizumab and 3 started ustekinumab. The other treatments received are indicated in Table 4.

None of the patients had an endoscopic dilation of stricture.

Description of patients who required surgery

Twenty-nine patients required surgery for stricture, among which 21 were operated within 6 months of diagnosis of the stricture. Median delay between diagnosis of stricture and surgical removal was 2.6 months (IQR 25-75: 1.1 – 6.2). Among the 29 patients that were operated, 11 (38%) had surgery for an acute reason, and 18 (62%) for a stricture that was refractory to medical treatments. Surgical procedure performed was resection-anastomosis in 27 (93%) and ileostomy in 2 (7%).

Predictors of delay until surgery for small bowel stricture during pediatric follow-up

Delay until surgery depending on clinical and biological variables was modeled using Kaplan Meier analyses (Supplementary Table 1, Figure 1). The variables associated with delay until surgery in the univariate analyses were anti-TNF α treatment before diagnosis of stricture (p=0.03), PCDAI >22.5 at the time of diagnosis of stricture (p=0.01), and among treatments received after diagnosis of stricture: steroids (p=0.03), immunomodulators (p=0.02) and anti-TNF α agents (p=0.01).

Imaging characteristics associated with response to treatment

Among the imaging characteristics, the only variable associated with delay until surgery was a dilatation proximal to the stricture of more than 30 mm, on MRE and/or on US (p=0.01). However this information could be collected only for 12 patients and therefore was not retained for the multivariable model. The other imaging characteristics of the stricture studied were: enhancement on T1-w, T2-w or diffusion sequences, length of stricture, comb sign, presence of a fistula. None were associated with group (surgery vs no surgery) in univariate analyses (Table 3).

Independent predictors of surgery

Using a multivariable Cox model, the variables that were associated independently with surgery were: PCDAI > 22.5 at diagnosis of stricture (OR 6.73 (95% CI: 2.27 – 19.91) p=0.0006) and anti-TNF α treatment after diagnosis of stricture (OR 0.21 (95% CI: 0.08 – 0.59) p=0.003). Study center was included in the model and did not affect final results, showing the absence of significant center effect.

Feasibility of the CREOLE criteria in routine care of pediatric stricturing small bowel disease

Based on the MRE reports that were collected, full CREOLE score could be calculated for 5 patients out of 50. Pooling the results from all imaging reports available at the time of the stricture (MRE, TDM, US), and substituting the criteria “marked enhancement on delayed T1-weighted sequence” by “delayed enhancement of the stricture after injection of contrast media on CT-scan” increased the

number of patients for whom a modified CREOLE score could be calculated to 12 patients. In these patients, the median modified CREOLE score was 4 [IQR 25-75: 3 – 4.5].

Taking into account the missing data by extrapolating the scores to number of items available for each patient, the mean CREOLE score was 3.59 in the group that did not require surgery compared to 3.58 in the surgery group. Comparing sub-groups based on the number of items available, did not yield evidence of a difference between surgery and non-surgery group. Individual detailed answers by item were not different between groups either (Figure 2).

Data at last news

Among the patients who had not been operated, 12 were aged less than 18 years at last follow-up (Median follow-up after diagnosis of stricture: 14.5 months [IQR 25-75: 9.0 – 17.3]). Nine were in clinical remission at last follow-up. Five out of 9 who had had a recent imaging did not have persistent evidence of a small bowel stricture.

Among the 9 patients who reached the age of 18 years during study period without being operated (Median follow-up after diagnosis of stricture: 34.2 months [IQR 25-75: 23.5-51.1]), 7 were in remission at last news. Two patients out of 5 who had had a recent imaging did not have persistent evidence of a small bowel stricture.

Discussion

Defining optimal care for small bowel strictures is a challenge for pediatric gastroenterologists. Although these patients have well been identified as a high-risk group requiring rapid optimization of treatment, the strategy for choice of drug, delay before optimization, criteria for surgery are made on a case-by-case basis (16, 17). Studies focusing on the particular question of risk factors for surgery in stricturing small bowel disease in pediatrics are rare (18). Based on retrospective registry data, several pediatric teams have studied the risk factors for first surgery for Crohn's disease in pediatric IBD, but the focus was on all indications for surgery and not restricted to stricturing small bowel disease (14, 15, 19). In addition, a substantial proportion of patients were diagnosed before TNF α antagonists became available in Pediatrics, which might have impacted on therapeutic strategies.

The cumulative probability of CD-related surgery in pediatric IBD has been reported between 13 and 50% at 5 years of disease, in a systematic review of population-based studies including all causes for surgery (20). In our series focusing on pediatric patients with small bowel strictures the surgery rate was much higher (58% of patients, with a delay of 2.6 months (IQR 25-75: 1.1 – 6.2) after diagnosis of stricture). Our surgery rate is similar to the pediatric series reported by Aloï *et al.* , in which 44% of children with stricturing CD required surgery (18). We deliberately chose to restrict our inclusions to the past 10 years, in order to reflect as much as possible the current treatment paradigms (17). The delay before surgery seems short, which would lead to think that once a stricture is diagnosed the outcome of medical treatment, whether “success” or “failure” requiring surgery, is rapidly determined. The delay of 6 months is in keeping with the primary outcome of the CREOLE study, which was absence of surgery within 6 months of initiating adalimumab (11). Although surgery early in the course of CD has been shown to have beneficial effects of growth and reduced immunosuppressant use in population-based registries (7), this was once again pooling all indications for surgery; the impact of delay between diagnosis of the stricture and its surgical removal has not been studied.

We found that a PCDAI score > 22,5 at diagnosis of stricture was significantly associated with surgery. A higher PCDAI might reflect increased disease activity, although some items of the PCDAI such as abdominal pain, abdominal mass, weight loss and general well-being might not discriminate between an inflammatory flare and obstructive symptoms due to a stricture. Indeed, we did not find

an association between the inflammatory markers ESR, CRP and platelets and the risk for surgery. Schaefer *et al.* and Rinawi *et al.* both found that greater disease severity at diagnosis of CD increased the risk for surgery during pediatric follow-up, but in their series surgery was performed predominantly for chronic disease refractory to medical treatment and not specifically for strictures (15, 19).

The role of anti-TNF α agents as disease-modifying treatments has been much debated. A recent review pointed out the fact that the decrease of surgery rate in adult IBD began 6 decades ago, and might be due to several other factors related to patient care and early diagnosis and not only to biologics (21). In our study, treatment with anti-TNF α agents before diagnosis of stricture did not modify the outcome (surgery vs no surgery). However receiving an anti-TNF α once the stricture had been diagnosed was a protective factor from surgery, and as a mirror image not receiving anti-TNFs after diagnosis of stricture increased the odds of surgery by almost 5-fold. Since all the strictures were diagnosed after 2010 it is unlikely that an anti-TNF α treatment was not available. Since we excluded from the analyses patients with an abscess at diagnosis of stricture, it is not likely either that the anti-TNF α treatment was contra-indicated. We suspect that patients with severe symptomatic strictures might have been treated preferably with enteral or parenteral nutrition, and that anti-TNF α treatment might have been withheld in the perspective of surgery in the short term, since it is recommended to avoid anti-TNF α drugs 4 to 6 weeks pre-operatively (16). In this case absence of anti-TNF α prescription would rather reflect the physician's appreciation of severity of disease and prediction of need for surgery on a personalized basis.

Among the imaging criteria collected routinely in our study centers, the only one associated with necessity for surgery was dilation proximal to the stricture of > 3 cm. In a pediatric study by Barkmeier *et al.* comparing MRE characteristics to histologic inflammation and fibrosis scores on surgically resected small bowel strictures, there was no correlation between length of stricture, marked enhancement on T1-weighted sequence, presence of a fistula and histologic fibrosis or inflammation (4). However a dilatation above the stricture of a diameter > 3 cm was significantly correlated with

transmural histologic fibrosis but not histologic inflammation, with OR of 43.4 (6.1->999.9) (4). In our study a proximal dilation of more than 3 cm, albeit in a subgroup of 12 patients, was also associated with surgery. Several teams have shown, on surgical specimens, that small bowel strictures combine to various degrees histologic inflammation and fibrotic tissue (3, 4). In the light of these observations, width of the pre-stenotic dilation might reflect severity of stricture and relative fibrotic load of the stenosis, which could indicate less chance of success of anti-inflammatory treatments to reverse stricture. We cannot exclude the fact that knowledge of the poor prognosis of response to medical treatment in case of a > 3 cm proximal dilation might have influenced the decision to operate the patient. However, the first pediatric guidelines on surgical management of Crohn's disease were published in 2017 and do not mention imaging characteristics of the stricture as an indication for surgery per se (16).

In another pediatric study by Rosenbaum *et al.* on patients with distal ileal CD and comparing imaging characteristics of those who were operated and those who were not, the MRE characteristics of the "surgery group" were: increased bowel wall thickness, increased mean T2 ratio, different enhancement patterns and increased mesenteric edema (3). Contrarily to our study they did not include only stricturing disease, therefore having a stricture on MRE was a significant risk factor for surgery (OR 8.2).

Several teams have studied abdominal ultrasound as an alternative to MRE for evaluating small bowel disease, as it is often used in pediatrics (22% of patients in our series had both an MRE and an ultrasound), and has been shown to have a good agreement with MRE regarding disease location and activity (22). However, its diagnostic accuracy in stricturing CD is slightly lower than MRE: sensitivity 74-100% vs 75-100%, and specificity 89-93% vs 91-100% for US and MRE respectively (23). In our series ultrasound tended to underestimate the length of strictures although bowel wall thickness was in adequacy with MRE measurements.

In order to be able to predict response to medical treatment, the CREOLE prognostic score was established in a multicenter adult study (11). We were not able to implement the CREOLE score in our pediatric population. The main reason was missing data, since information such as diameter of the dilation proximal to stricture and presence of a marked enhancement on delayed T1-weighted

sequence were missing in 80% and 32% of MRE reports respectively. Heterogeneity between reporting methodology for MRE has been noted in systematic reviews and limits comparison between studies (24). In our study, centralized reading of the MRI images was not possible, due to significant differences between imaging protocols. Developing a scoring system would be an aid for everyday decision-making and for future prospective or retrospective studies. Structured MRE reports have been developed for small bowel Crohn's disease and using them increases the report of several key factors among which description of strictures (25, 26). In pediatric IBD standardized MR enterography reports are not yet routinely used in all centers. MRE damage indexes have been created, such as the PICMI (pediatric MRE-based activity index) (27) and the pMEDIC (pediatric Crohn's disease intestinal damage score) developed by the ImageKids program. These scores have been used to assess mucosal healing *vs* endoscopy or to evaluate severity of terminal ileum disease when ileocecal valve could not be intubated (28), but have not yet been used as prognostic factors of response to treatment in strictures.

Finding biomarkers of personalized disease natural history is of great interest. Population-based registries have given us valuable information on natural history of CD in children, but the risk factors studied are generally fixed demographic or clinical factors present at diagnosis of CD and do not capture the changing course of disease in an individual. Our study, although retrospective and observational, has the advantage of studying factors at the time of diagnosis of stricture, as well as past history of disease, and their impact on outcome of the stricturing complication. For the time being, we can only focus on predicting the risk for surgery. Ideally, we would want to be able to predict the response to medical treatments, for instance anti-TNF α agents or other biotherapies such as vedolizumab or ustekinumab. For this, prospective studies will be necessary. This real life study points out that imaging results and protocols are inherently heterogeneous between study centers, and developing standardized imaging reports could be an aim of collaborative research. We acknowledge that the decision to operate might have been influenced by external factors that could not be captured by a study. The strength of this multicenter study was to reflect current care while controlling statistically for the center effect.

Conclusion

In conclusion, we have shown by focusing specifically on outcome of small bowel strictures in pediatric CD, that more than half of the patients will require surgery, and that having a high PCDAI at diagnosis of stricture was associated with increased odds of surgery while receiving anti-TNF α therapy after diagnosis of stricture was a protective factor. We were not able to include imaging characteristics of the stricture into the multivariable model, due to heterogeneity in MRE reporting in routine. For the same reason, we were not able to test feasibility and applicability to pediatrics of the CREOLE criteria. Similar to CREOLE, we found in univariate analysis that a dilation of over 30 mm proximal to stricture was associated with increased risk for surgery. Further research should aim to develop the use of standardized imaging reports in routine care, and to evaluate the role of other imaging techniques to guide medical decisions regarding small bowel strictures in pediatric CD.

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Figure legends

Figure 1. Kaplan Meier curves of time until surgical treatment of small bowel stricture. Panel A: whole study group (n=50), Panel B: by anti-TNF α treatment after diagnosis of stricture, Panel C: by PCDAI at diagnosis of stricture. The number at risk are indicated in bottom line of the figures.

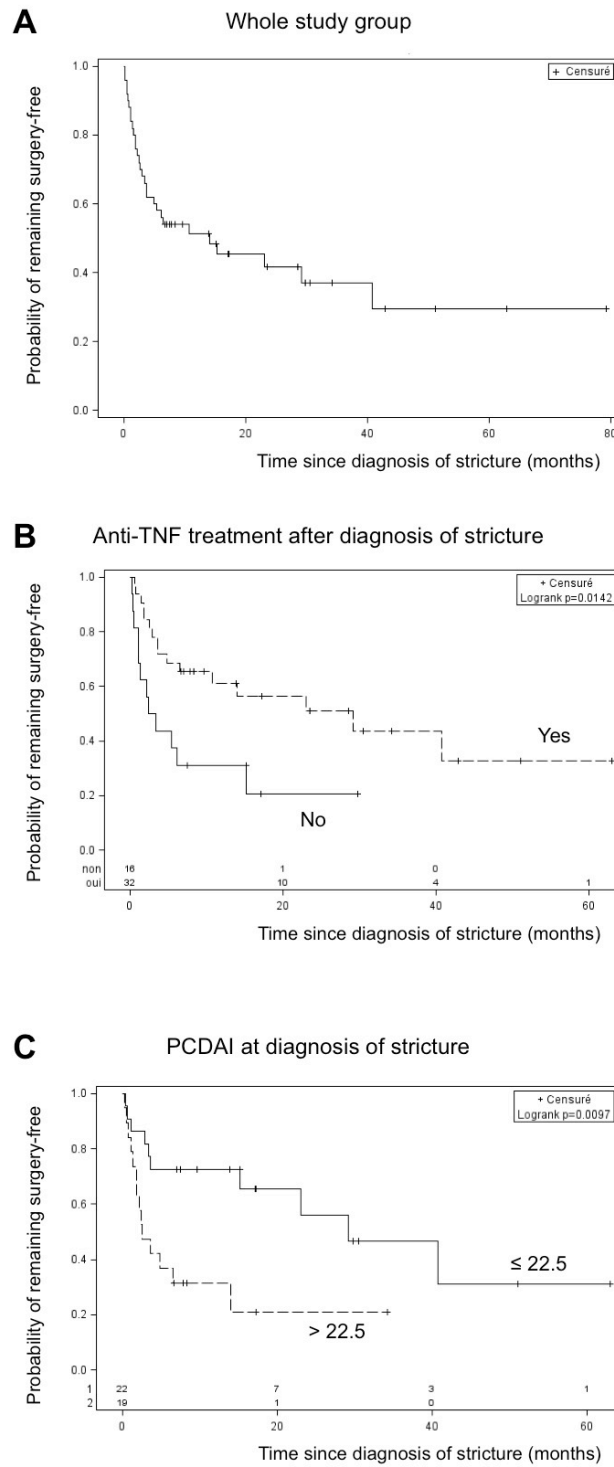
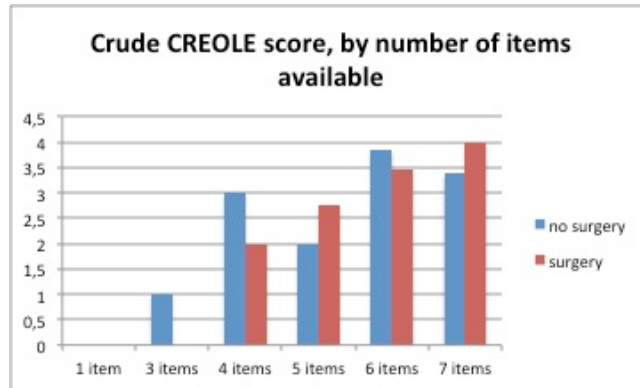
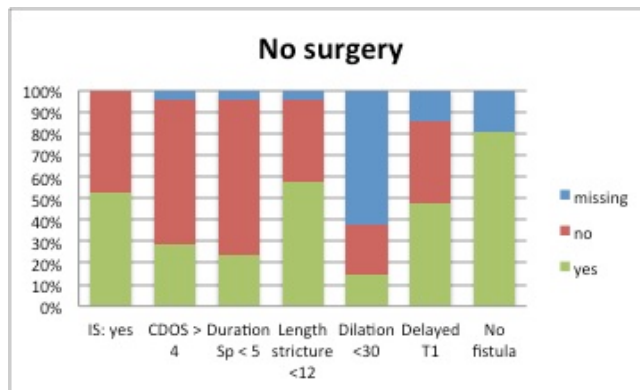


Figure 2. Comparison of CREOLE scores by group (surgery vs no surgery)

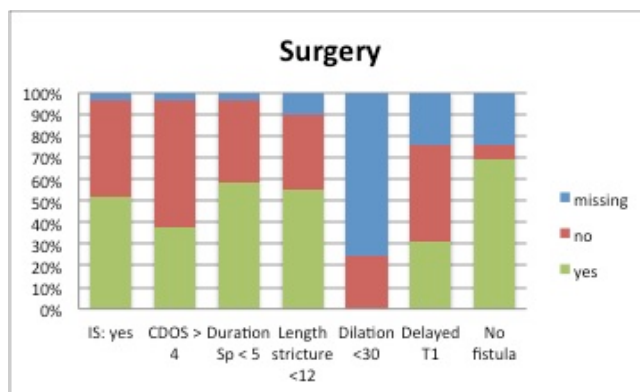
Panel A: CREOLE score depending on surgery or no surgery, by sub-group of patients having the same number of available items for calculation



Panel B: Detailed modalities of answer to each item of the CREOLE score, among patients who did not require surgery for small bowel stricture



Panel C: Detailed modalities of answer to each item of the CREOLE score, among patients who required surgery for small bowel stricture



Tables

Table 1. Comparison of the baseline characteristics of patients who required surgery following diagnosis of small bowel stricture with those who did not

Characteristic	No surgery (n=21)	Surgery (n=29)
Gender - male	16 (76%)	15 (52%)
Age at diagnosis of CD (yrs)	12.0 ± 2.9 (min-max: 6.0 – 16.6)	12.6 ± 2.7 (min-max: 5.6 – 16.7)
Weight at diagnosis of CD (kg)	34.2 ± 11.6 (min-max 16 – 56)	32.7 ± 12.5 (min-max: 15 – 65)
Height at diagnosis of CD (cm)	148.0 ± 21.3 (min-max: 116 – 185)	144.5 ± 16.1 (min-max: 118 – 171)
Paris classification at diagnosis of Crohn's disease		
A1a	7 (33%)	5 (17%)
A1b	14 (67%)	24 (83%)
L1	6 (29%)	11 (39%)
L2	2 (10%)	0
L3	12 (57%)	15 (54%)
No lower GI disease	1 (5%)	2 (7%)
L4a	5 (25%)	7 (26%)
L4b	5 (25%)	3 (11%)
No upper GI disease	10 (50%)	17 (63%)
B1	17 (81%)	18 (64%)
B2	3 (14%)	8 (29%)
B3	1 (5%)	2 (7%)
p1 (perianal disease)	2 (12%)	5 (19%)
G1 (growth delay)	6 (38%)	7 (28%)
ASCA / positive	5 (71%)*	9 (90%)*
ANCA / positive	3 (50%)*	0***
Granulomas / present	10 (63%)**	8 (31%)
Treatments received for CD, before diagnosis of small bowel stricture		
None	2 (10%)	2 (7%)
Aminosalicylates	6 (30%)	5 (18%)
Antibiotics	3 (15%)	7 (25%)
Corticosteroids or budesonide	13 (62%)	17 (61%)
Immunomodulators (azathioprine or methotrexate)	11 (52%)	15 (54%)
Anti-TNFα agent	8 (38%)	16 (57%)
IM and anti-TNFα	4 (19%)	8 (28%)
Other class of biologics	0	2 §
MODULEN IBD®	14 (70%)	17 (61%)
Enteral nutrition, other	0	1 (4%)
Parenteral nutrition	0	1 (4%)

* 14 missing data, ** 5 missing data, *** 19 missing data

§: ciclosporine (n=1), ustekinumab (n=1)

Table 2. Patient characteristics at diagnosis of small bowel stricture

Characteristic	No surgery (n=21)	Surgery (n=29)
Age at diagnosis of stricture (yrs)	14.6 ± 2.3 (min-max 7.8 – 18.1)	14.4 ± 2.0 (min-max 8.5 – 17.3)
Time since diagnosis of CD (months)	median 19.7 [IQR 25-75: 2.0 – 55.5]	median 7.6 [IQR 25-75: 2.3 – 23.6]
Weight at diagnosis of stricture (kg)	45.5 ± 11.0 (min-max 22.0 – 73.8)	39.7 ± 12.8 (min-max 22 – 69)
Height at diagnosis of stricture (cm)	160.8 ± 15.1 (min-max 124.5 – 185.0)	152.2 ± 14.1 (min-max 117.6 – 177.6)
PCDAI at diagnosis of stricture	17.5 [IQR 25-75: 15.0 – 27.5]	25 [IQR 25-75: 20.0-31.2]
CD activity at diagnosis of stricture (PGA)		
Active - severe	0	3 (11%)
Active - moderate	4 (20%)	6 (22%)
Active - mild	12 (60%)	14 (52%)
Remission	4 (20%)	4 (15%)
Duration of obstructive symptoms		
< 5 weeks	5 (25%)	17 (61%)
≥ 5 weeks	9 (45%)	8 (29%)
No obstructive symptoms	7 (30%)	4 (10%)
CDOS	2.9 ± 2.3 (min-max 0-6)	3.4 ± 2.3 (min-max 0 – 6)
Most recent laboratory results at time of stricture		
Leucocytes (G/L)	10.0 ± 3.8	8.0 ± 2.8
Hemoglobin (g/dL)	12.1 ± 1.9	12.0 ± 1.4
Hematocrit (%)	37.4 ± 5.9	36.0 ± 4.3
MGV (fL)	74.9 ± 7.7	77.4 ± 7.0
Platelets (G/L)	408 ± 114	423 ± 132
CRP (mg/L)	15.4 [IQR 25-75: 2.6 – 34.5]	20.5 [IQR 25-75: 7.4 – 42.0]
ESR (mm)	15 [IQR 25-75: 9.8 – 41.0] (10 missing)	20 [IQR 25-75: 11 – 45] (6 missing)
Albumin (g/L)	35.7 ± 4.8	37.7 ± 6.1
Fecal calprotectin (mg/g)	800 [IQR 25-75: 573-800])(n=7)	673 [IQR 25-75: 75 – 800] (n=6)

Table 3. Imaging results at the time of diagnosis of stricture, comparing patients who required surgery subsequently and those who did not. Data presented as median [IQR 25-75] or n (%).

	MR-enterography (n=41)			Small bowel ultrasound (n=18)			CT scan enterography (n=5)	
	No surgery (n=16)	Surgery (n=25)	p	No surgery (n=9)	Surgery (n=9)	p	No surgery (n=2)	Surgery (n=3)
Number of strictures	1 [1-2]	1 [1-1]	NS	1.5 [1.0-2.5]	1 [1-1]	NS	1	1
Length of stricture (cm)	6.0 [4.0 – 15.0] 1 missing	9.8 [5.0 – 15.0] 5 missing	NS	5.0 [1.3 – 10.0]	10.0 [5.0 – 12.0] 4 missing	NS	5 (n=1)	10 (n=1)
Maximum wall thickness (mm)	8.0 [5.3 – 9.0] 7 missing	8.0 [6.0 – 8.0] 14 missing	NS	5 [5-5] 4 missing	7 [6-7] 4 missing	NS	2 missing	10 [9-12]
Presence of a proximal dilatation	12 (80%)	19 (95%)	NS	7 (100%)	3 (50%) 3 missing	0.07	2 (100%)	3 (100%)
Diameter of dilation proximal to stricture (mm)	24.5 [22 – 32.5] 12 missing	47.5 [25.1 – 51.5] 21 missing	NS	4.9 [3.9-5.5] 5 missing	9 missing		6 (n=1)	4.6 [2.5 – 4.7]
Hypersignal on T2-weighted sequences	3 (50%) 10 missing	7 (58%) 13 missing	NS					
Hypersignal on diffusion sequences with restriction of ADC	5 (83%) 10 missing	8 (89%) 16 missing	NS					
Time of enhancement on T1w: delayed phase	8 (89%) 7 missing	11 (100%) 14 missing	NS					
Degree of enhancement on T1w or inflammatory aspect on US or CT-scan	None: 2 (22%) Moderate: 5 (56%) Marked: 2 (22%) Missing: 7	None: 2 (11%) Moderate: 10 (53%) Marked: 7 (37%) Missing: 6	NS	6 (67%)	5 (83%)	NS	2 (100%)	2 + 1 missing
Comb sign	9 (90%) 6 missing	11 (73%) 10 missing	NS					
Fistula	0	0	NS	0	1 (13%)	NS		

Table 4. Patient outcome after diagnosis of small bowel stricture. Treatments received include new treatments and treatments continued from before diagnosis of stricture

Treatments received after diagnosis of stricture	No surgery (n=21)	Surgery (n=29)
Corticosteroids (oral or budesonide)	13 (65%)	9 (32%)
Immunomodulators (azathioprine or methotrexate)	15 (71%)	14 (50%)
Anti-TNF α agent	16 (80%)	16 (57%)
Both IM and anti-TNF α		
Other biologic	vedolizumab: 1	vedolizumab: 1, ustekinumab: 3
MODULEN IBD [®]	3 (15%)	11 (39%)
Other enteral nutrition	0	1 (4%)
Indication for surgery:		
Acute		11 (38%)
Refractory stricture		18 (62%)
Type of surgery:		
Resection-anastomosis		27 (93%)
Ileostomy		2 (7%)
Delay between diagnosis of stricture and small bowel surgery (months)		2.6 [IQR 25-75: 1.1 – 6.2]
Delay between diagnosis of CD and small bowel surgery (months)		24.5 [IQR 25-75: 9.1-42.1]

Supplementary Table 1. Kaplan Meier analyses of time until surgical resection among 50 pediatric patients with stricturing small bowel Crohn's disease, and multivariable Cox model

Variable	Stratas of the variable	Log-rank p	Multivariable Cox model HR (95%CI)
Gender	Male / Female	0.28	
Paris classification at diagnosis of Crohn's disease			
	Age (A1a, A1b)	0.29	
	Lower location (L4a, L4b)	0.73	
	Upper location (L1, L2, L3)	0.85	
	Behavior (B1, B2, B3)	0.35	
	Perianal modifier (p)	0.71	
	Growth delay (G0, G1)	0.61	
Treatments received for CD, before diagnosis of small bowel stricture			
	None	0.90	
	Aminosalicylates	0.28	
	Antibiotics	0.10	NS
	Corticosteroids	0.81	
	Immunomodulators	0.96	
	Anti-TNF α agent	0.03	NS
	Both IM and anti-TNF	0.20	
	MODULEN IBD [®]	0.41	
Characteristics at the time of diagnosis of the stenosis			
Age	0-5, 6-12, 13-17 years	0.99	
Time since diagnosis of CD	Continuous (months)	0.21	
Weight	<-2SD, -2 to 2SD, >2SD	0.06	NS
Height	<-2SD, -2 to 2SD, >2SD	0.33	
PCDAI	≤ 22.5 , > 22.5	0.01	6.73 [95%CI: 2.27-19.91], p=0.0006
CDOS	≤ 3 , > 3	0.05	NS
Most recent laboratory results at time of stricture			
	Leucocytes (G/L) ≤ 8.55 / > 8.55	0.05	NS
	Hemoglobin (g/dL)	0.70	
	Hematocrit (age and sex adjusted cutoffs from PCDAI)	0.72	
	MGV (fL)	0.64	
	Platelets (≤ 450 , > 450 G/L)	0.70	
	CRP (≤ 10 , > 10 mg/L)	0.58	
	ESR (< 20 , $20-50$, > 50 mm)	0.60	
	Albumin (≤ 30 , $31-34$, ≥ 35 g/L)	0.36	

Suppl Table 1 (continued)

Variable	Stratas of the variable	Log-rank p	Multivariable Cox model HR (95%CI)
MRE characteristics (n=41)			
	Length of stricture	0.66	
	Stricture wall thickness	0.41	
	Proximal dilatation	0.24	
	Diameter of dilation	0.19	
	Ileo-coecal valve stenosis (Y/N)	0.83	
	T2-w enhancement (Y/N)	0.49 (n=19)	
	T1w gadolinium enhancement (Y/N)	0.83 (n=28)	
Composite imaging data (merged from MRE and US)			
	Dilation proximal to stricture > 30 mm (Y/N)	0.01 (n=12)	Not included
Treatments received after the diagnosis of small bowel stricture			
	Corticosteroids	0.03	NS
	Immunomodulators	0.02	NS
	Anti-TNF α	0.01	0.21 [95%CI: 0.08- 0.59] p=0.003
	IM and anti-TNF α	0.12	
	MODULEN IBD [®]	0.21	

NS: not significant

Article 3 : Modèle de colite aigue chez le rat pré-pubère

**MR-colonography assessment of Acute Trinitrobenzene Sulfonic Acid Colitis
in pre-pubertal Rats, and effect of a Polymeric Diet enriched in TGF-beta 2**

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All authors drafted the work or revised it critically for important intellectual content.

All authors approved the final version to be published.

All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

ABSTRACT

Objectives: 1/ To validate a model of acute experimental colitis in pre-pubertal rats and determine whether magnetic resonance colonography characteristics could be correlated to histologic inflammation. 2/ To test this model by treating the rats with MODULEN-IBD® to reverse inflammation.

Methods: 24 rats were randomized at weaning to one of 3 groups: TNBS group (n=8) fed a standard diet, who received one intra-rectal 60 mg/kg dose of TNBS-ethanol; Control group (n=8) fed standard diet and received one dose of intra-rectal PBS; TNBS+MODULEN group (n=8), received one dose of TNBS and were exclusively fed MODULEN IBD® after induction of colitis. All rats were assessed by MR-colonography one week after induction of colitis. Colons were analysed by histology and measures of inflammation markers (IL-1 β , TNF- α , NOS2 and COX-2).

Results: Intra-rectal instillation of TNBS induced acute colitis in pre-pubertal rats compared to controls, with typical features of acute colitis observed on MR-colonography (increased colon wall thickness, increased colon intensity on T2-weighted images, target sign, ulcers) confirmed on histology. Treatment with MODULEN IBD® significantly reduced colon weight/length ratio. MODULEN IBD® did not ameliorate the MRI signs of colitis. We failed to show a difference in inflammatory marker expression among study groups.

Conclusion: MR-colonography correlates with acute inflammation. Treatment with MODULEN IBD® reduced some signs of inflammation. There was a mild signal of fibrosis in the TNBS group that suggests that the fibrotic process might initiate very early, which requires further validation.

Key words: magnetic resonance imaging, inflammatory bowel disease, growth and development.

What is known

- TNBS-induced colitis is a widely used animal model mimicking features of IBD, with transmural inflammation and which elicits a predominantly Th1 immune response at the acute phase of inflammation.
- There is no standardized protocol for inducing TNBS colitis in rats, and pre-pubertal models that could take into account specificities such as growth and susceptibility due to young age are lacking.
- MR-colonography has been validated by our team to assess signs of bowel wall damage following TNBS administration in adult rats, correlated to inflammatory signs on histology and to an increase of pro-inflammatory cytokines and markers.
- Modulen IBD[®], a polymeric diet enriched in TGF-beta 2, is recommended for induction of remission in paediatric IBD. When administered as sole source of nutrition for 8 weeks, it has been shown to induce clinical remission in 79% of children and mucosal healing in 31%.

What is new

- MR-colonography is feasible in pre-pubertal rats and shows images of colitis similar to those of adult rats, correlated to histology.
- Feeding young rats exclusively with MODULEN IBD[®] during the acute inflammatory stage reduced several signs of inflammation.
- There were mild signs of fibrosis in the TNBS group, which suggests that the fibrotic process might initiate as early as the first inflammatory hit.

INTRODUCTION

Crohn's disease beginning in childhood has several specificities compared to adult-onset disease: a higher incidence of complicated (fistulizing, stenosing) phenotypes, more growth failure at diagnosis and restricted adult height in some cases (1-3).

Animal models of intestinal inflammation mimicking IBD are widely used to better understand the different cellular and molecular pathways of inflammation and fibrosis, and to target these pathways to develop new drugs. Different types of IBD models exist, which can be grouped into 4 categories: chemically induced, biologically induced, genetic and cell transfer models (4). Among the chemically induced models, the 2,4,6 Trinitrobenzene Sulfonic Acid Colitis (TNBS), first described by Morris in 1989 is frequently used and many variations exist (5). Briefly, TNBS is instilled intra-rectally mixed with ethanol. The ethanol acts as a mucosal barrier breaker allowing the hapten TNBS to interact with colonic proteins and elicit an immunological response by rendering these proteins immunogenic to the host immune system. The resulting colonic inflammation resembles Crohn's disease, with transmural inflammation, ulcers and granulomas. Most studies on acute TNBS colitis in rats have been carried out on adult animals. However weanling and pre-pubertal rats have several distinct features that require adapting the TNBS model, such as rapid growth and susceptibility to growth failure, increased mucosal permeability and increased susceptibility to drugs. For example, in models of DSS colitis in pre-pubertal animals, symptoms appeared earlier than in adults and were more severe for the same dose of DSS (6, 7).

In order to follow non-invasively the effect of different interventions and reduce the number of animals needed for longitudinal studies, small animal imaging techniques have been developed. We previously used a model of acute TNBS colitis in adult rats and showed that magnetic resonance colonography (MRC) could accurately evaluate inflammation, compared to histology and colonic cytokine expression (8).

In children, exclusive enteral nutrition with a polymeric diet has shown equal efficacy in inducing remission as corticosteroids, without the side effects. In this setting, the ECCO/ESPGHAN guidelines recommend exclusive enteral nutrition as first-choice treatment for inducing remission in children,

over corticosteroids (9). Several enteral diets have been compared: elemental diet, semi-elemental diets, polymeric diet and a polymeric diet enriched in TGF-beta 2, MODULEN IBD[®] (Nestle, Vevey, Switzerland). Administered as sole food source for 8 weeks in children, MODULEN IBD[®] has been shown to induce clinical remission in 79% children and mucosal healing in 31% (10).

The objectives of our study were: 1/ to adapt a model of acute experimental colitis to pre-pubertal rats and determine whether MR-colonography (MRC) characteristics could be correlated to histology; 2/ To test this model by treating the rats with MODULEN IBD[®] to reverse inflammation.

METHODS

Ethics

Animal care and experimentation complied with both French and European Community regulations (Directive 2010/63/UE). Study protocol was approved by the Institutional Care and Use Committee (Comité d’Ethique Normande en Matière d’Expérimentation Animale, CENOMEXA). Rachel Marion-Letellier is authorized by the French Government to use the present rat model (Authorization no. 76-106). Painful procedures were carried out under general anaesthesia and all efforts were made to minimize suffering.

Study design

Twenty-four Sprague Dawley male rats were purchased at weaning (post natal day 21) from Janvier labs (Le Genest-Saint-Isle, France). They were randomly allocated to one of three study groups: Control (n=8), TNBS (n=8) and TNBS + MODULEN (n=8) (Figure 1, Panel A). The rats were housed 4 per standard cage to provide for their interaction needs, were exposed to light / dark cycles of 12 hours each and provided with water *ad libitum*. After induction of colitis, the TNBS + MODULEN group received MODULEN IBD[®] powder as sole food source. The other groups received a standard rat breeding diet (A03, SAFE) in a powdered form (SDC Table 1: Composition of A03 vs MODULEN IBD[®]).

Induction of colitis

After one week of acclimation, colitis was induced by intra-rectal instillation of 2,4,6-trinitrobenzene sulfonic acid (TNBS, Sigma-Aldrich, Saint-Quentin-Fallavier, France). After fasting for 24 hours, rats were anesthetized by intra-peritoneal injection of ketamine 8 mg/kg (Panpharma) and Chlorpromazine 1 mg/kg (Sanofi-Aventis). A polyurethane cannula was inserted 3 cm into the rectum and TNBS – ethanol injected, at a dose of 60 mg/kg of body weight. TNBS was mixed with a 50% Ethanol vehicle, for a total volume of 250 µL. The rats were maintained in a head-down position for 5 minutes after the injection to minimize leaks. Rats in the control group received an intra-rectal instillation of 250

μ L of phosphate-buffered saline (PBS). During the week following induction of colitis, the rats were observed for signs of pain or significant weight loss.

Magnetic resonance colonography

A magnetic resonance colonography was performed within the week following TNBS or PBS administration (between Day 4 and Day 7). Rats were anesthetized by intra-peritoneal injection of thiopental (Panpharma) at a dose of 90 mg/kg. Cardiac rhythm was monitored during MRI by surface electrodes. Rats were installed in the cradle in a supine position. Hypothermia was prevented by a tube of circulating warm water around the rat during MRI and a warming plate during recovery phase. MRI imaging was performed with a small animal machine: Bruker BioSpec 47 / 40 USR, 4.7 Tesla (Bruker Biospin, Ettlingen, Germany). There was no injection of antispasmodic or contrast agent. Respiratory movements were corrected using the Intradate™ technique. The parameters for the T2 RARE (rapid acquisition with relaxation enhancement) sequence were: TR 5443 ms, TE 34 ms, matrix 320x224, slice 1 mm, NEX 3, flip angle 180°, field of view (FOV) 5,0 x 5,3 cm, NEX 4, acquisition time 10 minutes, RARE factor 8. Parameters for T2 RARE with fat suppression (FAT-SAT): TE 39 ms, TR 6027 ms, RARE factor 8, NEX 3, acquisition time 8 minutes, flip angle 180°, slice 1 mm, FOV 5,0 x 5,3 cm, matrix 320x224. T1 sequence with intragate FLASH (fast low angle shot); TR 413 ms, TE 2,8 ms, matrix 256x256, slice 1,1 mm, flip angle 80°, FOV 4,5 x 4,5 cm; acquisition time: 14 minutes. MRI images were analysed in DICOM, using the ParaVision 5.0 software. A senior radiologist (C. S.-C.) who was blind to the allocation group interpreted all images.

Image quality taking into account respiratory and bowel wall movements was assessed on a scale of 0 (poor quality) to 3 (excellent quality). Measures were made in the descending colon.

In order to assess inflammation, the criteria used, similarly to our previous work (8) were: maximal colon wall thickness (average of 3 measures), minimal colon wall thickness (average of 3 measures), colon wall thickness at splenic angle (average of 3 measures), colon wall signal intensity in the Regions of Interest (ROI) on T2w sequences (average of 2 measures), target sign (present or absent), colon mucosa irregularities suggesting ulcerations, spontaneous colon wall T1w enhancement.

The imaging criteria suggestive of fibrosis were: narrowing of the colon lumen ((maximal diameter - minimal diameter) x 100 / maximal diameter), stenosis defined as a narrowing to colon lumen associated with proximal colon dilation, tubular aspect defined as a loss of haustrations with a tubular aspect of the lumen, detachment of the mucosa defined as the presence of a mucosal flap in the lumen.

Killing and samples

On Day 8 after colitis induction, rats were killed by a lethal dose of intraperitoneal thiopental and then decapitated. Tail was measured as a proxy for growth. Colon was sampled, washed with PBS to remove faeces, measured and weighed. Six one-centimetre samples were taken from the colon, starting from the rectum, one of which was fixed in 10% neutral buffered formalin (Sigma-Aldrich) for histology, the others stored at – 80°C for Western-Blot and PCR.

Histology

Histologic analyses were made by a senior pathologist (M.A.), blinded to allocation group, on a sample of distal colon taken 4 cm from the anus. The formalin-fixed samples were embedded in paraffin, and 5-micrometer sections were coloured with hematoxylin/eosin (H&E; Merck, Darmstadt, Germany) for standard histology. Samples were studied on 3 levels of cut. Inflammation was scored using a semi-quantitative score previously used by our team: from 0 (no inflammation) to 3 (severe inflammation) (8, 11). Fibrosis was scored from 0 (no fibrosis) to 3 (severe fibrosis). Images were taken by standard light microscopy using a Leica microscope.

Colon expression of cyclooxygenase-2 (COX-2) by Western Blot

Frozen colon samples were homogenized in PBS with 0.1% protease inhibitor cocktail (Sigma) and phosphatase inhibitor cocktail (Sigma). Homogenates were centrifuged (12 000g, 15 min, 4°C) and supernatants were collected and stored at -80°C. Protein concentration was determined following Bradford's colorimetric method and Western blot for COX-2 was performed as previously (12). Protein expression was quantified by densitometry with the ImagQuantTL software (GE Healthcare,

USA). To check equal loading, the blots were analysed for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression.

mRNA levels for Interleukin-1 β (IL-1 β), TNF- α cytokines and inflammatory marker NOS2

RT-qPCR was performed as previously (13). Briefly, colon samples were frozen in liquid nitrogen and stored at -80°C before RNA preparation. Total RNA was isolated using guanidium isothiocyanate method and reverse transcribed into cDNA. PCR was performed with CFX96 Real-Time System (Bio-Rad, Marnes-la-Coquette, France).

Data analyses

Characteristics of the rats were compared between TNBS and Control group, MODULEN and TNBS group and MODULEN and Control group. Since the group sizes were < 30, non-parametric methods were used for analyses: qualitative variables were compared using Fisher's exact test, quantitative variables were compared using Wilcoxon Mann-Whitney with exact correction for small samples. Missing data (following premature death of an animal) was excluded from analyses.

Statistical analyses were performed using SAS 9.2 (Cary, NC, USA) and Graph Pad Prism 5 (San Diego, CA, USA). A two-sided p-value < 0.05 was considered significant for all analyses.

RESULTS

Validation of the acute colitis model

Growth and histology

Body weight growth curve during study protocol and tail length at the end of the protocol did not differ between TNBS and Control groups (Figure 1, Panel B, C). Mortality rate was 25% (2/8) in the control group, and 38% (3/8) in the TNBS group.

The histologic inflammation score was significantly higher in the TNBS group compared to the control group ($p=0.015$) (Figure 2, Panel D). TNBS group had a higher fibrosis score but did not reach statistical significance ($p=0.06$) (Figure 2, Panel E). Colon weight/length, a marker of inflammation, did not differ between TNBS group and Control group ($p=0.08$) (Figure 2, Panel F), although colon weight was significantly increased in the TNBS group compared to the Control group (2.3 ± 1.1 g vs 1.4 ± 0.3 g, $p=0.009$).

Magnetic resonance colonography

Bowel wall thickness measured in descending colon on axial plane, T2 sequence, was significantly increased in the TNBS group compared to controls (Table 1 and Figure 3, Panel A). MRI signs suggestive of inflammation, such as a target sign, wall ulcers, and increased wall signal intensity on T2w images were significantly more prevalent in the TNBS group than in the control group (Table 1). There was no significant increase of spontaneous T1w enhancement, luminal stenosis or mucosal flap. Image quality was good, except for 1 rat from the TNBS group that had wall artefacts.

Inflammatory markers and cytokine expression

The mRNA levels of 3 inflammatory marker genes (IL1 β , TNF α and NOS2) were not significantly different between TNBS and control groups (SDC Figure 1). Colon expression of COX-2 was not significantly different between groups (SDC Figure 1).

Effect of MODULEN IBD[®] in an acute TNBS colitis model

Growth

At the end of the study, rats in the TNBS+MODULEN group had a significantly lower weight than the rats in the TNBS group (154.6 ± 12.9 g vs 183.8 ± 14.6 , $p=0.004$) and the rats in the control group (mean weight 193.6 ± 11.9 g, $p=0.0007$) (Figure 1, Panel B). Tail length, a marker of growth, did not differ significantly between groups (Figure 1, Panel C).

Histology

The histologic inflammation score and fibrosis score did not differ significantly between TNBS+MODULEN and TNBS group. However, inflammation score in the TNBS+MODULEN group was not different from Control group (Figure 2, Panel D). In addition, colon weight/length ratio, a marker of inflammation, was significantly lower in the TNBS+MODULEN group than in the TNBS group ($p=0.03$) (Figure 2, Panel F).

Magnetic resonance colonography

There was no significant difference in MRI characteristics between the TNBS and TNBS+MODULEN groups (Table 1). Rats from the TNBS+MODULEN group differed from controls on the following characteristics: increased wall thickness, increased wall intensity on T2w images, presence of ulcers (100% of rats) and luminal stenosis (100% of rats). A mucosal flap was observed in 33% of rats from the TNBS+MODULEN group, but not in the other groups. Image quality was good, except for one rat that had wall artefacts and one that had respiratory artefacts.

Inflammatory marker and cytokine expression

The colon mRNA expression of IL-1 β , TNF- α and NOS2 was not significantly different between TNBS+MODULEN and TNBS or control groups (SDC Figure 1). Colon expression of COX-2 was not significantly different between groups.

DISCUSSION

In this study we have shown that a single intra-rectal instillation of TNBS was able to induce acute colitis in pre-pubertal rats, and that typical features of acute colitis could be observed on MR-colonography and confirmed by histology. We failed to show a difference in inflammatory markers among study groups.

The TNBS-hapten model of colitis first described by Morris *et al.* (5) has been shown to mimic IBD by eliciting a Th1 T-cell based response. Clinical features observed are diarrhoea, weight loss, histologic inflammation and ulcers. Weight loss reaches a peak 3 days after induction of colitis but can be recovered by Day 7 (14), which could explain why we did not observe different final weights between TNBS and control groups.

There is no standardized protocol for inducing TNBS colitis in rats, and various doses of TNBS have been used, putting into balance the severity of colitis induced with the mortality rate (15). Indeed, low doses of 50 mg/kg cause mild colitis (Wallace score of 2), whereas high doses of up to 150 mg/kg cause severe colitis (minimal Wallace score of 5) but are associated with a mortality rate of 30% caused by excessive inflammation. The only pre-pubertal rat models reported to date used a fixed dose of 8 mg of TNBS (between 80 and 133 mg/kg depending on the animal's weight) (16, 17). In our experimental protocol we chose an individually weight-adapted dose of 60 mg/kg, to take into account the increased susceptibility caused by young age of the rats (6). Using this dose, we induced mild to severe histologic colitis in 4/5 rats from the TNBS group. Despite the low dose chosen, our team's expertise using this model with older animals and precautions manipulations, there was significant mortality in all 3 groups (between 2 and 3 rats per group) which underlines the challenge of developing a reproducible model of acute bowel inflammation in pre-pubertal rats with the least invasive procedures possible.

MRI is an important imaging technique to follow progression of inflammation and tissue damage in IBD. Compared to endoscopy, it has several advantages: it may be performed without sedation, does not expose to X-ray radiation, and may show transmural inflammation, abscesses or fistulas. There has been concern about long-term brain toxicity in children (9) of contrast agents used during MRI, such as gadolinium, for which reason efforts are made to develop contrast-free MRI techniques (18).

Our team showed the feasibility of MR-colonography to assess acute TNBS colitis in adult rats (8). Similarly to our previous study, we obtained good quality images, without requiring anti-spasmodic or contrast agents, and observed typical signs suggestive of colon inflammation in the TNBS-treated rats, but not in the controls: ulcers, target sign and bowel wall thickening.

We were not able to replicate in this pre-pubertal study our previous findings of increased colon production of IL-1 β and COX-2 following TNBS administration. A possible explanation for this discrepancy was that we chose to wait one week after induction of colitis instead of 2 days, hoping to observe the maximum severity of lesions before recovery of the lesions. However, this delay might explain why the pro-inflammatory markers had normalized, contemporary to recovery process, although the histology might take longer to.

Along with the absence of inflammatory markers, we were surprised by the signal of fibrosis we observed on histology, since rats in the TNBS had a higher although not significant histologic fibrosis score than controls. Intestinal fibrosis is a dynamic process, which is thought to arise as a consequence of chronic uncontrolled inflammation. Fibrosis in TNBS models has been observed after 4 to 8 doses of TNBS (17, 19). Presence of fibrosis as soon as 1 week after the first dose of TNBS suggested that tissue repair pathways might be activated very early in inflammation or might be an independent phenomenon. Brenna *et al.* showed temporal changes in gene expression in acute TNBS colitis between 0 and 12 days after induction of colitis, with a decrease in regulation of metabolism and an increase of the regulation of tissue remodelling genes (14). They conclude that mucosal cells might be exposed to pro-fibrotic signalling cascades during inflammatory response.

In the second part of the study, several anti-inflammatory effects were observed in the young rats treated with MODULEN IBD[®]. The anti-inflammatory effect of MODULEN IBD[®] has been shown in children based on clinical, biological and endoscopic endpoints (20-23). In our pre-pubertal rat model we showed that the TNBS rats treated with MODULEN IBD[®] did not have significantly more inflammation on histology than controls even though they did not differ significantly from the TNBS group either. In addition, the reduced colon weight/length ratio in the TNBS+MODULEN group is in favour of an anti-inflammatory effect, even if we lacked power to replicate the results on the

biological level. It must be noted that reduced weight/length ratio cannot be attributed to growth failure, since colon lengths were not different between groups nor tail lengths. However, final weight was lower in the TNBS + MODULEN group than in the two others, indicating that the intake of MODULEN IBD[®] might have been insufficient to meet the energy requirements and have caused caloric deprivation. The macronutrient composition of MODULEN IBD[®] has several differences with a standard growing rodent diet (SDC Fig 1). The energy content of MODULEN IBD[®] is 5000 kcal / kg of powder, of which 42% is supplied by lipids, 44% by carbohydrates and 14% by protein. In contrast, the standard A03 “rodent diet for growing and breeding” (SAFE) nutritional composition is: 13.5% lipids, 61.3% carbohydrates, 25.2% protein with a total of 3395 kcal / kg in powder form. Given as a sole source of nutrition, and ad libitum instead of by gavage, the effect might have been hampered by low palatability. In order to control for feeding difficulties due to the powdered form of the feed, we fed the control and TNBS groups the standard rodent diet in a powdered form and not as pellets.

Treatment with MODULEN IBD[®] did not reverse the intestinal damage seen on MR-colonography. This might have been affected by the timing of MRI. Rimola *et al.* showed in adult Crohn’s disease patients persistent damage on MR-enterography despite endoscopic remission after one year of anti-TNF α or stem cell transplantation treatment (24). The MRE abnormalities that persisted were: mural enhancement, mural thickness and strictures. One might argue that the persistence of strictures reflects fibrotic sequelae of inflammation. In our study, stenosis was present in the TNBS group and in the TNBS+MODULEN group. In this acute setting, the inflammatory process per se could have caused bowel wall thickening and stenosis, although there was a non-significant increase in the histologic fibrosis score in the TNBS group but not the TNBS+MODULEN group.

In conclusion, we have shown that a single intra-rectal instillation of TNBS in pre-pubertal rats causes colonic inflammation that can be observed on histology and non-contrast MR-colonography. Treatment with MODULEN IBD[®] showed a mild anti-inflammatory effect on histology, but not on MRI. Optimal timing of MRI remains to be determined before implementing MRI as a routine non-invasive assessment tool for determining efficacy of therapeutic interventions in TNBS colitis.

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FIGURE LEGENDS

Figure 1. Experimental design for induction of acute colitis in pre-pubertal rats, and treatment with MODULEN IBD[®].

A. Experimental design. B. Body weight by group, in the days following randomization (** P<0.01 compared to the 2 other groups). C. Tail length at the end of the study, by group (The groups did not differ statistically). CTL: controls, TNBS: 2,4,6-trinitrobenzene sulfonic acid colitis group, MOD: TNBS + MODULEN group.

Figure 2. Validation of the acute colitis model: histology, hematoxylin-eosin stain.

A. H&E histology from the control group showing normal histology. B. Representative histology from the TNBS group. The arrow indicates severe transmural inflammation. C. Representative histology from the TNBS + MODULEN. The arrow indicates an ulceration. D. Histology inflammation score by group. E. Histology fibrosis score by group. F. Colon weight/length ratio by group. CTL: controls, TNBS: 2,4,6-trinitrobenzene sulfonic acid colitis group, MOD: TNBS + MODULEN group. * P < 0.05.

Figure 3. Validation of acute colitis model: MR-colonography

A. Colon wall thickening: maximum thickness, by group (** P < 0.01). B. MRC representative image from the TNBS group. The arrow indicates an irregular colon wall suggestive of an ulcer. C. MRC representative image from the TNBS group: the arrow indicates increased wall signal intensity on T2-weighted sequence. D. MRC representative image from the TNBS group: the arrow indicates narrowing of the colon lumen suggestive of a stenosis and the star a pre-stenotic dilation. MRC: magnetic resonance colonography. CTL: controls, TNBS: 2,4,6-trinitrobenzene sulfonic acid colitis group, MOD: TNBS + MODULEN group.

SUPPLEMENTAL DIGITAL CONTENT:

SDC Figure 1. Expression of inflammatory markers in colonic tissue homogenates, TNBS-induced acute colitis model in pre-pubertal rats. Gene expression measured by RT-PCR: IL-1 β (Panel A), NOS2 (Panel B) and TNF- α (Panel C); Expression of COX-2 measured by Western Blot (Panel D). None of the groups were statistically different. CTL: controls, TNBS: 2,4,6-trinitrobenzene sulfonic acid colitis group, MOD: TNBS + MODULEN group.

SDC Table 1. Nutritional composition of MODULEN IBD[®] (Nestlé Health Science) and AO3 (SAFE diets).

Table 1. Magnetic resonance colonography – TNBS induced acute colitis model

	CTL (n=8)	TNBS (n=5)	MOD (n=6)	TNBS/ CTL (p)	MOD/ TNBS (p)	MOD/ CTL (p)
Colon wall thickness (axial T2):						
Maximum thickness (mm)	0.36 ±0.04	1.03 ±0.36	1.26 ±0.48	0.002	NS	<0.001
Minimal thickness (mm)	0.36 ±0.05	0.63 ±0.14	0.69 ±0.19	0.002	NS	<0.001
Thickness at kidney hilum level (mm)	0.42 ±0.06	0.72 ±0.14	0.53 ±0.22	0.002	NS	<i>0.06</i>
Increased wall signal intensity on T2w (n, %)	0 (0)	4 (0.80)	5 (0.83)	0.007	NS	0.003
Target sign - yes (n, %)	0 (0)	4 (0.80)	3 (0.50)	0.007	NS	<i>0.055</i>
Spontaneous T1w hypersignal intensity (n, %)	0 (0)	2 (0.40)	1 (0.17)	NS	NS	NS
Irregular patterns of mucosal wall (ulcers) (n, %)	0 (0)	5 (1.0)	6 (1.0)	<0.001	-	<0.001
Stenosis (n, %)	0 (0)	3 (0.60)	6 (1.0)	0.04	NS	<0.001
Mucosal flap (n, %)	0 (0)	0 (0)	2 (0.33)	-	NS	NS

Figure 1

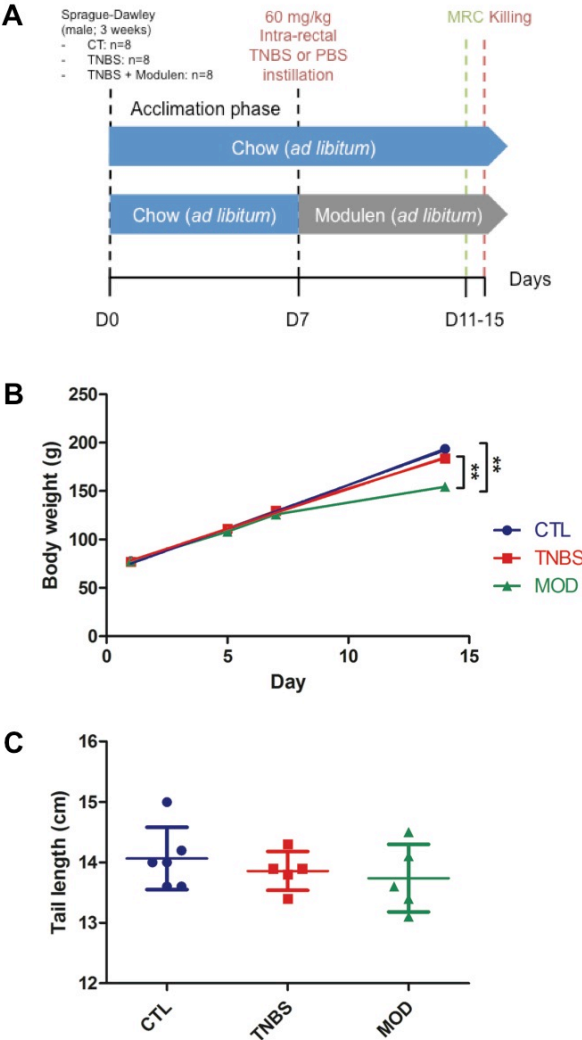


Figure 2

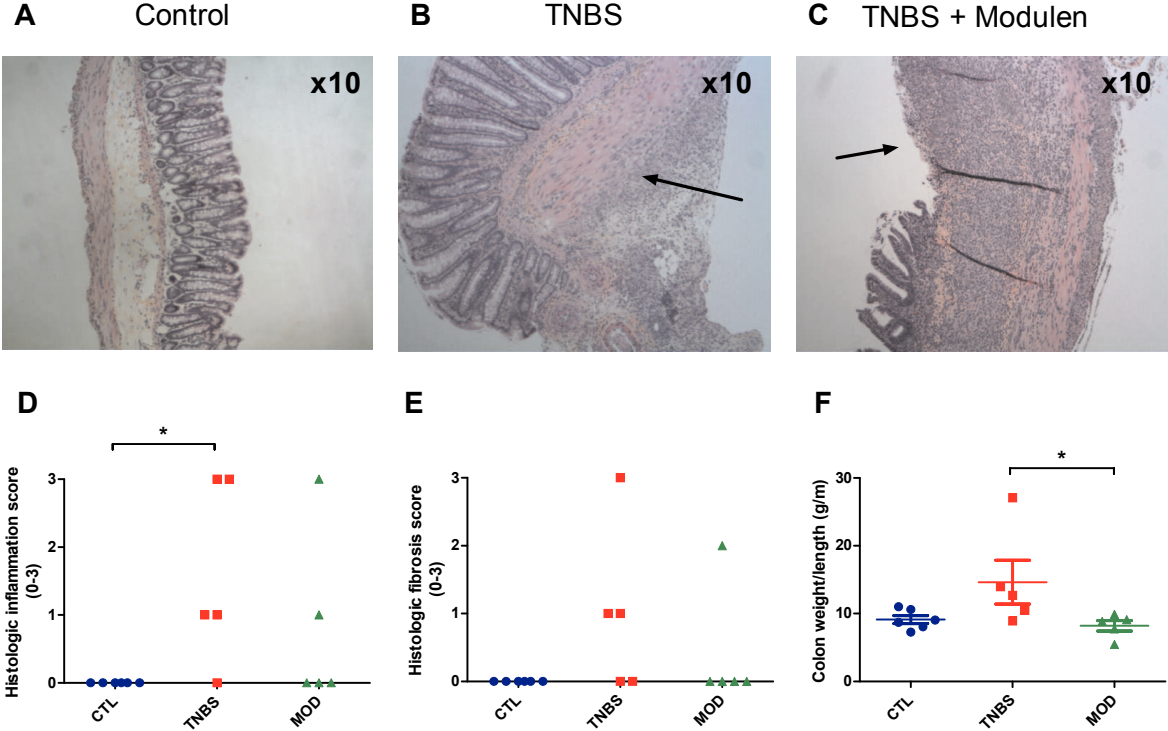
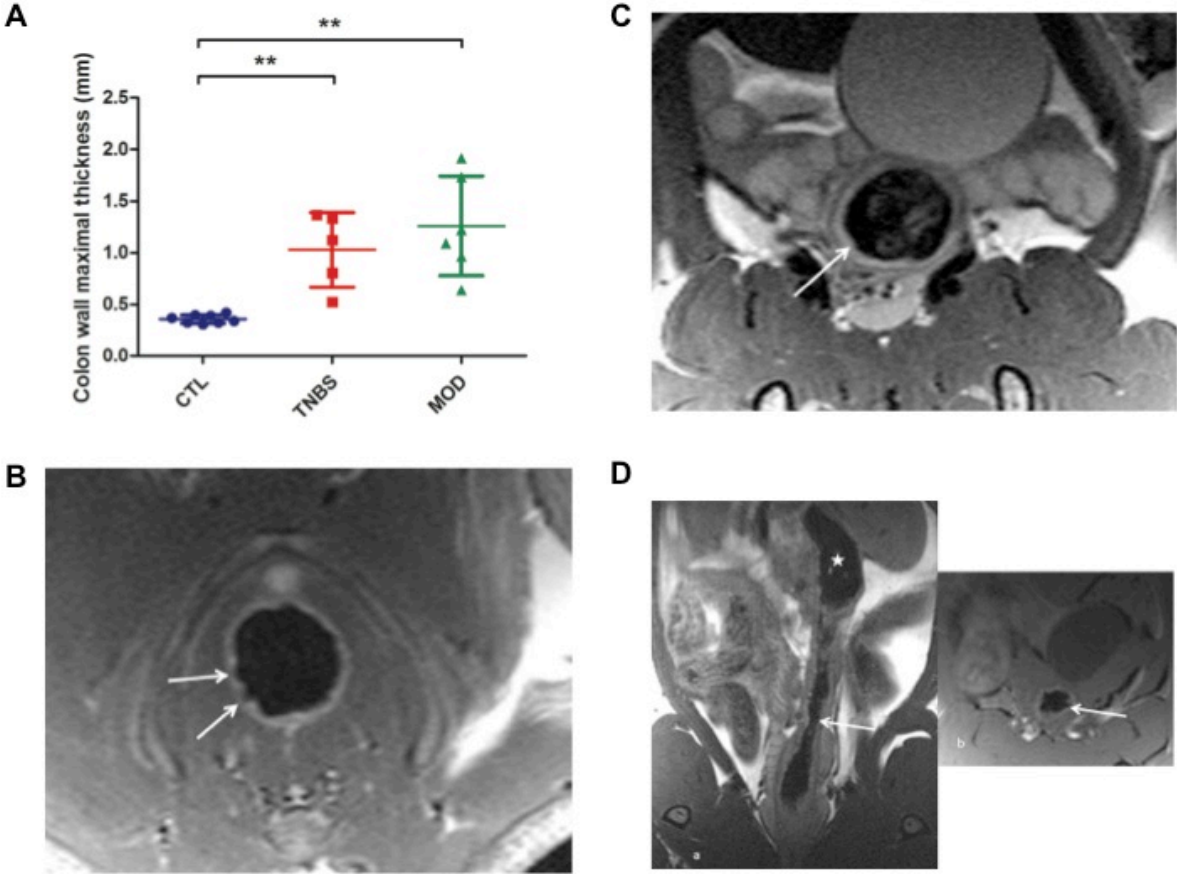
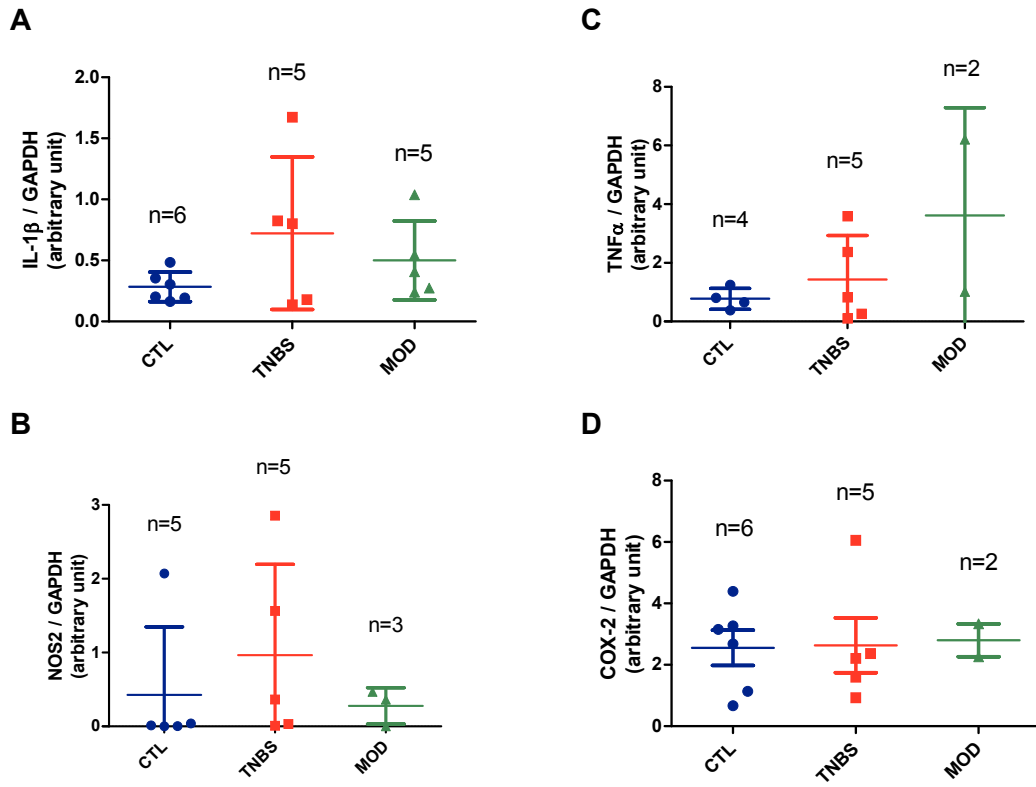


Figure 3



SDC Fig 1



Supplementary Table 1. Nutritional composition of MODULEN IBD® (Nestlé Health Science) and AO3 (SAFE diets)

	MODULEN IBD® (per 100g)	AO3 (per 100g)
Energy kJ/kcal	2070 / 500	1421 / 340
Fat (% kcal) g	(42%) 23	(13,5%) 5.1
of which saturates g	13	
<i>Palmitic acid g</i>		0.76
<i>Stearic acid g</i>		0.15
of which monounsaturates g	3.9	
<i>Oleic acid g</i>		1
<i>Palmitoleic acid g</i>		0.05
of which polyunsaturates g	2.4	
α-linolenic acid g	0.2	0.28
Linoleic acid g	2.1	2.5
MCT	6.0	
Choline mg	35	210
Cholesterol mg	33	
Carbohydrates (% kcal) g	(44%) 54	(61,3%) 52 (NFE*)
of which sugars g	21	4.4
of which lactose g	< 0.5	
Starch	0 g	33.5
Hemicellulose	0 g	oui
Pectin	0 g	oui
Protein (% kcal) g	(14%) 18	(25,2%) 21.4
Arginine g		1.4
Cystine g		0.32
Lysine g		1.15
Methionine g		0.44
Tryptophane g		0.26
Glycine g		1.2
Minerals		
Sodium mg/mmol	170/7.4	280
Chloride mg/mmol	365/10	410
Potassium mg/mmol	600/15	860
Calcium mg/mmol	445/11	820
Phosphorus mg	300	590
Phosphate mmol	9.7	
Magnesium mg/mmol	100/4.1	200
Iron mg	5.4	28
Zinc mg	4.7	6.4
Copper mg	0.49	1.8
Iodine µg	49	
Selenium µg	17	
Manganese mg	0.98	9
Chromium µg	25	
Molybdenum µg	37	
Fluoride mg	<0.01	
Vitamins		
A µg RE	410	1400 UI
D µg	4.9	120 UI
K µg	27	K3 : 570
C mg	47	0
B1 (Thiamin) mg	0.59	0.8
B2 (Riboflavin) mg	0.64	1.3
B6 mg	0.83	0.35

Niacin mg / mg NE	5.8 / 9.8	9
Folic acid µg	120	90
B12 µg	1.6	4
B5 (Pantothenic acid) mg	2.4	2.7
Biotin µg	16	7
E mg α-TE	6.5	5
Other nutrients / ingredients		
Fibres g	0 g	4
Mineral ash	0 g	5.4
Moisture g		12.1

*NFE (nitrogen-free extract) : %NFE = 100% - (%Moisture + %Crude Fibres+ %Crude Protein + %Lipids + %Mineral Ash)

Article 4 : Modèle de colite chronique chez le rat pré-pubère

Effect of a polymeric diet rich in transforming growth factor beta 2 on chronic 2,4,6-trinitrobenzene sulfonic acid colitis in pre-pubertal rats assessed by magnetic resonance colonography

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Short title: Chronic colitis in pre-pubertal rats

Non-standard abbreviations:

- α -SMA: alpha- smooth muscle actin
- COL1A1: alpha-1 type I collagen
- CTGF: connective tissue growth factor
- COX-2: cyclooxygenase 2
- FLASH: fast low angle shot
- FOV: field of view
- GAPDH: Glyceraldehyde 3-phosphate dehydrogenase
- MR-colonography: magnetic resonance colonography

- TNBS: 2,4,6-trinitrobenzene sulfonic acid
- PBS: phosphate-buffered saline
- RARE: rapid acquisition with relaxation enhancement

Conflict of interest statement:

None declared

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Conference presentation: N/A

Authors' contributions:

Substantial contribution to:

- conception and design: CDL, GS, RML
- acquisition of data: AA, AD, CBF, CDL, CG, EC, LN, MJ, MP, RML
- analysis and interpretation of data: CDL, CSC, GS, MA, MP, RML

All authors drafted the article or revised it critically for important intellectual content.

All authors approved the final version to be published.

All authors agreed to be accountable for all aspects of the work thereby ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

All authors had full access to all of the data in the study and had final responsibility for the decision to submit for publication.

Abstract:

Background and aims: Paediatric Crohn's disease patients have a higher incidence of stricturing disease than adults. Murine models help to better understand the dynamic process of intestinal fibrosis and test therapeutic interventions. Pre-pubertal models are lacking. We aimed to develop a model of chronic colitis in pre-pubertal rats and confirm colitis on MR-colonography. We then tested if a polymeric diet rich in TGF- β 2 could have an effect on TNBS-induced intestinal inflammation and fibrosis.

Methods: Colitis was induced in 20 five-week-old Sprague Dawley male rats by weekly rectal injections of increasing doses of TNBS (90 mg/kg, 140 mg/kg and 180 mg/kg) for 3 weeks, while 10 controls received phosphate-buffered saline (PBS). Starting from first administration of TNBS, 10 rats were fed exclusively MODULEN IBD[®] powder, while remaining rats were fed breeding chow. Colitis was assessed one week after last dose of TNBS by histology, MR-colonography and measures of inflammatory and fibrosis markers.

Results: Histologic inflammation and fibrosis scores were higher in TNBS group than controls ($p < 0.05$ for both). MR-colonography showed increased colon wall thickness in TNBS group compared to controls ($p < 0.01$), as well as stenosis and a target sign ($p < 0.05$). Colon expression of COL1A1, CTGF, α -SMA and COX-2 did not differ between TNBS rats and controls. TNBS colitis was not associated with growth failure. Treatment with MODULEN IBD[®] caused growth failure, increased colon weight/length ratio ($p < 0.01$), but did not affect histological scores or MRI characteristics.

Conclusions: Our pre-pubertal model developed significant colitis on MR-colonography and histology. Treatment with MODULEN IBD[®] did not reverse inflammation or fibrosis.

Key words: transforming growth factor beta 2, fibrosis, growth and development.

1. Introduction

Crohn's disease is a chronic inflammatory bowel disease (IBD) characterized by flares and periods of remission, leading to progressive tissue damage. Complicated phenotypes of disease include fistulizing or stricturing bowel disease. Bowel stenosis, defined as luminal narrowing with obstructive symptoms or pre-stenotic dilation on imaging, occurs in 20 to 40% of patients during the first ten years of disease and is the main cause for surgery in IBD^{1,2}. Paediatric-onset IBD has a more severe phenotype than adult IBD with a higher prevalence of stenosis and penetrating disease³. In addition, Crohn's disease beginning before puberty can lead to significant growth failure and delayed puberty. Ongoing efforts are made to reverse the complex inflammatory process, and drugs targeting multiple steps of the inflammatory process have been developed. Intestinal fibrosis occurs in the context of inflammation leading to tissue damage and altered tissue reconstruction with excessive extracellular matrix deposition⁴. Intestinal fibrosis is a dynamic process, and mechanisms of initiation and perpetuation of the process are not yet fully understood. Evidence from other diseases tends to show reversibility of tissue fibrosis (i.e. liver, kidney or lung fibrosis). However, to date, there is no effective treatment for reversing intestinal fibrosis. Efforts are thus made to prevent the fibrotic process through several targets⁵. In order to test the drugs, murine models have been developed. Since fibrosis is a dynamic procedure, non-invasive techniques are needed to follow progression of fibrosis. Our team has previously described a model of chronic colitis in adult rats that had specific characteristics on MR-colonography⁶. Based on the hypothesis that colitis might develop more quickly and severely in pre-pubertal rats, as in humans, the primary aim of this study was to develop a model of chronic colitis in young pre-pubertal rats and confirm the colitis on MR-colonography.

Enteral nutrition with a polymeric diet given exclusively for 6 to 8 weeks is the recommended treatment for induction of remission of Crohn's disease in children with mild to severe disease, according to the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Crohn's and Colitis Organization⁷. In particular, MODULEN IBD[®], a polymeric diet rich in TGF- β 2 has shown rates of clinical remission of up to 79% in children⁸. Several teams have shown promising results with MODULEN IBD[®] as Maintenance therapy in paediatric Crohn's disease. The secondary aim of our study was thus to evaluate if a maintenance

treatment with MODULEN IBD[®] in a pre-pubertal rat model of chronic colitis could have an effect on inflammation and/or prevent fibrosis.

2. Material and methods

Rats and study design

Animal care and experimentation complied with the European directive for the use and care of laboratory animals (Directive 2010/63/UE) and received the approval of the Institutional Care and Use Committee (Comité d’Ethique Normande en Matière d’Expérimentation Animale, CENOMEXA-registration number: APAFIS#16184-2018071711054339). Rachel Marion-Letellier is authorized by the French Government to use the present rat model (Authorization no. 76-106). Animal welfare was monitored daily by visual inspection and all interventions were done during the light cycle. Painful procedures were carried out under general anaesthesia and all efforts were made to minimize suffering.

Thirty 4-week-old Sprague Dawley male rats were purchased from Janvier labs (Le Genest-Saint-Isle, France). After one week of acclimation they were randomly allocated to one of three study groups: Control (n=10), TNBS (n=10) and TNBS + MODULEN (n=10) (Figure 1, Panel A). The rats were housed 5 per standard cage to provide for their interaction needs, were exposed to light / dark cycles of 12 hours each and provided with water *ad libitum*. After induction of colitis, the TNBS + MODULEN group received MODULEN IBD[®] powder as sole food source. The other groups received a standard rat breeding diet (A03, SAFE) in a powdered form (Suppl Table 1: Composition of A03 vs MODULEN IBD[®]).

Induction of colitis

Starting at the age of 5 weeks, colitis was induced by weekly intra-rectal instillation of 2,4,6-trinitrobenzene sulfonic acid (TNBS, Sigma-Aldrich, Saint-Quentin-Fallavier, France) for 3 weeks. After fasting for 24 hours, rats were anesthetized by intra-peritoneal injection of ketamine 8 mg/kg (Panpharma) and Chlorpromazine 1 mg/kg (Sanofi-Aventis). A polyurethane cannula was inserted 3 cm into the rectum and TNBS – ethanol injected, at weekly increasing doses of 90, 140 and 180 mg/kg of body weight. TNBS was dissolved in a 50% Ethanol vehicle, for a total volume of 250 µL. The

rats were maintained in a head-down position for 5 minutes after the injection to minimize leaks. Rats in the control group received an intra-rectal instillation of 250 μ L of phosphate-buffered saline (PBS). During the study protocol, rats were weighed twice a week and observed for signs of pain or significant weight loss.

Magnetic resonance colonography

One week after the third intra-rectal injection, a magnetic resonance colonography was performed. Rats were anesthetized by intra-peritoneal injection of Thiopental (Panpharma) at the dose of 90 mg/kg. Heart rate was monitored during the MRI by surface electrodes. Rats were installed in the cradle in a supine position. Hypothermia was prevented by a tube of circulating warm water around the rat during MRI and a warming plate during recovery phase.

MRI was performed with a small animal machine: Bruker BioSpec 47 / 40USR, 4.7 Tesla (Bruker Biospin, Ettlingen, Germany). There was no injection of antispasmodic or contrast agent. Respiratory movements were corrected using the Intradate™ technique. Four sequences were performed; the total acquisition time was 42 minutes per animal. Parameters for the T2-weighted (T2w) RARE (rapid acquisition with relaxation enhancement) sequence were: TR 5443 ms, TE 34 ms, matrix 320x224, slice 1 mm, NEX 3, flip angle 180°, field of view (FOV) 5,0 x 5,3 cm, NEX 4, acquisition time: 10 minutes, RARE factor 8. Parameters for T2-w RARE with fat suppression (FAT-SAT): TE 39 ms, TR 6027 ms, RARE factor 8, NEX 3, acquisition time 8 minutes, flip angle 180°, slice 1 mm, FOV 5,0 x 5,3 cm, matrix 320x224. T1-weighted sequence with intragate FLASH (fast low angle shot): TR 413 ms, TE 2,8 ms, matrix 256x256, slice 1,1 mm, flip angle 80°, FOV 4,5 x 4,5 cm; acquisition time 14 minutes. Diffusion sequence: TE 2,6 ms, TR 5,2 ms, number of segments 44, NEX 1, flip angle 50°, slice 1 mm, FOV 4 cm, matrix 128 x 128, acquisition time 10 minutes, b values: 0 s/mm², 100 s/mm², 200 s/mm².

MRI images were analyzed in DICOM, using the ParaVision 5.0 software. A senior radiologist (C. S.-C.) who was blinded to the allocation group interpreted all images.

Image quality taking into account respiratory and bowel wall movements was assessed on a scale of 0 (poor quality) to 3 (excellent quality). Measures were made in the descending colon.

In order to assess inflammation, the criteria used, similarly to our previous work ⁶ were: maximal colon wall thickness (average of 3 measures), minimal colon wall thickness (average of 3 measures), colon wall thickness at splenic angle (average of 3 measures), colon wall signal intensity in Regions of Interest (ROI) on T2w sequences (average of 2 measures), target sign (present or absent), ulcerations, spontaneous colon wall hypersignal on T1w sequences. Signal intensity in ROI was measured in the descending part of the colon in axial plane, which anatomically allowed a cross section of the bowel. Size of ROI was adapted to the wall thickness of the rat, usually 0.0002 cm². The results presented are the mean value of measurements performed.

The MRI signs suggesting fibrosis, according to our previous work ⁶, were: narrowing of the colon lumen ($((\text{maximal diameter} - \text{minimal diameter}) \times 100 / \text{maximal diameter})$), stenosis defined as a narrowing of colon lumen associated with proximal colon dilation, and detachment of the mucosa defined as the presence of a mucosal flap in the lumen.

Killing and samples

After completion of the MR-colonography, rats were killed by a lethal dose of intraperitoneal thiopental and then decapitated post-mortem. Tail was measured as a proxy for growth. Colon was resected, washed with PBS to remove faeces, measured and weighed. Six one-centimetre samples were taken from the colon, starting from the rectum, one of which was fixed in 10% neutral-buffered formalin (Sigma-Aldrich) for histology, the others stored at -80°C for Western-Blot and PCR.

Histology

Histologic analyses were done by a single pathologist (M.A.), blinded to allocation group, on a sample of distal colon taken 4 cm from the anus.

The formalin-fixed samples were embedded in paraffin, and 5-micrometer sections were coloured with hematoxylin and eosin (H&E; Merck, Darmstadt, Germany) for standard histology. Samples were studied on 3 levels of cut.

Inflammation was scored using a semi-quantitative score previously used by our team: from 0 (no inflammation) to 3 (severe inflammation) ⁶. Fibrosis was scored from 0 (no fibrosis) to 3 (severe fibrosis). Images were taken by standard light microscopy using a Leica microscope.

Colon expression of cyclooxygenase-2 (COX-2) and alpha Smooth Muscle Actin (α -SMA) by Western Blot

Western blot of COX-2 and α -SMA was performed as previously ⁶.

Alpha-1 type I collagen (COL1A1), Connective Tissue Growth Factor (CTGF) and alpha Smooth Muscle Actin (α -SMA) mRNA

Colon samples were frozen in liquid nitrogen and stored at -80°C before RNA preparation. Total RNA was isolated using the guanidium isothiocyanate method and reverse transcribed into cDNA. Colon mRNA expression of COL1A1 (primer sequences F:ACTCAGCCCTCTGTGCCT, R: CCTTCGCTTCCATACTCG), CTGF (F: GGCAGGGCCAACCACTGTGC, R: CAGTGCACTTGCCTGGATGG), α -SMA (F:CTTCTATAACGAGCTTCGC, R: TCCAGAGTCCAGCACAAT) and the internal control (GAPDH) was measured by qPCR as previously ⁹.

Statistics

Distribution of variables was described by mean \pm SD for normally distributed quantitative variables, and percentage for qualitative variables. We used non-parametric Wilcoxon rank sum tests for 2-group comparison with Wilcoxon exact test correction for small numbers and Fisher's exact test for qualitative variables. Growth curves were compared between groups using a mixed model studying the effects of time, group and time*group. Analyses were replicated using an Analysis of Variance for repeated measures. Statistical analyses were performed using SAS 9.2 and Graph Pad Prism 5. All tests were two-tailed and a p-value < 0.05 was considered significant.

3. Results

Survival and growth

Growth curves of TNBS rats did not differ from controls during the protocol, and final weight and tail length were not significantly different between the TNBS group and the control group: 346.7 ± 29.7 g vs 373.1 ± 42.5 g and 19.8 ± 1.3 cm vs 20.3 ± 1.0 cm respectively (Figure 1, Panel B and C). The survival rate was 50% (5/10) in the TNBS group: 3 rats died during the anaesthesia for TNBS injections n°1 and 2, 1 rat had to be euthanized due to intestinal occlusion after 2nd dose of TNBS, 1 died during anaesthesia for MRI but the colon could be analysed for histology. In the control group, 2 rats died at PBS injection n°2, the overall survival rate in the control group was 80%.

Validation of the chronic colitis model: histology

Colon weight/length ratio (g/m) did not differ significantly between the TNBS and the control group. However, colon length was shorter in the TNBS group than in the control group ($p=0.02$) (Figure 2, Panel F).

Eighty-three per cent (5/6) rats from the TNBS group had mild to severe inflammation based on H&E histology, compared to 13% (1/8) in the control group ($p=0.04$) (Figure 2, Panel D). Sixty-seven per cent (4/6) rats in the TNBS group had mild to severe fibrosis based on histologic score, compared to none in the control group ($p=0.015$) (Figure 2, Panel E).

MR-colonography characteristics of the TNBS chronic colitis

Image quality was deemed satisfactory for analyses, although wall artefacts occurred in 5/7 rats from the control group. Colon wall thickness in T2-weighted images in the axial plane was significantly higher in the TNBS group than in the control group, with a mean maximal thickness of 1.14 ± 0.44 mm in the TNBS group vs 0.56 ± 0.10 mm in the control group ($p=0.003$) (Table 1, Figure 3 Panel A). Colon stenosis was observed in 60% of rats in the TNBS group vs none in the control ($p=0.046$) (Figure 3, Panel D). A target sign was observed in 60% of rats in the TNBS group vs none in the control ($p=0.046$) (Figure 3, Panel B). There was no difference between TNBS group and control

group regarding increased wall signal intensity on T2w sequences, spontaneous T1w hypersignal intensity or colon wall ulcers. Diffusion analyses could not be interpreted due to excessive noise in proportion to wall thickness.

Biological markers of inflammation and fibrosis

There was no difference in gene expression levels of COL1A1, CTGF and α -SMA relative to GAPDH, between controls and TNBS group. Mean level of COL1A1 was 7.30 ± 5.91 in the TNBS group vs 24.92 ± 18.63 in the control group (NS) (Figure 4, Panel A). CTGF was 3.05 ± 1.38 in TNBS group vs 5.39 ± 2.03 in the control group (NS) (Figure 4, Panel B). α -SMA was 1.90 ± 0.85 in the TNBS group vs 4.91 ± 2.40 in the control group (NS) (Figure 4, Panel C). Protein expression of α -SMA and an inflammatory marker, COX-2, measured by Western Blot did not differ between TNBS and control group (Figure 4, Panel D and E).

Effect of MODULEN IBD[®] in a model of chronic colitis in pre-pubertal rats

Survival and growth

The growth curve for body weight of rats in the TNBS + MODULEN group was lower than those of the other groups ($p < 0.0001$ mixed model), and final weight and tail length were significantly lower in the TNBS + MODULEN group than in the TNBS group: 259.1 ± 56.4 g vs 346.7 ± 29.7 g ($p = 0.004$) and 18.5 ± 1.1 cm vs 19.9 ± 1.3 cm ($p = 0.04$) (Figure 1, Panel B and C). Final body weight and tail length were also lower than the control group ($p = 0.0003$ and $p = 0.003$ respectively). Rats in the TNBS + MODULEN group survived colitis induction and sedation procedures better: only one rat died prematurely in the TNBS + MODULEN group, in the 24 hours following the 3rd dose of TNBS.

Effect of MODULEN IBD[®] on TNBS chronic colitis by histology

Colon weight/length ratio (g/m) did not differ significantly between the TNBS + MODULEN group and the TNBS group: 22.5 ± 11.2 vs 15.3 ± 4.7 g/m ($p = \text{NS}$), but was significantly higher than the control group ($p = 0.0002$) (Figure 2, Panel F).

Eighty-nine per cent (8/9) of rats from the TNBS + MODULEN group had mild to severe inflammation based on histology, compared to 83% (5/6) in the TNBS group (p=NS), and compared to 13% (1/8) in the control group (p=0.006) (Figure 2, Panel D).

Seventy-eight per cent (7/9) of rats from the TNBS + MODULEN group had mild to severe fibrosis based on histology, compared to 67% (4/6) of rats in the TNBS group (p=NS) and compared to none in the control group (p=0.006) (Figure 2, Panel E).

Effect of MODULEN IBD[®] on TNBS colitis by MR-colonography

MRI characteristics of rats from the TNBS + MODULEN group did not differ from those of the TNBS group for any of the studied characteristics (Table 1). When compared to the control group, rats from the TNBS+MODULEN group had an increased colon wall thickness (p=0.0003), and developed more frequently a mucosal flap (p=0.001) or ulcers (p=0.03).

Effect of MODULEN IBD[®] on TNBS colitis by biological markers

Colon COL1A1, CTGF and α -SMA mRNA levels did not differ between TNBS + MODULEN group and TNBS group (Figure 4, Panel A, B, C). When compared to controls, the rats in the TNBS + MODULEN group had a lower α -SMA gene expression (p=0.005). This result was confirmed by protein expression of α -SMA by Western Blot (p=0.003 compared to controls). Colon expression of COX-2 did not differ between the 3 groups (Figure 4, Panel E).

4. Discussion

In this study we have shown that a protocol of 3 intra-rectal TNBS injections successfully causes colitis in pre-pubertal rats, characterized by inflammation and fibrosis on histology, and MR-colonography, although the rats did not exhibit growth delay.

The TNBS chronic colitis model in mice developed by Lawrance *et al.* showed interesting properties mimicking natural history of IBD in humans, with a progressive reduction in the proportion of mice having severe inflammation between the 2nd and 6th dose of TNBS, and a progressive increase of severe fibrosis incidence (50% of mice at the 6th dose)¹⁰. They also showed the dynamic nature of fibrosis, persisting 2 to 4 weeks after the last dose of TNBS.

There have been very few published studies on chronic TNBS colitis in pre-pubertal rats^{11,12}. Hence our first objective was to adapt the TNBS chronic colitis model to young rats, with low mortality and significant fibrosis before reaching adult age. The window of opportunity was narrow, since in Sprague Dawley rats the perinatal period lasts until Postnatal Day 21 (PND 21), the pre-pubertal period until PND 42 (6 weeks) and the pubertal period until PND 63 (9 weeks)¹³. We chose to induce colitis starting at 5 weeks of age and all rats were analysed at 8 weeks of age, before the end of the pubertal period.

Among the signs of colitis expected in a TNBS model, weight loss and linear growth failure are classical features^{11,12}. In our model there was no significant growth failure based on weight and tail length in the TNBS group, although significant histologic inflammation indicates that the dose of TNBS was sufficient. A possible explanation results from the fact that the rats were fed a high energy high protein (25.2% crude protein) rat breeding diet during the whole study instead of transitioning to normal diet as they increased in age, in order to allow between-group comparisons with the MODULEN IBD[®] fed rats. In comparison, rats in other chronic TNBS models are fed a standard rat chow with normal protein intake (generally 17-19% crude protein). In this case, the rat breeding diet might have had the effect of nutritional supplementation that could have counterbalanced the detrimental nutritional effect of colitis. Ballinger *et al.* showed, in a model of acute TNBS colitis in pre-pubertal Wistar rats with pair-fed controls that colitis caused impaired linear growth, caused in

part by inflammation and in part by undernutrition¹². Nutritional supplementation in the colitic group increased weight almost to the value of controls¹².

The other possible explanation for the absence of growth failure in the TNBS group could be that the most severely colitic rats died during the protocol and those that survived had a better nutritional status. Indeed, there was a significant mortality rate of 5/10 in the TNBS group vs 2/10 in the control group. We adapted and lowered the doses of TNBS compared to those previously reported, but maybe insufficiently for these young animals. In the absence of comparable studies in this age group, it might be necessary in future studies to lower the doses 2 and 3 of the protocol.

Choosing the correct dose of TNBS is a challenge when putting into balance colonic inflammation, fibrosis and morbidity. Among the animal models of intestinal fibrosis¹⁴, the chronic TNBS colitis requires repeated doses of TNBS to induce fibrosis. The effect of TNBS also has a high variability between species and between animals in an experimental group^{10,15}. Previous studies have shown that TNBS models based on 2 to 3 doses of TNBS cause severe but transient colitis. Fibrosis, as confirmed by elevated expression of collagen and components of the extra-cellular matrix, becomes particularly evident after 4 doses¹¹, 6 doses⁶ and even 8¹⁰ repeated administrations of TNBS.

Our study is the first to assess pre-pubertal colitis in rats using MR-colonography. The MR-colonography characteristics of our model were similar to our previously-reported adult rat chronic TNBS colitis model, with a significant increase of colon wall thickness and stenosis with no spontaneous T1w hypersignal⁶. There is persisting need for imaging techniques that could quantify the amount of fibrous tissue in strictures and assist the decision of medical or surgical management. Diffusion MR techniques are promising, but could not be applied to our model due to the small size of the animal colons and the “noise” due to the diffusion technique. Other MRI techniques applied in mice or rodents have ambitioned to better identify the fibrous nature of a stricture, such as dynamic contrast enhanced MRI studies (with injection of Gd-DTPA)¹⁶ or non-contrast MRI magnetization transfer and T2-weighted signal intensity ratio (comparing bowel wall intensity at the area of greatest thickening, to paraspinous muscle signal intensity)¹⁷.

Moving on to the second objective of our study, feeding rats exclusively with MODULEN IBD[®] during study protocol did not have an anti-inflammatory effect based on histologic scores and MRI images.

Compared to the TNBS group, rats in the MODULEN group had significant growth failure starting within 6 days after first dose of TNBS, without subsequent catch-up growth. This growth failure could be explained by the colitis in itself, although nutritional factors could have amplified the weight loss. Indeed, although the rat breeding diet that the TNBS group was fed with has a high protein content, MODULEN IBD[®] is on the contrary low in protein and high in fat, with 42% energy from fat, 44% from carbohydrates and 14% from protein. The growth curve of the MODULEN IBD[®] group is very similar to that of a group of rats fed a protein-restricted diet (12% crude protein), in a model studying compensatory growth¹⁸.

Protein restriction alone does not seem to explain why the severity of colitis in the MODULEN group was not less than in TNBS group. The majority of rats from the MODULEN group had moderate to severe inflammation and coexisting fibrosis on histology. MRI showed signs of intense inflammation with increased thickness and signs such as ulcers and a mucosal flap that were not seen in the TNBS group, but did not show significant stenosis. This would tend to suggest a pro-inflammatory effect in the MODULEN group.

Once again, nutritional aspects of the diet could explain in part these observations. Xue Li *et al.* showed that a high fat diet (60% energy from fat compared to 10% in controls) given for 4 weeks before induction of TNBS colitis in C57BL/6 mice increased severity of weight loss and histologic damage, and increased pro-inflammatory cytokines¹⁹. They showed that high fat diet caused an increase in oxidative stress, tight junction dysfunction and increased mucosal permeability.

We chose to use MODULEN IBD[®] in its original form and not as a supplementation to the rodents' standard diet as other teams have done^{20,21} in order to best reproduce the effect of an exclusive enteral nutrition by MODULEN IBD[®] as recommended in paediatric IBD induction therapy. We also chose not to administer the MODULEN IBD[®] by gavage since the model was to be the least invasive possible. We had observed in a previous study of MODULEN IBD[®] treatment in acute TNBS colitis that the rats ate the powder and did not develop growth failure (tail length) over a period of 10 days.

Admitting the alternative hypothesis that difference in macronutrient composition is not significant in explaining the increased severity of colitis in the MODULEN group, another explanation would be that a component of the diet had a pro-inflammatory effect. A characteristic of MODULEN IBD[®] is its enrichment in TGF-beta 2 (>24 ppm)²². TGF- β has several isoforms. TGF- β 1 is the prominent cytokine involved in fibrotic procedures in multiple organs, involved in the signalling cascade leading to differentiation of fibroblasts to a myofibroblast phenotype which in turn causes collagen deposition in the extracellular matrix. TGF- β 2 is classically associated with an anti-inflammatory effect. However, there have been recent reports of increased TGF- β 2 levels in colon strictures in a rat model of 2,4,6-trinitrobenzene sulfonic acid (TNBS) colitis²³. In our study, not only were there less strictures in the MODULEN group, but also a decrease in all fibrosis markers compared to controls, although only alpha-SMA decrease was significant.

As in other animal models of intestinal fibrosis, we used histology as the gold standard for confirming fibrosis and therefore validity of the chronic model^{24,25}. One reason we did not observe an increase in “classical” fibrosis markers such as CTGF, alpha-SMA and COL1A1 could be the location of the sample. Indeed, in patients with stricturing ileal Crohn’s disease there has been shown an increase in alpha-SMA and COL I – III expression only in stenotic tract, and not in non-involved ileum²⁶.

However, considerations such as timing of analyses are also paramount, due to the dynamic nature of the fibrotic process. Briefly, fibrosis in the intestine is based on increased extracellular matrix production by activated myofibroblasts, and decreased degradation of these extracellular matrix components^{4,27,28}. Myofibroblasts can originate from fibroblasts and from multiple other cellular sources, via epithelial to mesenchymal transition (EMT) and endothelial to mesenchymal transition (EndoMT). Many growth factors such as TGF-beta and CTGF are implicated in activation of mesenchymal cells, either alone or by cooperative interaction²⁹. Upon activation, myofibroblasts express alpha-SMA, which is commonly used in studies as a marker of activated myofibroblast presence. Once activated, myofibroblasts produce extracellular matrix components according to a certain temporality. For example, Zhu *et al.*, in a chronic (5 doses of TNBS) colitis model in adult Sprague-Dawley rats showed increased expression of COL1A and COL3 mRNA as soon as the 2nd

week of colitis, and collagen deposition starting at 3rd week³⁰. During the final resolution of fibrosis phase, myofibroblasts are reduced by apoptosis³¹.

When applying these theoretical considerations to our model, we can hypothesize that the timing at which the colons were harvested coincided with the beginning of resolution of colon fibrosis. Indeed, histology showed submucosal fibrosis, but the markers of myofibroblast activation (CTGF and COL1A1) were not increased compared to controls suggesting the myofibroblasts were no longer activated. On the contrary, the decrease of α -SMA in the MODULEN group could be interpreted as a sign of myofibroblast apoptosis and possibly, as an anti-fibrotic effect of MODULEN IBD[®]. This would need to be confirmed by serial analyses, with a longer follow-up after last dose of TNBS in order to confirm accelerated resolution of fibrosis in the MODULEN group compared to the TNBS group.

In conclusion, this model of chronic colitis in pre-pubertal rats caused significant inflammation and fibrosis based on histology and MR-colonography, and could be suitable for future therapeutic studies on developing animals. Absence of growth failure was probably explained by nutritional supplementation effect of rodent breeding diet. MODULEN IBD[®] administered a sole source of nutrition did not ameliorate colitis, although there was an interesting decrease in pro-fibrotic markers that would require further study.

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Figure legends:

Figure 1. Experimental protocol and growth

A. Experimental protocol for induction of chronic colitis in pre-pubertal rats, and treatment with MODULEN IBD[®]. B. Rat body weight by group (** $p < 0.01$ for between-group effect and time effect, 2-group comparison, mixed model). C. Tail length at the end of the study, by group (** $p < 0.01$ vs controls, 2-tailed).

Figure 2. Validation of the pre-pubertal chronic colitis model: histology

A. Representative images of H&E from the Control group showing normal histology. B. Representative images of H&E from the TNBS group showing severe transmural inflammation with glandular regeneration and severe fibrosis. C. Representative images of H&E from the TNBS+MODULEN group showing severe inflammation and severe fibrosis. D. Histologic inflammation score by group. E. Histologic fibrosis score by group. F. Colon weight/length ratio by group. * $p < 0.05$, ** $p < 0.01$, significant comparisons are vs Control group unless otherwise specified.

Figure 3. Validation of the pre-pubertal chronic colitis model: MRI-colonography

A. Colon wall thickening: maximum thickness by group (** $p < 0.01$ vs controls, 2-tailed). B. MRI axial plane T2w image of the descending colon, TNBS group. The arrow indicates a Target sign. C. MRI T2w image of the descending colon, TNBS+MODULEN group. The arrow indicates irregularities of the colon wall suggestive of an ulcer, with increased T2w signal intensity. D. MRI image of the descending colon, TNBS group. The arrow indicates colon stenosis, without T2w hypersignal, which could suggest a fibrotic stricture.

Figure 4. Expression of inflammatory markers in colonic tissue homogenates, TNBS-induced chronic colitis model

Gene expression of COL1A1 (Panel A), CTGF (Panel B) and α -SMA (Panel C) measured by RT-PCR (** $p < 0.01$ vs controls, 2-tailed); Protein expression of COX-2 (Panel D) and α -SMA (Panel E) measured by Western Blot (** $p < 0.01$ vs controls, 2-tailed).

Tables

Table 1. MR-colonography characteristics, by study group. TNBS-induced chronic colitis in pre-pubertal rats

	Control (n=7)	TNBS (n=5)	MODULEN (n=8)	TNBS / CTL (p)	TNBS / MOD (p)	MOD /CTL (p)
Colon wall thickness (axial T2)						
Maximum thickness (mm)	0.56 ± 0.10	1.14 ± 0.44	1.40 ± 0.41	0.003	NS	0.0003
Minimal thickness (mm)	0.50 ± 0.07	0.97 ± 0.23	1.09 ± 0.24	0.003	NS	0.0003
Thickness at kidney hilum level (mm)	0.54 ± 0.07	1.03 ± 0.34	1.07 ± 0.16	0.003	NS	0.0003
Stenosis (n, %)	0 (0)	3 (0.60)	4 (0.50)	0.046	NS	<i>0.08</i>
Mucosal flap (n, %)	0 (0)	2 (0.40)	7 (0.88)	NS	NS	0.001
Increased wall signal intensity on T1w (n, %)	0 (0)	1 (0.20)	2 (0.25)	NS	NS	NS
Irregular patterns of mucosal wall (ulcers) (n, %)	0 (0)	1 (0.20)	5 (0.63)	NS	NS	0.03
T2w wall intensity (mean of 2 ROI measures)	1303,7 ± 375,3	1413 ± 208	3210 ± 2531	NS	NS	NS
Increased wall signal intensity on T2w (n, %)	0 (0)	2 (0.40)	4 (0.50)	NS	NS	<i>0.08</i>
Target sign - yes (n, %)	0 (0)	3 (0.60)	3 (0.38)	0.046	NS	NS

Figures

Figure 1

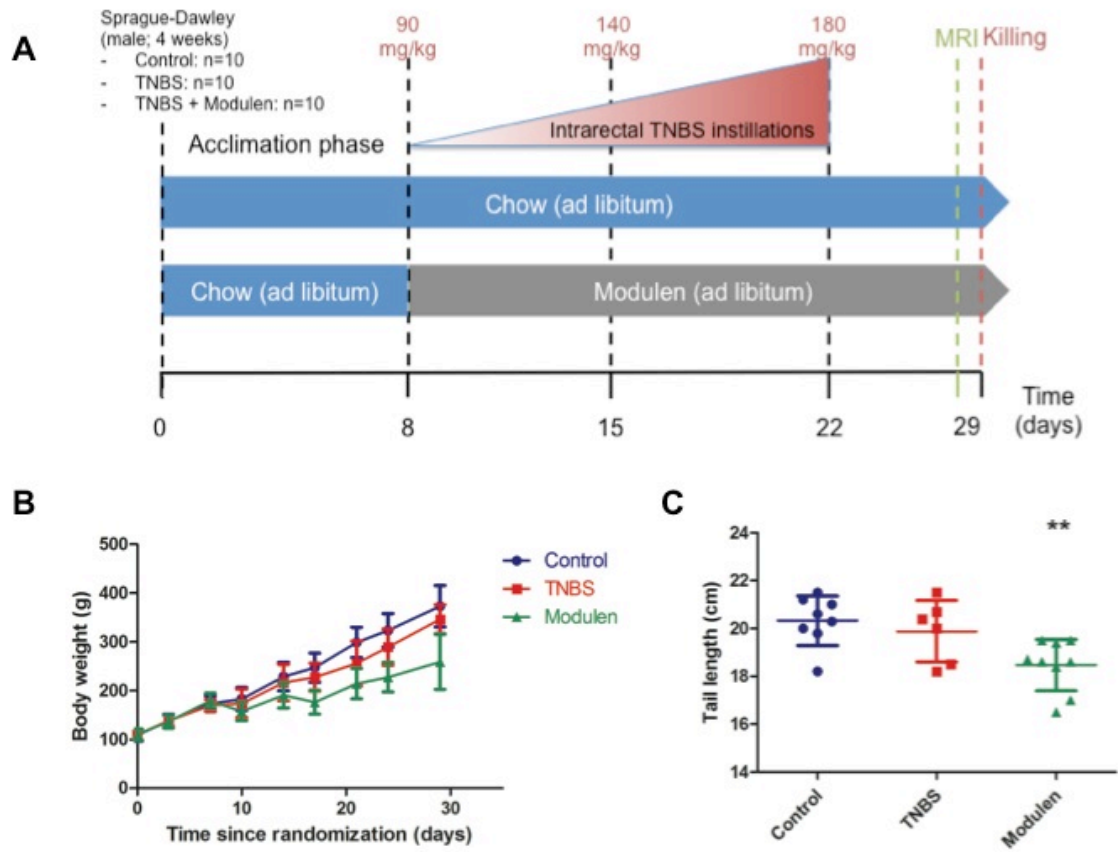


Figure 2

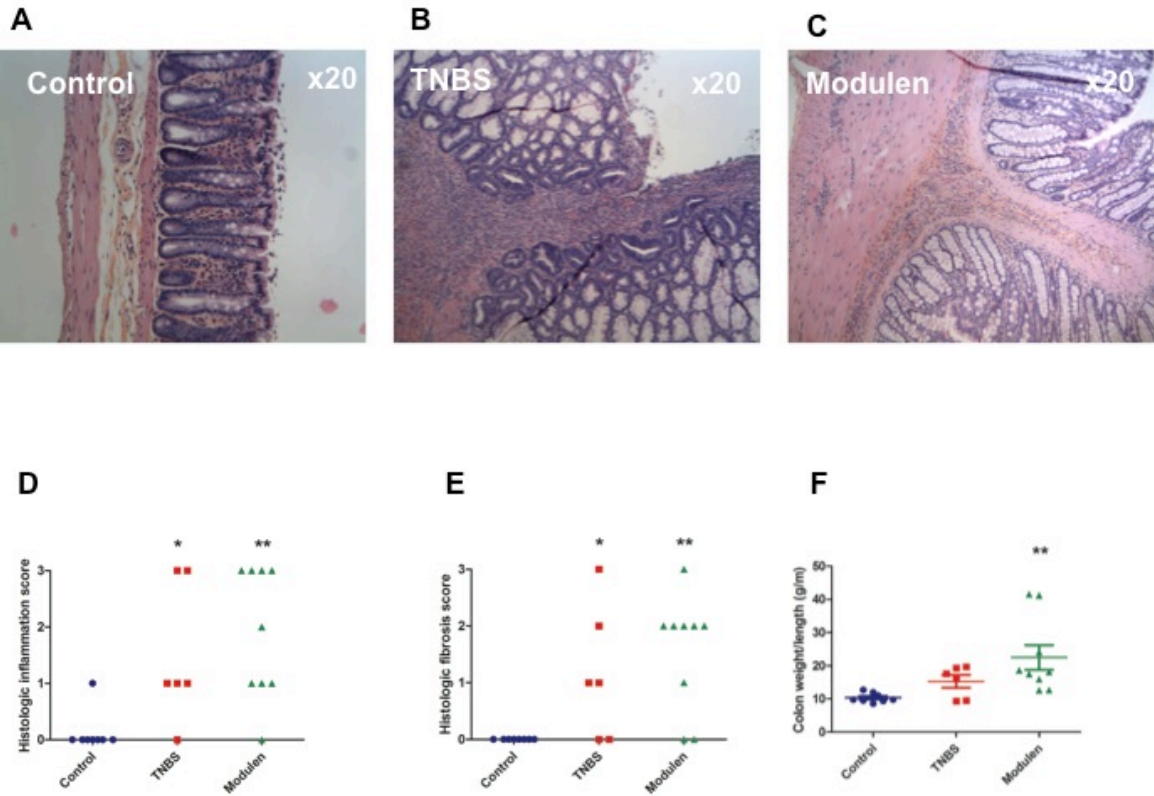


Figure 3

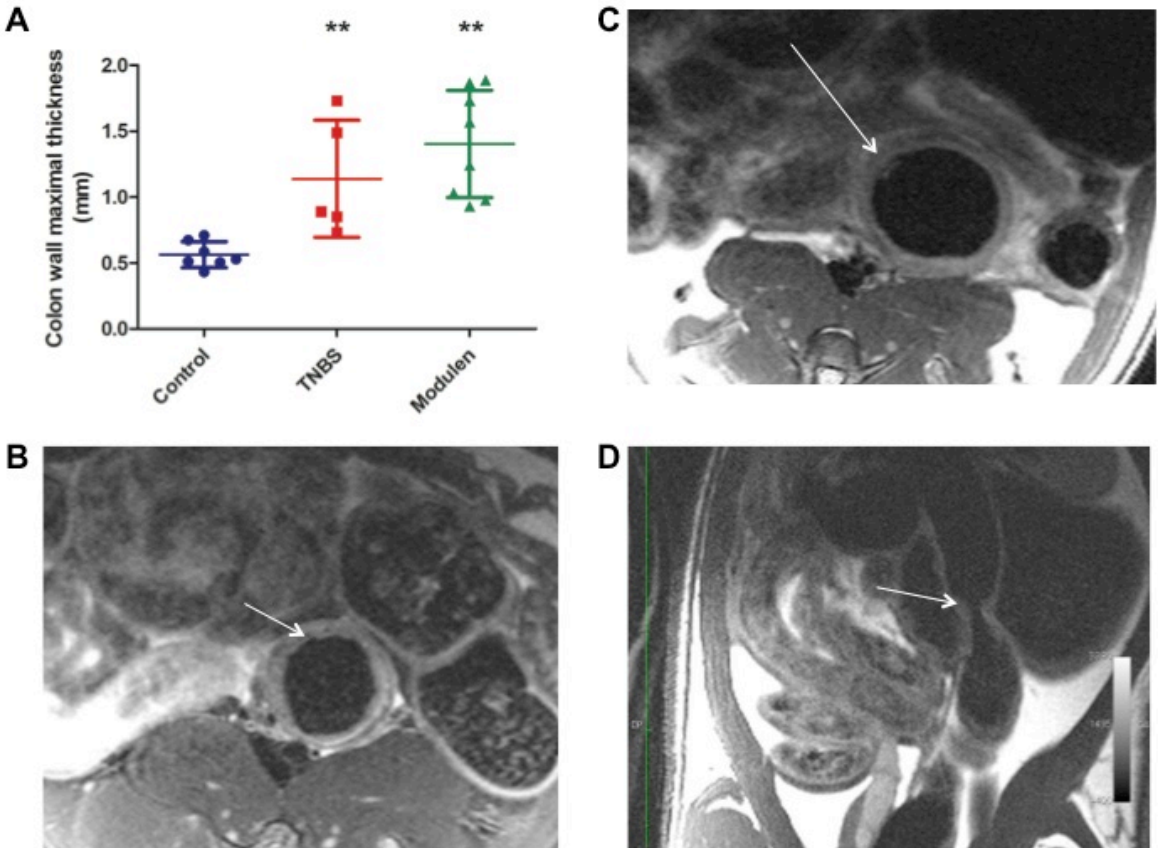
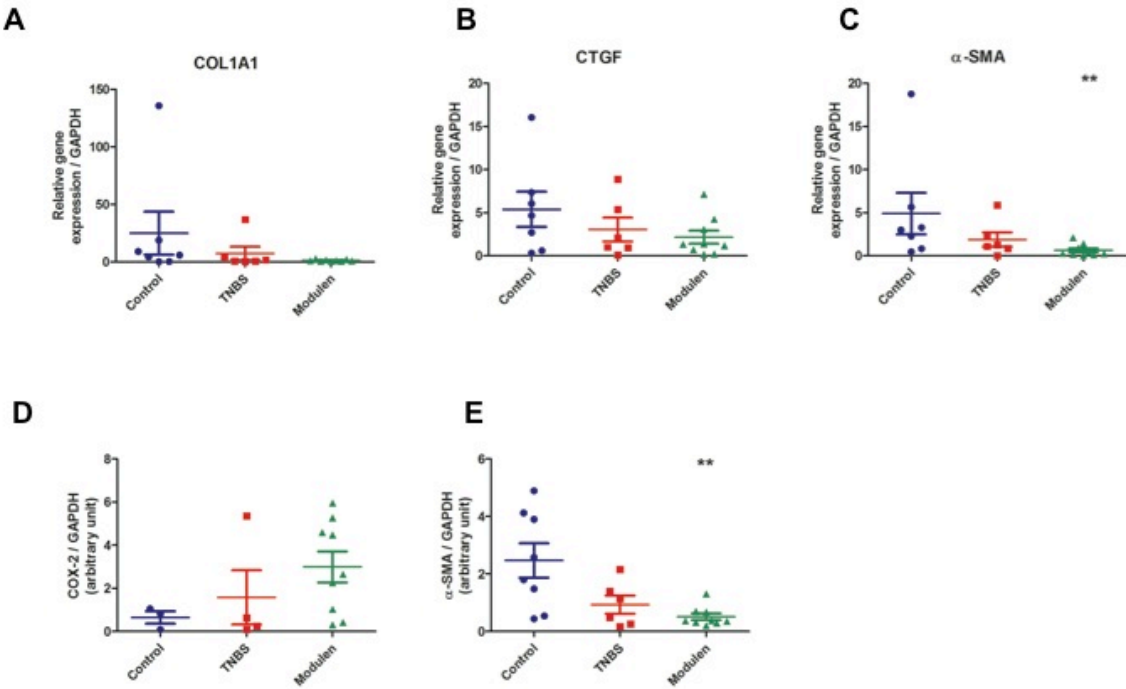


Figure 4



Supplementary Table 1 : Nutritional composition of MODULEN IBD[®] (Nestlé Health Science) and AO3 (SAFE diets)

	MODULEN IBD [®] (per 100g)	AO3 (per 100g)
Energy kJ/kcal	2070 / 500	1421 / 340
Fat (% kcal) g	(42%) 23	(13,5%) 5.1
of which saturates g	13	
<i>Palmitic acid g</i>		0.76
<i>Stearic acid g</i>		0.15
of which monounsaturates g	3.9	
<i>Oleic acid g</i>		1
<i>Palmitoleic acid g</i>		0.05
of which polyunsaturates g	2.4	
α-linolenic acid g	0.2	0.28
Linoleic acid g	2.1	2.5
MCT	6.0	
Choline mg	35	210
Cholesterol mg	33	
Carbohydrates (% kcal) g	(44%) 54	(61,3%) 52 (NFE*)
of which sugars g	21	4.4
of which lactose g	< 0.5	
Starch	0 g	33.5
Hemicellulose	0 g	oui
Pectin	0 g	oui
Protein (% kcal) g	(14%) 18	(25,2%) 21.4
Arginine g		1.4
Cystine g		0.32
Lysine g		1.15
Methionine g		0.44
Tryptophane g		0.26
Glycine g		1.2
Minerals		
Sodium mg/mmol	170/7.4	280
Chloride mg/mmol	365/10	410
Potassium mg/mmol	600/15	860
Calcium mg/mmol	445/11	820
Phosphorus mg	300	590
Phosphate mmol	9.7	
Magnesium mg/mmol	100/4.1	200
Iron mg	5.4	28
Zinc mg	4.7	6.4
Copper mg	0.49	1.8
Iodine µg	49	
Selenium µg	17	
Manganese mg	0.98	9
Chromium µg	25	
Molybdenum µg	37	
Fluoride mg	<0.01	
Vitamins		
A µg RE	410	1400 UI
D µg	4.9	120 UI
K µg	27	K3 : 570
C mg	47	0
B1 (Thiamin) mg	0.59	0.8
B2 (Riboflavin) mg	0.64	1.3
B6 mg	0.83	0.35
Niacin mg / mg NE	5.8 / 9.8	9

Folic acid μg	120	90
B12 μg	1.6	4
B5 (Pantothenic acid) mg	2.4	2.7
Biotin μg	16	7
E mg α -TE	6.5	5
Other nutrients / ingredients		
Fibres g	0 g	4
Mineral ash	0 g	5.4
Moisture g		12.1

*NFE (nitrogen-free extract) : $\%NFE = 100\% - (\%Moisture + \%Crude\ Fibres + \%Crude\ Protein + \%Lipids + \%Mineral\ Ash)$

III) Discussion générale de la thèse

L'objectif général de la thèse était de caractériser les séquelles anatomiques et fonctionnelles des MICI chez l'enfant, et de rechercher un lien avec les caractéristiques de la maladie. Pour cela nous avons utilisé deux types d'approches : clinique et expérimentale. Les études réalisées nous ont amené aux constatations suivantes :

1) Y a-t-il un lien entre la MICI et le développement de TFI chez l'enfant ?

Dans l'étude TFI-MICI, nous avons montré que la prévalence des TFI chez les enfants et adolescents ayant une MICI en rémission était similaire à celle de la population générale (72, 92) et n'était pas liée à la gravité de la maladie antérieure.

Cette conclusion doit cependant être considérée avec réserve, car il a pu y avoir un biais de recrutement, les patients les plus anxieux et déprimés ayant pu ne pas répondre aux questionnaires. Toutefois, les patients étant suivis dans des CHU et centres experts, il était attendu que les cas les plus sévères soient représentés, ce qui aurait dû tendre à surestimer la prévalence si le lien entre sévérité et douleurs fonctionnelles séquellaires était vérifié. Par ailleurs, il n'est pas possible de répondre formellement à la question de la différence de prévalence des TFI chez les enfants ayant une MICI vs chez des témoins sains, étant donné que la méthodologie de l'étude n'était pas une étude cas-témoins. Pour tenir compte des différences socio-culturelles et géographiques qui ont été rapportées dans une méta-analyse mondiale des TFI de l'enfant (72), il aurait été nécessaire d'apparier les témoins pour l'âge et le sexe, voire de les recruter dans la même zone géographique.

Comment expliquer la différence de prévalence des TFI-MICI par rapport aux adultes (74, 76, 77), et l'absence de lien avec les caractéristiques de la MICI ? Une des caractéristiques des populations des études de MICI adulte est leur durée d'évolution de la maladie plus longue, et un taux moins élevé d'immunosuppresseurs et d'anti-TNF que chez les enfants. Parmi les enfants qui ont rempli les questionnaires, malgré une durée de suivi de 3 ans, la plupart n'avaient pas

été hospitalisés en dehors de la période diagnostique, et très peu avaient été opérés. Tandis que le phénotype initial de la maladie était similaire aux données de population générale (localisation, phénotype...), environ 30% avaient un traitement immunosuppresseur et presque 70% un traitement anti-TNF. Ceci est différent des chiffres de prescription d'immunosuppresseur et d'anti-TNF dans les séries de population générale (61% avaient eu un immunosuppresseur et 24% un anti-TNF dans la série EPIMAD pédiatrique 1988-2002) (93).

Nous assistons à une diminution du délai d'introduction des immunosuppresseurs et anti-TNF au cours des MICI de l'enfant. La précocité d'introduction pourrait être liée à la sérénité des prescripteurs par rapport au rapport bénéfice/risque des traitements et les obtentions d'AMM pour les anti-TNF en pédiatrie. Il y a sans doute aussi eu un effet des recommandations ESPGHAN/ECCO qui identifiaient des patients à haut risque de complication (patients présentant sténose, retard de croissance, fistule, ulcérations creusantes...) et conseillaient un step-up rapide dans ce cas (sans préciser de débiter immédiatement les anti-TNF)(94).

Dans notre série, il est possible que les patients aient été mis sous anti-TNF de manière proactive étant donné la présence de facteurs de risque et non suite à la survenue de complications. La précocité d'introduction des anti-TNF a pu accélérer la cicatrisation de la muqueuse. Cependant, nous ne pouvons pas certifier l'absence de toute inflammation résiduelle parmi les patients analysés, car la rémission endoscopique n'a pas été vérifiée pour tous et un résultat de calprotectine fécale n'était disponible que pour un tiers des patients.

Dans des modèles expérimentaux de douleurs fonctionnelles post inflammatoires, des troubles de la perméabilité intestinale sont souvent rapportés. L'augmentation de la perméabilité intestinale favorise l'activation de mécanorécepteurs, dont l'activation persiste pendant plusieurs semaines dans les modèles animaux et est responsable d'une hypersensibilité viscérale.

Le même mécanisme physiopathologique est évoqué dans le syndrome de l'intestin irritable post infectieux (95). La rapidité de cicatrisation intestinale et de restauration de l'intégrité de la

barrière épithéliale intestinale pourrait influencer l'intensité d'activation des mécanorécepteurs et la durée de l'hypersensibilité séquellaire.

Dans ce cadre, il est possible que l'introduction précoce de traitements anti-inflammatoires et immunosuppresseurs puissants puisse avoir eu un effet sur l'axe intestin-cerveau et stoppé le développement de douleurs fonctionnelles séquellaires mais aussi eu un effet bénéfique sur les conséquences psychosociales.

L'introduction précoce des anti-TNF pourrait-elle avoir un effet sur les atteintes psychologiques ou neurologiques centrales liées à l'inflammation ? Dans les modèles animaux de colite TNBS, il a été montré la présence d'une hypersensibilité viscérale à distance d'une colite chronique TNBS (20). L'inflammation colique est associée à l'augmentation d'IL-6 et de TNF- α cérébral, et des brèches de la barrière hémato-encéphalique ont été constatées (96). Une étude en IRM fonctionnelle chez des adultes ayant une MICI traitée par anti-TNF a montré une amélioration de plusieurs paramètres psychologiques avant et après les perfusions d'anti-TNF, avec une corrélation au niveau de l'activité cérébrale (97).

Par ailleurs, notre étude a mis en évidence que la fatigue est une conséquence importante des MICI de l'enfant, comme cela avait été décrit chez l'adulte (98). Nous n'avons pas trouvé de lien entre les traitements administrés et la fatigue, mais notre étude n'était pas conçue dans ce but. Une série adulte a montré que 61% des patients débutant une biothérapie (infliximab, adalimumab, vedolizumab ou ustekinumab) rapportaient une fatigue significative. La fatigue diminuait progressivement au cours de l'année de traitement, avec des taux de fatigue inférieurs chez les patients atteignant la rémission. Cependant, à un an de suivi, 28% des patients qui étaient en rémission rapportaient la persistance de la fatigue (99). La fatigue doit pouvoir être prise en compte, d'autant qu'elle est associée à une diminution de qualité de vie.

En conclusion les TFI ne sont pas un problème fréquent dans notre cohorte, et il est possible que la stratégie de traitement précoce ait pu avoir un effet bénéfique via l'axe intestin-cerveau. Il

nous faut donc nous concentrer sur les séquelles anatomiques, et principalement la fibrose intestinale.

2) Il n'y a pas d'association entre la durée et la sévérité de l'inflammation préalable et le développement de sténoses nécessitant une résection chirurgicale

Malgré le progrès des traitements anti-inflammatoires et immunosuppresseurs, la proportion de patients qui nécessiteront une chirurgie du grêle pour sténose ne diminue pas, et le délai avant première chirurgie reste stable (12, 93). Ceci renforce l'hypothèse que la fibrose évolue pour son propre compte (100). Nous avons voulu savoir si la précocité d'introduction des traitements anti-inflammatoires pouvait avoir un effet. Dans STENOPED, 48% des patients avaient reçu un anti-TNF avant le diagnostic de sténose du grêle. Le fait d'avoir reçu un anti-TNF avant le diagnostic de sténose était associé à la nécessité de chirurgie de celle-ci, en analyse univariée. Par contre la durée de la maladie antérieure avant diagnostic de la sténose n'influçait pas la nécessité de chirurgie pour sténose. Cette constatation va à l'inverse de l'hypothèse anciennement admise que la fibrose nécessiterait un terrain d'inflammation chronique pour se développer (42). Ainsi, dans notre modèle de colite aigue chez le rat pré-pubère nous avons été surpris de voir un signal de fibrose à l'histologie, une semaine après une dose unique de TNBS. Afin de confirmer cette suspicion de début précoce de la fibrose, il serait intéressant de confirmer l'activation de voies de signalisation pro-fibrotiques en mesurant l'IL-13, le TGF- β 1 ou le COL1A et de suivre leur cinétique au cours de la résolution de l'inflammation et après.

3) La précocité d'introduction du traitement anti-inflammatoire ne semble pas permettre de stopper le phénomène pro-fibrosant

Si comme nous le suspectons le phénomène pro-fibrosant débute dès le début de l'inflammation, et évolue ensuite pour son propre compte, il paraît indispensable de réverser le processus fibrosant dès que possible. Les traitements anti inflammatoires et immunosuppresseurs

puissants semblent n'avoir que peu d'effet sur les sténoses du grêle constituées. Par exemple dans l'étude STENOPED, parmi les 32 patients (64% de la population) qui ont été traités par anti-TNF après le diagnostic de la sténose, 16 (50%) ont nécessité une résection chirurgicale de la sténose.

S'il semble nécessaire d'avoir une signalisation pro-inflammatoire pour déclencher la signalisation pro-fibrotique, il n'est pas connu si le fait de supprimer le signal inflammatoire très tôt peut stopper la signalisation pro-fibrotique. Dans nos deux modèles de colite chez des rats en développement, nous avons administré le MODULEN IBD® en exclusif, à but anti inflammatoire dès le début de l'induction de l'inflammation colique par TNBS. Le traitement par MODULEN n'a pas diminué la proportion de rats ayant des sténoses à l'IRM ni les signes histologiques de fibrose dans le modèle aigu ou le modèle chronique. Cependant le MODULEN n'a pas eu d'effet anti-inflammatoire significatif dans notre modèle, et les rats du groupe MODULEN ont développé une dénutrition qui a pu impacter les résultats. Il aurait été intéressant de tester un anti-inflammatoire puissant tel qu'un anti-TNF dès le début de l'induction de la colite afin d'évaluer l'impact sur le développement ultérieur de la fibrose. Des équipes ont montré un effet anti-inflammatoire de l'infliximab administré en sous cutané dans des modèles de colite TNBS chez le rat. Mais il n'a pas été étudié d'effet sur la fibrose, et l'infliximab était administré après l'induction de la colite (après la dose 1 ou la dose 3 de TNBS du protocole) et non de façon concomitante (101, 102).

Dans l'étude CREOLE, l'adalimumab a permis d'éviter la chirurgie chez 50,7% des patients dans les 4 ans qui ont suivi l'inclusion, cependant il n'y a pas eu de contrôle d'imagerie pour s'assurer de la persistance ou non des sténoses (103). Le traitement anti-TNF a pu ne traiter que la partie inflammatoire des sténoses, sans qu'il ne puisse y avoir d'élément sur une éventuelle régression de la fibrose. L'amélioration des lésions IRM pourrait devenir un objectif thérapeutique dans le traitement des sténoses, et non pas simplement l'absence de nécessité de chirurgie ou de dilatation endoscopique dans les 24 à 48 semaines, qui est le critère de jugement principal actuel des études pharmacologiques anti-fibrosantes (65).

4) Place de l'IRM dans la caractérisation des sténoses intestinales

L'IRM pourrait avoir une place primordiale dans la compréhension de l'histoire naturelle de la fibrose intestinale de la maladie de Crohn. En effet elle permet une évaluation transmurale et non invasive de l'intestin, et peut être répétée dans le temps permettant un suivi longitudinal (104, 105).

Cependant à ce jour il n'est pas possible de prédire l'évolution des sténoses simplement par les séquences d'entéro-IRM faites en routine. Une des raisons de cette difficulté est la coexistence de fibrose et d'inflammation dans les zones sténosées, confirmée sur des études sur pièces d'exérèse intestinale (106, 107). Il nous faut donc dépasser notre volonté de caractériser fibrose et inflammation par IRM et déterminer d'autres signes permettant de déterminer le stade évolutif de la sténose et son degré de réversibilité. Dans l'étude STENOPED, le seul critère d'imagerie qui était associé avec la nécessité de chirurgie était une taille de dilatation d'amont à la sténose > 30 mm. La dilatation pré-sténotique est associée de manière inconsistante avec la nécessité de chirurgie : oui dans l'étude CREOLE (103), non dans l'étude de Zappa *et al.* (108). Sur pièce anatomopathologique, les sténoses associées à une dilatation pré-sténotique de plus de 3 cm avaient une composante fibreuse majoritaire dans une étude pédiatrique (107), mais pas dans une étude adulte (108). Un consensus international en secteur adulte visant à donner un cadre aux futures études thérapeutiques sur la fibrose intestinale des MICI propose que les critères diagnostiques d'une sténose du grêle « naïve » soient l'association de : un diamètre de dilatation sus-jacent à la sténose supérieur à 30 mm, un rétrécissement de la lumière de plus de 50% et l'épaississement de la paroi de plus de 25% par rapport au grêle sain adjacent (65). Dans les recommandations ESPGHAN concernant les indications de chirurgie dans les MICI pédiatriques, la dilatation pré-sténotique ne fait pas partie des critères de chirurgie (109). Il est donc peu probable que la présence seule d'une dilatation pré-sténotique ait influencée la décision chirurgicale dans STENOPED.

Il serait possible que la dilatation sus-sténotique soit un témoin de l'épuisement de l'intestin à lutter contre l'obstacle organique. Une manière de visualiser le retentissement fonctionnel de la

sténose serait par des séquences d'IRM dynamique évaluant la motricité de l'intestin grêle pré-sténotique (110). Ces séquences peuvent montrer des ondulations péristaltiques de « lutte ».

En faisant un parallèle avec l'œsophage de lutte sus-jacent à un défaut de relaxation musculaire du sphincter inférieur de l'œsophage dans l'achalasia, la dilatation du corps œsophagien est un facteur associé à l'échec d'un traitement endoscopique de l'achalasia et orientant le choix thérapeutique vers une chirurgie première (111).

L'inflammation intestinale est un processus évoluant par poussées. La fibrose elle aussi a une nature dynamique, même si son histoire naturelle n'est pas encore bien comprise (100). Evolue-t-elle aussi par poussées ou par progression par pallier avec aggravation progressive de la charge de fibrose ? La composition de la matrice et sa quantité sont sous contrôle d'enzymes protéolytiques telles les MMP et leurs inhibiteurs, les TIMP. Il y a quelques pistes concernant les voies qui stimulent les TIMP et les MMP (52). Mais il n'est pas connu si ces voies permettent de diminuer la quantité de collagène dans la matrice extra-cellulaire.

La réversibilité de la fibrose a été montrée dans d'autres organes (foie, poumons...)(112). Dans l'intestin, il y a eu des séries rapportant un taux de succès de 53% des dilatation endoscopique de sténose, sans nécessité de chirurgie ultérieure avec un suivi de 49 mois (113). Parmi ces patients, certains avaient été améliorés par une seule dilatation tandis que d'autres en ont nécessité jusqu'à 5 (113). Cependant l'amélioration des symptômes et l'absence de chirurgie ne signifient pas la résolution de la fibrose. Une revue de la littérature concernant 1112 patients qui avaient eu une ou plusieurs stricturoplasties pour des sténoses du grêle a montré que le taux de récurrence à 5 ans était de 28%. Élément intéressant, la récurrence survenait dans un site autre que celui de la stricturoplastie initiale dans 90% des cas, ce qui faisait suspecter que la fibrose du site de sténose initial ait pu être réversée par la stricturoplastie (114).

Chez l'Homme le turnover de la fibrose intestinale pourrait nécessiter plusieurs mois ou années (40). L'avantage des modèles murins est de pouvoir suivre la cinétique de l'inflammation et de fibrose, dans une temporalité beaucoup plus courte que chez l'humain (66).

Bien que nos modèles de colite n'aient pas été élaborés dans le but d'un suivi longitudinal, il est possible de comparer les caractéristiques des rats pré-pubères ayant reçu une seule dose de TNBS avec ceux qui en ont reçu 3. Dans les deux cas un délai de 1 semaine a été laissé entre la dernière dose de TNBS et la réalisation de l'IRM et de l'histologie du colon. Sous réserve que la première dose de TNBS était différente entre les 2 modèles, la sémiologie IRM observée était différente entre le modèle aigu et chronique. Autant dans le modèle aigu que chronique, nous avons constaté une augmentation de l'épaisseur colique des rats TNBS par rapport aux témoins, et des sténoses chez 60% des rats TNBS. Ces signes IRM ne sont donc pas discriminants. Par contre dans le modèle aigu, l'IRM montrait des ulcères coliques (100% des rats TNBS), un signe de la cible (80% des rats TNBS) et un hypersignal T2 (80%), tandis que l'hypersignal T1 spontané de la paroi était peu fréquent (40% des rats TNBS) de même que le décollement muqueux (0%). Dans le modèle chronique au contraire, l'IRM montrait peu d'ulcères (20%), peu d'hyper T2 (40%), peu d'augmentation du signal sur les séquences T1 (20%) mais plus de décollement muqueux (40%). Il faut noter que les caractéristiques IRM étudiées sur les modèles de rats pré-pubères étaient les caractéristiques du colon descendant en entier, et non pas seulement les sténoses.

Notre équipe a publié précédemment un modèle de colite chronique TNBS (6 doses) chez des rats Sprague-Dawley adultes (115). Les rats recevaient la première dose de TNBS à l'âge de 5 semaines et avaient un âge de 11 semaines à la fin de l'expérience (période post-pubertaire). Les caractéristiques de la colite chronique ont été décrites par IRM : augmentation de l'épaisseur colique par rapport aux témoins, sténose chez 78% des rats (7/9), décollement muqueux chez 44% (4/9), augmentation du signal T2 par rapport aux témoins. Le décollement muqueux serait donc une caractéristique plutôt associée avec la chronicité, mais présente chez < 50% des animaux.

Le suivi longitudinal de colite a déjà été fait dans un modèle de colite DSS chez la souris (116). Les souris ont reçu le DSS dans l'eau de boisson de J0 à J5. Les IRM faites à J6, J11 et J21 ont montré une augmentation d'épaisseur du colon de J6 à J11 chez les souris DSS versus les

contrôles, suivi d'une amélioration à J21. L'augmentation d'épaisseur était plus importante dans le groupe DSS 3% que le groupe DSS 2%. L'IRM était corrélée au score clinique de colite ainsi qu'à l'endoscopie. Il n'y a pas eu de suivi longitudinal de fibrose colique par IRM.

5) Impact d'une nutrition enrichie en TGF-beta 2 sur l'inflammation et la fibrose : facteur protecteur, délétère ou neutre ?

Nous avons montré que l'alimentation exclusive par MODULEN IBD® pendant toute la durée d'induction de la colite TNBS chronique chez des rats pré-pubères causait un retard de croissance, n'améliorait pas les signes d'inflammation ni de fibrose colique par rapport aux rats du groupe TNBS, selon l'histologie.

Cependant nous avons observé le signal intéressant d'une diminution d'expression d' α -SMA (en ARNm et en expression protéique) sur le broyat de colon, prélevé 1 semaine après la 3^{ème} dose de TNBS intra rectal. Alpha-SMA est une isoforme de l'actine, exprimé par les myofibroblastes activés. Le taux d' α -SMA ou le nombre de cellules exprimant α -SMA est couramment utilisé dans les modèles de fibrose de différents organes (peau, cornée, rein, foie, poumon) comme un marqueur de l'activité des myofibroblastes. La diminution d'expression d' α -SMA *in vitro* est utilisée comme biomarqueur d'efficacité de traitements anti fibrosants. Par exemple dans un modèle cutané de cicatrisation de plaie, l'application de Basic fibroblast growth factor sur un derme artificiel promeut l'apoptose et inhibe l'expression d' α -SMA, menant à la réduction de la contraction de la plaie (117).

Le fait que la diminution d' α -SMA puisse faire suite à l'apoptose des myofibroblastes serait une hypothèse séduisante, d'autant qu'il y avait bien une fibrose sous muqueuse constatée en histologie. Il n'y a pas d'augmentation de CTGF, pouvant faire suspecter que les signaux pro-fibrosants ont été suspendus.

Pour étayer l'hypothèse, il faudrait s'assurer que les signaux observés correspondent bien à la temporalité suspectée d'un début de résolution de fibrose. Pour cela il faudrait suivre le modèle

dans le temps pour s'assurer de la stabilité, régression voire résolution complète de la fibrose. Afin de confirmer l'hypothèse que la diminution d' α -SMA témoigne d'une apoptose des myofibroblastes, il serait possible de mesurer des marqueurs d'apoptose tels que l'activité caspase ou un marquage à l'annexine V (67). De l'inactivation et l'apoptose des myofibroblastes à la résolution de la fibrose, il y a encore plusieurs étapes nécessaires, notamment la dégradation des composants de la matrice extra-cellulaire en excès via les enzymes qui dégradent la MEC tels les MMP (Matrix metalloproteinases), dont l'activité peut être mesurée par zymographie (40, 118).

Rôle de TGF-beta 2 dans la cascade pro-fibrosante

Le composant principal qui distingue le MODULEN IBD® des autres préparations polymériques est le TGF- β 2. Les isoformes de TGF-beta, TGF-beta 1, 2 et 3 ont un rôle central dans la résolution de l'inflammation mais aussi dans la cascade pro-fibrosante dans de nombreux organes et modèles cellulaires (61, 119-121).

Historiquement, il avait été montré que TGF- β 1 avait un rôle majeur dans l'activation des myofibroblastes du tissu sous-cutané, soit par injection sous cutanée par pompe, soit et en culture, démontré par une augmentation d'expression d' α -SMA (59).

TGF- β 2 a été mesuré en quantité augmentée dans les sténoses du grêle, sans pouvoir distinguer s'il s'agissait d'une réponse anti-inflammatoire locale ou d'un médiateur pro-fibrosant (122).

La découverte de la transdifférenciation d'autres types cellulaires vers des cellules mésenchymateuses par EndoMT et EMT a permis de découvrir un rôle pro-fibrosant de TGF- β 2. Par exemple, TGF- β 1 et TGF- β 2 stimulent l'EMT et la fibrogenèse sur modèle cellulaire rénal. L'effet de TGF- β 2 peut être bloqué par le miR200a qui diminue l'expression de SMAD 3, diminue les protéines de MEC et prévient l'EMT dépendante de TGF- β 2 (123).

Dans un modèle de fibrose intestinale, un autre micro ARN de la famille miR200b a été montré comme ayant un effet anti fibrosant : il inhibe l'EMT en ciblant ZEB1 et ZEB2 (des inhibiteurs de

E-cadhérine) dans les cellules IEC-6, dans un modèle de colite TNBS chronique (6 doses dans des rats SD adultes) (69).

TGF- β 2 a aussi un effet pro-fibrosant dans d'autres organes : par exemple sur culture cellulaire de cellules bovines de l'épithélium rétinien pigmentaire, l'administration de TGF- β 2 entraîne une augmentation de l'expression d' α -SMA (124) . Dans notre Unité, nous utilisons un modèle *in vitro* de fibrose intestinale en incubant des myofibroblastes intestinaux en présence de TGF- β 2 et les cellules sur-expriment alors des marqueurs de fibrose tels qu' α -SMA (résultat non publié). Talapka *et al.*, dans un modèle de colite chez le rat SD adulte ayant reçu 1, 2 ou 3 doses de TNBS séparés par des intervalles de 2 semaines, ont montré que tous les rats développaient des sténoses dans un délai de 60 jours (8 semaines). Le nombre et la taille des sténoses augmentaient avec le temps et le nombre d'injections de TNBS. Avec un recul de 90 jours à partir du début de la colite (13 semaines), ils ont montré que l'ARNm de TGF- β 2 était surexprimé, l'ARNm de TGF- β 3 sous exprimé et que la MMP9 était surexprimée, dans la sténose et le colon adjacent ; tandis que l'ARNm de TIMP1 était surexprimé dans les segments adjacents à la sténose mais sous exprimé dans la sténose. Des résultats similaires étaient notés au temps tardif de 120 jours (17 semaines) (125).

Influence possible de la dénutrition sur l'inflammation

Un élément qui limite l'interprétation des résultats du traitement par MODULEN IBD® dans notre modèle de colite aiguë et chronique chez le rat pré-pubère est la survenue d'un retard de croissance qui a fait suspecter une carence protéino-énergétique. Il a été montré que la colite expérimentale en elle-même pouvait causer une diminution des ingestas et que la perte de poids était un symptôme associé à la colite chez le rat (90, 91), cependant les rats du groupe TNBS seul n'ont pas développé de retard de croissance. Nous avons aussi constaté que la composition du MODULEN IBD® s'apparente à une alimentation hypoprotidique pour le rat en croissance, mais hyperlipidique. La malnutrition est associée chez l'enfant à une entéropathie caractérisée par une atrophie villositaire, une augmentation de la perméabilité intestinale, une infiltration

lymphocytaire de la muqueuse, une augmentation de l'activité des lymphocytes T CD3+ et des macrophages, et une diminution de l'expression d'IL-10 (126). Il est possible que les rats du groupe MODULEN aient eu une susceptibilité accrue à la colite dans un contexte de perméabilité intestinale accrue. Nous ne pouvons pas non plus exclure l'absence d'un micronutriment dans le MODULEN qui serait nécessaire aux besoins d'un rat en croissance et aurait pu aggraver la colite. Plusieurs facteurs alimentaires ont été montrés comme aggravant des colites expérimentales chez le rat : un déficit en vitamine A (127), en méthyl (128) ou un régime causant une hyperhomocystéinémie (121) par exemple.

6) Quelles sont les pistes de recherche ultérieures ?

1) Vers une meilleure compréhension du stade évolutif et de la réversibilité des sténoses intestinales : sur modèle animal

- La temporalité des séquences impliquées dans le développement de la fibrose intestinale suite à un facteur déclenchant inflammatoire, et la résolution de la fibrose par la suite restent mal connues. Nous proposons un modèle longitudinal de colite TNBS chez le rat pré-pubère, avec des mesures itératives des cytokines et marqueurs inflammatoires et fibrosants. De manière à avoir un modèle le moins invasif en réduisant le nombre d'animaux selon les recommandations du comité éthique, les anesthésies pourraient être faites par isoflurane, les prélèvements faits par biopsie de la muqueuse colique, et le suivi des lésions tissulaires par IRM.

- Afin de disséquer le rôle de l'inflammation et du processus pro-fibrosant dans le maintien de la fibrose intestinale, une étude sur modèle de colite chronique TNBS du rat pourrait comparer 4 bras expérimentaux : rats traités par anti-TNF, rats traités par un anti-fibrosant tel que la curcumine, la simvastatine (129) ou un inhibiteur de l'ACE (cibles anti-fibrogéniques résumées dans : (40)), rats traités par anti-TNF et anti-fibrosant simultanément, groupe contrôle.

2) Vers une meilleure compréhension du stade évolutif et de la réversibilité des sténoses du grêle : sur modèle humain

- Implémenter des techniques d'IRM dynamique, ou des techniques avancées pour quantifier la proportion de tissu fibreux (130): transfert de magnétisation (131), ciné-IRM(110), élastographie ultrasonore (132), ou tomographie par émission de positons quantitative (133), dans le cas d'étude pédiatrique multicentrique prospective, avec relecture centralisée des images, et en prévoyant une imagerie de contrôle après 24 semaines pour suivre l'évolution sous traitement (65).

- L'apport de l'IRM dans la caractérisation des sténoses a pu être limité par l'hétérogénéité des protocoles d'imagerie et l'absence de trame commune. Afin de faciliter des études ultérieures, il pourrait être bénéfique de tenter d'harmoniser les trames de CR d'entéro-IRM dans les centres participant au GETAID pédiatrique (projet de thèse d'interne en radiologie).

3) Puisque les TFI ne semblent pas un problème préoccupant dans notre population, se concentrer sur l'évaluation et la prévention/traitement de la fatigue.

- Puisque plusieurs arguments appuient l'implication de l'axe intestin-cerveau dans la physiopathologie des MICI par une modulation psycho-neuro-endocrino-immune (13), il serait intéressant d'étudier spécifiquement les dysfonctions de l'axe associés à la fatigue. Notamment il serait pertinent d'étudier si les patients ayant une fatigue ont plus de dysautonomie, d'insuffisance hypothalamo-hypophyso-surrénalien ou de faibles taux de corticotrophine que ceux qui n'ont pas de fatigue.

Conclusion

La MICI de l'enfant peut entraîner des conséquences pour la vie entière. Le développement de traitements immunosuppresseurs et de biothérapies et l'application récente de stratégies thérapeutiques basées sur l'estimation du risque permettent aujourd'hui de contrôler les symptômes de la maladie et d'obtenir la cicatrisation muqueuse chez beaucoup d'enfants. Il nous faut aller plus loin et tenter de prévenir les complications. Nous avons vu que les complications fonctionnelles ne semblent pas plus fréquentes chez les enfants ayant une MICI que dans la population générale ; à la condition de s'être assuré que les symptômes ne correspondent pas à de l'inflammation résiduelle, et avec la conviction que le fait de débiter rapidement des traitements anti-inflammatoires puissants et notamment des anti-TNF a pu permettre d'éviter d'installer une dysfonction de l'axe intestin-cerveau.

Plus préoccupantes sont les complications organiques, notamment le développement de fibrose pouvant aboutir à des sténoses nécessitant parfois une résection chirurgicale. Nous avons vu que la durée de la maladie et sa sévérité n'influencent pas la nécessité de chirurgie, confortant l'hypothèse selon laquelle la fibrose évolue pour son propre compte, indépendamment de l'inflammation. Nous sommes encore loin de pouvoir utiliser l'imagerie comme marqueur de risque individuel de nécessité de chirurgie, ou comme prédicteur de réponse aux nouvelles biothérapies.

Nous avons montré que l'administration précoce de traitement anti-inflammatoire chez le rat en croissance ne prévient pas le développement de la fibrose, même si la baisse d'un marqueur de fibrose est intrigante et nécessiterait d'être étudiée de façon plus approfondie.

Il paraît nécessaire de développer des molécules anti-fibrosantes, à administrer en parallèle des traitements anti-inflammatoires, chez les sujets à risque de développer de la fibrose significative. Les modèles animaux de fibrose ont un rôle important à jouer dans cet objectif, et le suivi longitudinal par IRM permettrait de suivre la cinétique des destructions tissulaires et de la régénération, de manière non invasive.

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Annexes

Annexe 1 : Critères diagnostiques des Troubles fonctionnels intestinaux de l'enfant et l'adolescent d'après la classification de Rome IV

Reproduit de Koppen *et al.* (134), d'après Hyams *et al.* (71)

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Appendix 2. Functional Gastrointestinal Disorders: children and Adolescents

Reference: Hyams JS, Di Lorenzo C, Saps M, Shulman RJ, Staiano A, van Tilburg M. Functional Disorders: children and Adolescents. *Gastroenterology*. 150 (6), 1456–68 (2016).

H1a. Diagnostic Criteria for Cyclic Vomiting Syndrome

Must include all of the following:

- (1) The occurrence of 2 or more periods of intense, unremitting nausea and paroxysmal vomiting, lasting hours to days within a 6-month period
- (2) Episodes are stereotypical in each patient
- (3) Episodes are separated by weeks to months with return to baseline health between episodes
- (4) After appropriate medical evaluation, the symptoms cannot be attributed to another condition

H1b. Diagnostic Criteria^a for Functional Nausea and Functional Vomiting

H1b1. Functional Nausea

Must include all of the following fulfilled for the last 2 months:

- (1) Bothersome nausea as the predominant symptom, occurring at least twice per week, and generally not related to meals
- (2) Not consistently associated with vomiting
- (3) After appropriate evaluation, the nausea cannot be fully explained by another medical condition

H1b2. Functional Vomiting

Must include all of the following:

- (1) On average, 1 or more episodes of vomiting per week
- (2) Absence of self-induced vomiting or criteria for an eating disorder or rumination
- (3) After appropriate evaluation, the vomiting cannot be fully explained by another medical condition

^aCriteria fulfilled for at least 2 months before diagnosis.

H1c. Diagnostic Criteria^a for Rumination Syndrome

Must include all of the following:

- (1) Repeated regurgitation and rechewing or expulsion of food that
 - a. Begins soon after ingestion of a meal
 - b. Does not occur during sleep
- (2) Not preceded by retching
- (3) After appropriate evaluation, the symptoms cannot be fully explained by another medical condition. An eating disorder must be ruled out

^aCriteria fulfilled for at least 2 months before diagnosis.

H1d. Diagnostic Criteria^a for Aerophagia

Must include all of the following:

- (1) Excessive air swallowing
- (2) Abdominal distention due to intraluminal air which increases during the day
- (3) Repetitive belching and/or increased flatus
- (4) After appropriate evaluation, the symptoms cannot be fully explained by another medical condition.

^aCriteria must be fulfilled for at least 2 months before diagnosis.

H2a. Diagnostic Criteria^a for Functional Dyspepsia

Must include 1 or more of the following bothersome symptoms at least 4 days per month:

- (1) Postprandial fullness
- (2) Early satiation
- (3) Epigastric pain or burning not associated with defecation
- (4) After appropriate evaluation, the symptoms cannot be fully explained by another medical condition.

^aCriteria fulfilled for at least 2 months before diagnosis.

Within FD, the following subtypes are now adopted:

- (1) Postprandial distress syndrome includes bothersome postprandial fullness or early satiation that prevents finishing a regular meal. Supportive features include upper abdominal bloating, postprandial nausea, or excessive belching
- (2) Epigastric pain syndrome, which includes all of the following: bothersome (severe enough to interfere with normal activities) pain or burning localized to the epigastrium. The pain is not generalized or localized to other abdominal or chest regions and is not relieved by defecation or passage of flatus. Supportive criteria can include (a) burning quality of the pain but without a retrosternal component and (b) the pain commonly induced or relieved by ingestion of a meal but may occur while fasting.

H2b. Diagnostic Criteria^a for Irritable Bowel Syndrome

Must include all of the following:

- (1) Abdominal pain at least 4 days per month associated with one or more of the following:
 - a. Related to defecation
 - b. A change in frequency of stool
 - c. A change in form (appearance) of stool
- (2) In children with constipation, the pain does not resolve with resolution of the constipation (children in whom the pain resolves have functional constipation, not irritable bowel syndrome)
- (3) After appropriate evaluation, the symptoms cannot be fully explained by another medical condition

(Continued)

(Appendix 2. Continued).

^a Criteria fulfilled for at least 2 months before diagnosis.

H2c. Diagnostic Criteria^a for Abdominal Migraine

Must include all of the following occurring at least twice:

(1) Paroxysmal episodes of intense, acute periumbilical, midline or diffuse abdominal pain lasting

(1) 1 h or more (should be the most severe and distressing symptom)

(2) Episodes are separated by weeks to months.

(3) The pain is incapacitating and interferes with normal activities

(4) Stereotypical pattern and symptoms in the individual patient

(5) The pain is associated with 2 or more of the following:

- a. Anorexia
- b. Nausea
- c. Vomiting
- d. Headache
- e. Photophobia
- f. Pallor

(6) After appropriate evaluation, the symptoms cannot be fully explained by another medical condition.

^a Criteria fulfilled for at least 6 months before diagnosis.

H2d. Diagnostic Criteria^a for Functional Abdominal Pain-NOS

Must be fulfilled at least 4 times per month and include all of the following:

(1) Episodic or continuous abdominal pain that does not occur solely during physiologic events (e.g. eating, menses)

(2) Insufficient criteria for irritable bowel syndrome, functional dyspepsia, or abdominal migraine

(3) After appropriate evaluation, the abdominal pain cannot be fully explained by another medical condition

^a Criteria fulfilled for at least 2 months before diagnosis

H3a. Diagnostic Criteria for Functional Constipation

Must include 2 or more of the following occurring at least once per week for a minimum of 1 month with insufficient criteria for a diagnosis of irritable bowel syndrome:

(1) 2 or fewer defecations in the toilet per week in a child of a developmental age of at least 4 years

(2) At least 1 episode of fecal incontinence per week

(3) History of retentive posturing or excessive volitional stool retention

(4) History of painful or hard bowel movements

(5) Presence of a large fecal mass in the rectum

(6) History of large diameter stools that can obstruct the toilet

After appropriate evaluation, the symptoms cannot be fully explained by another medical condition.

H3b. Diagnostic Criteria for Nonretentive Fecal Incontinence

At least a 1-month history of the following symptoms in a child with a developmental age older than 4 years:

(1) Defecation into places inappropriate to the sociocultural context

(2) No evidence of fecal retention

(3) After appropriate medical evaluation, the fecal incontinence cannot be explained by another medical condition

Titre de la thèse : Séquelles anatomiques et fonctionnelles des maladies inflammatoires chroniques de l'intestin (MICI) de l'enfant

Mots clés : Inflammation, Fibrose, Développement, Imagerie par Résonance Magnétique, Colite expérimentale, Axe intestin-cerveau

Résumé : Les maladies inflammatoires chroniques de l'intestin (maladie de Crohn, rectocolite hémorragique, colite inclassée) de l'enfant peuvent occasionner des séquelles anatomiques telles que la fibrose intestinale responsable de sténoses, ainsi que des troubles fonctionnels intestinaux (TFI) persistants lors de la rémission de la maladie. L'objectif de la thèse était d'estimer l'impact de ces complications et de rechercher une association avec les caractéristiques antérieures de la maladie. Nous avons utilisé pour cela deux séries pédiatriques et deux modèles animaux.

Dans l'étude TFI-MICI, nous avons montré que 20% des enfants et adolescents avec MICI en rémission clinique et biologique avaient des TFI, et 15% des douleurs abdominales fonctionnelles (DAF). Les DAF étaient associées à une fatigue accrue, à des symptômes dépressifs et une diminution de la qualité de vie, mais pas à de l'anxiété. Il n'y avait pas d'association entre la gravité de la MICI et la présence de DAF.

Dans l'étude STENO-PED, nous avons montré que les seuls critères cliniques et radiologiques associés à la nécessité de résection chirurgicale des enfants ayant une maladie de Crohn sténosante du grêle étaient une dilatation sus-sténotique > 30 mm et un PCDAI > 22,5 au diagnostic de sténose. L'administration d'un traitement anti-TNF après le diagnostic de sténose était un facteur protecteur par rapport au risque de chirurgie.

Il manque de modèles expérimentaux permettant de suivre l'histoire naturelle de la fibrose intestinale en lien avec l'inflammation et l'effet des traitements, notamment chez l'animal pré-pubère. Nous avons adapté au rat pré-pubère un modèle de colite aiguë (1 dose de TNBS) et chronique (3 doses de TNBS) et avons montré que les rats développaient une inflammation significative dans les 2 modèles, basé sur un score histologique. L'IRM montrait un épaississement de la paroi colique et des sténoses dans les 2 modèles, et des ulcérations de la muqueuse dans le modèle aigu. En histologie, il y avait une fibrose légère à modérée dans le modèle TNBS chronique et une ébauche non significative de fibrose dans le modèle aigu. Le traitement des rats par MODULEN IBD® exclusif dès le début de l'induction de l'inflammation n'a pas entraîné de diminution de l'inflammation ni de la fibrose histologiques dans les deux modèles.

La prise en charge des séquelles anatomiques et fonctionnelles des MICI pédiatriques devient un enjeu majeur même si la prévalence des douleurs fonctionnelles nous est apparue comme non augmentée par rapport à la population générale. Les modèles animaux pré-pubères pourraient permettre de mieux analyser la cinétique de la fibrogénèse et l'impact d'une suppression précoce de l'inflammation et/ou de nouvelles thérapies anti-fibrosantes.

Title : Functional and structural sequelae of pediatric inflammatory bowel disease

Key words : Inflammation, fibrosis, growth and development, magnetic resonance imaging, experimental colitis, brain-gut axis

Summary : The course of pediatric-onset inflammatory bowel disease (Crohn's disease, ulcerative colitis and IBD-unclassified) can be complicated by structural or functional sequelae. Structural complications include intestinal fibrosis which can cause bowel strictures. IBD can be associated with functional abdominal pain persisting despite remission of inflammation. The purpose of our work was to determine the burden of these complications and search for association with previous severity of inflammation, based on two clinical studies and two animal models.

In the first study, TFI-MICI, we showed that 20% of children and adolescents with IBD in clinical and biochemical remission had functional gastrointestinal disorders, among which 15% had functional abdominal pain disorders (FAPD). FAPD was associated with increased fatigue, depressive symptoms and reduced quality of life, but not with anxiety. There was no association between the severity of IBD and presence of FAPD.

The second study, STENO-PED, focused on imaging and clinical predictors of response to treatment in children and adolescents with stricturing small bowel Crohn's disease. We showed that the predictors for surgery were a dilation proximal to the stricture of > 30mm, and a PCDAI score at diagnosis of stricture > 22.5. Receiving an anti-TNF α treatment after diagnosis of stricture was a protective factor from surgery.

Experimental models allowing to follow the progression of fibrosis along with inflammation and assess response to treatment are lacking, in particular for pre-pubertal animals. We adapted to Sprague-Dawley pre-pubertal rats a model of acute (1 dose of TNBS) and chronic (3 doses of TNBS) hapten-induced colitis. The rats in both models developed significant inflammation, based on histology and magnetic resonance colonography. Rats in the chronic colitis model developed histologic fibrosis. There was a non-significant trend to fibrosis in the acute model. We treated rats in both models with MODULEN IBD® from induction of colitis to collection of colonic samples. This treatment did not reverse inflammation nor fibrosis.

Fibrosis and functional abdominal pain in pediatric-onset IBD are two important problems, although functional pain appeared to be not more frequent than in the general population. Animal models could be of great assistance in order to better decipher the link between inflammation and fibrosis, and see if an effective early suppression of inflammation along with new anti-fibrotic therapies could halt the progression of fibrosis.