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MUDr. Ivana Čopová

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Methods of Biomedical Informatics in the study of Inflammatory Bowel Disease in children

Disertační práce

Školitel: doc. MUDr. Ondřej Hradský, Ph.D.

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Abstrakt

Zánětlivá střevní onemocnění (IBD) představují skupinu chronických, polygenně podmíněných onemocnění postihujících primárně gastrointestinální trakt, jejichž incidence v dospělé i dětské populaci globálně narůstá. Mezi tyto nemoci řadíme Crohnovou nemoc (CD), ulcerózní kolitidu (UC) a tzv. neklasifikovatelné IBD (IBD-U). Fekální kalprotektin (FC) je markerem zánětu u IBD a jeho hladiny korelují s aktivitou onemocnění definovanou klinickými parametry, endoskopickým nálezem a histologií. Současná lékařská praxe je spojena s dostupností velkého množství klinických dat a snahou jejich efektivního uplatnění v procesu medicínskeho rozhodování takovým způsobem, aby bylo dosaženo maximální možné redukce rizika nepříznivého průběhu onemocnění a výskytu s onemocněním a/nebo léčbou asociovaných komplikací.

Primárním cílem disertační práce je využití metod biomedicínské informatiky v oblasti pediatrických IBD v procesu ověření FC v predikci aktivity onemocnění a odpovědi na léčbu, hledání dalších potencionálních prediktivních faktorů a tvorbu predikčních modelů pro konkrétní klinické situace.

Zjistili jsme, že na základě vývoje hladin FC v časné fazi indukční léčby pomocí výhradní enterální výživy nelze rozhodovat o dalším vedení terapie. Ověřením domácího testu na stanovení koncentrace FC ve stolici jsme poukázali na jeho možné benefity pro urychlení procesu rozhodování, nicméně s nutností konfirmace výsledku klasickými laboratorními metodami. Definováním vyváženého cut-off metabolitů azathioprinu k předpovědi dosažení efektivních hladin infliximabu jsme získali vhodný nástroj pro optimalizaci kombinované terapie u pacientů s CD. Srovnáním dvou preparátů první linie biologické léčby jsme upozornili na potřebu zohlednění nalezených rizikových faktorů při volbě terapie. Zjistili jsme, že indukční léčba nehraje zásadní roli v délce trvání remise onemocnění u pacientů s nekomplikovanou CD současně užívajících azathioprin. Zdůvodnili jsme vhodnost suplementace vitaminu D u dětských pacientů s IBD a poukázali na možnost redukce dříve indikovaných vyšetření u asymptomatických jedinců.

Nové poznatky vyplývající z realizovaných studií s sebou přináší možnost exaktnějšího a individuálně zaměřeného přístupu v procesu klinického rozhodování.

Klíčová slova: fekální kalprotektin, predikce, zánětlivá střevní onemocnění

Abstract

Inflammatory bowel diseases (IBD) are a group of chronic, polygenic diseases primarily affecting the gastrointestinal tract, with an increasing incidence in both adult and paediatric populations globally. These diseases include Crohn's disease (CD), ulcerative colitis (UC) and so-called IBD unclassified (IBD-U). Faecal calprotectin (FC) is a marker of inflammation in IBD and its levels correlate with disease activity as defined by clinical parameters, endoscopic findings and histology. Current medical practice is associated with the availability of a large amount of clinical data and the desire to apply it effectively in the medical decision-making process in such a way as to achieve the maximum possible reduction in the risk of adverse disease course and the occurrence of disease- and/or treatment-associated complications.

The primary goal of this dissertation is to apply biomedical informatics methods to paediatric IBD in the process of validating FC in predicting disease activity and response to treatment, searching for additional potential predictive factors, and developing prediction models for specific clinical situations.

We found that the development of FC levels in the early phase of induction therapy with exclusive enteral nutrition cannot be used as a basis for further management. By validating a home test for the determination of FC concentration in stool, we pointed out its potential benefits for speeding up the decision-making process, however, with the need to confirm the result by conventional laboratory methods. By defining a balanced cut-off of azathioprine metabolites to predict the achievement of effective infliximab levels, we obtained a suitable tool for optimizing combination therapy in CD patients. By comparing two first-line biologic therapy agents, we highlighted the need to consider the identified risk factors in the choice of therapy. We found that induction therapy does not play a major role in the duration of disease remission in patients with uncomplicated CD concomitantly taking azathioprine. We justified the appropriateness of vitamin D supplementation in paediatric patients with IBD and highlighted the possibility of reducing previously indicated testing in asymptomatic individuals.

The new findings from the conducted studies bring with them the possibility of a more exact and individualized approach in the clinical decision-making process.

Key words: faecal calprotectin, prediction, inflammatory bowel diseases

Seznam použitých zkratek

25-OHD	25-hydroxyvitamin D
5ASA	5-aminosalicylates; 5-aminosalicyláty
6-TGN	6-thioguanine nucleotide; 6-thioguanin nukleotid
ADA	adalimumab
AI	arteficial intelligence; umělá imteligence
ANCA	anti-neutrophil cytoplasmic antibody; protilátky proti cytoplasmě neutrofilů
ASCA	anti-*Saccharomyces cerevisiae* antibody; protilátky proti *Saccharomyces cerevisia*e
aTNF	protilátka proti tumor-nekrotizujícímu faktoru
AZA	azathioprin
BKV	BK polyomavirus; lidský polymavirus 1
CD	Crohn´s disease; Crohnova choroba
CDED	Crohn´s diesease exclusion diet; dieta pro Crohnovou chorobu založená na vyloučení konktrétních potravin
CI	confidence interval; konfidenční interval
CMV	cytomegalovirus
CRP	C-reaktivní protein
EBV	Epstein-Barrové virus
ECCO	European Crohn´s and Colitis Organisation; Evropská organizace pro Crohnovou chorobu a kolitidu
EDTA	ethylendiamintetraacetic acid; kyselina etylendiamnintetraoctová
EEN	exclusive enteral nutrition; výhradní enterální výživa
ELISA	enzyme-linked immuno sorbent assay
EPP	events per predictor parameter; počet událostí na parametr prediktoru
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition; Evropská společnost pro dětskou gastroenterologii, hepatologii a výživu
ESR	erythrocytes sedimentation rate; sedimentace erytrocytů
FC	faecal calprotectin; fekální kalprotektin
GLM	golimumab
IBD	inflammatory bowel disease; zánětlivé střevní onemocnění

IBD-U	inflammatory bowel disease unclassified; neklasifikovatelné zánětlivé střevní onemocnění
ICR	ileocékální resekce
IFX	infliximab
JAK	Janus kináza
JCV	John Cunninghan polyomavirus; lidský polymavirus 2
MH	mucosal healing; slizniční hojení
MINI	mucosal inflammation noninvasive index; neinvazivní index slizničního zánětu
ML	machine learning; strojové učení
MRE	magnetická rezonanční enterografie
NLR	negative likelihood ratio
OOB	out of bag error, chyby na testovacím souboru modelu
(wP)CDAI	(wieghted pediatric) Crohn´s disease activity index; (vážený dětský) index aktivity Crohnovy choroby
PCR	polymerase chain reaction; polymerázová řetězová reakce
pQCT	peripheral quantitative computed tomography; periferní kvantitativní výpočetní tomografie
PUCAI	pediatric ulcerative colitis activity index; index aktivity ulcerózní kolitidy
RCT	randomized controlled trial; randomizovaná kontrolovaná studie
ROB	risk of bias; riziko skreslení
ROC	reciever operating characteristic
RZB	rizankizumab
SARS-CoV-2	severe acute respiratory syndrome-related coronavirus 2; těžký akutní respirační syndrom vyvolaný koronavirem 2
SVM	support vector machine; metoda podpůrných vektorů
TRIPOD	transparent reporting of a multivariable prediction model for individual prognosis or diagnosis
UC	ulcerózní kolitida
UST	ustekinumab
VDZ	vedolizumab

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1. Úvod

1.1. Rozhodování v medicíně

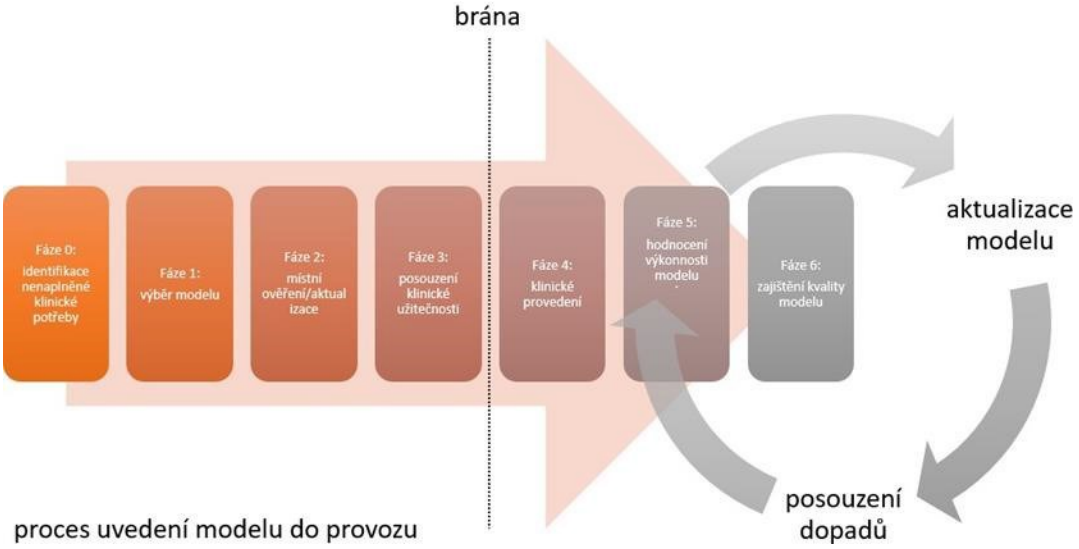
Lékařské rozhodování závisí na znalostech, zkušenostech a úsudku lékaře. Kromě znalostí o základním chorobném procesu a nejaktuálnějších dostupných možnostech léčby by měl lékař formulovat své rozhodnutí na základě porozumění vědecké literatuře. Navíc je ve své praxi často postaven do situace, kdy by bylo výhodné „nahlédnout do budoucnosti“. Jednak chce co nejlépe předpovědět prognózu konkrétního pacienta charakterizovaného mnohými dalšími faktory než jen samotným onemocněním. Rovněž chce vědět, který z diagnostických nebo terapeutických přístupů by tomuto konkrétnímu pacientovi přinesl největší prospěch. Tradičně v těchto situacích postupuje na základě poměrně velkého množství informací o pacientovi v časově omezených podmínkách, kdy může být obtížné je vyhodnotit v odpovídajícím klinickém kontextu a formulovat závěr a adekvátní doporučení. Z tohoto důvodu je snahou identifikovat specifické znaky pro jednotlivé klinické situace, které by umožnily standardizaci rutinního rozhodovacího procesu v okamžiku, kdy je možné ovlivnit budoucí výsledek. Cílem tohoto přístupu je naplňování principů precizní medicíny umožňující minimalizaci rizika nepříznivého průběhu onemocnění a rozvoji s onemocněním a/nebo léčbou asociovaných komplikací na úrovni konkrétního pacienta. Všechny původní práce sdělení spojuje primární záměr přispět ke zlepšení péče o dětské pacienty se zánětlivými střevními onemocněními díky nově identifikovaným možnostem predikce konkrétního klinického jevu za využití metod biomedicínské informatiky.

1.1.1. Prediktivní modelování

Prediktivní modelování slouží k vytvoření nástroje k předpovědi buď konkrétní číselné hodnoty určité veličiny, nebo k odhadu, zda dojde nebo nedojde k určité události. K vytvoření predikčního modelu lze použít různé metody, které lze obecně rozdělit do dvou kategorií: matematické/ statistické modelování a počítačové modelování s využitím metod strojového učení. Než je možné vyvinout jakýkoli nový predikční nástroj, je třeba retrospektivně nebo prospektivně shromáždit data o jednotlivých pacientech stran prediktorů a odpovědi. Informace

z jednotlivých proměnných jsou v modelu využity k odpovědi na konkrétní hlavní otázku. Ke zjištění, které proměnné jsou nejreprezentativnější, a jejich spojení s předpokládaným výsledkem se používá konkrétní statistický algoritmus.

Collins et al. vytvořil doporučení pro tvorbu klinických predikčních modelů – TRIPOD (Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis)[1]. Navíc Steyerberg v roce 2019 aktualizoval svoji práci popisující tzv. sedm kroků modelování [2]. Ideální studie s cílem tvorby predikčního modelu by dle jeho doporučení měla dodržet následující pravidla: výběr správného designu studie, určení potřebné velikosti souboru, při tvorbě modelu pak vhodnou transformaci proměnných, vhodný způsob, jak se vypořádat s neúplnými údaji, a především správný výběr prediktorů. V rámci samotného modelování neopomenout hodnocení funkčnosti modelu a jeho validace. Vnitřní validace modelu se pak vypořádává s problémem přesycení modelu a zevní s otázkou zobecnitelnosti modelu.



Obr. 1 Převedení predikčních modelů z raného vývoje do širokého klinického použití [3].

Proces zahrnuje progresivní fáze:

- definice nenaplněné klinické potřeby; způsob identifikace prediktorů;
- výběr prediktorů a vývoj modelu;
- validace; ověření, jak model funguje ve stále více zobecnitelných podmínkách;
- aktualizace/zlepšení; kdy se do modelu přidávají nové parametry a/nebo větší velikost vzorku ve snaze zlepšit jeho přesnost a zobecnitelnost;

- posouzení vlivu modelu na klinické pracovní postupy a hodnocení zdravotních a ekonomických dopadů v kontrolovaném prostředí;
- provádění; implementace modelu do širokého použití a sledování jeho dlouhodobých dopadů v běžné klinické praxi.

1.1.1.1. Návrh studií pro vývoj predikčního modelu a velikost souboru

Pro vývoj prognostického predikčního modelu je vhodným designem studie prospektivní kohortová. Prospektivní sběr dat je důležitý z toho důvodu, že mohou být do zpracování přidány kandidátní prediktory, které nejsou součástí rutinní klinické péče. V retrospektivní studii by mohly některé kandidátní prediktory z různých důvodů chybět (nejsou součástí běžné praxe; data byla dříve shromážděna pro jiné účely, než je vývoj predikčního modelu; apod.). U tvorby diagnostických modelů (tj. předpovídá stav, který je již přítomný nebo nepřítomný), může stačit studie průřezová. V takovém návrhu jsou kandidátní prediktory i výsledek měřeny najednou.

Definování velikosti souboru pro tvorbu predikčních modelů je volen dle techniky, která je k tvorbě modelu používána. Jedním z pravidel používaných k odhadu potřebné velikosti souboru je poměr událostí a prediktorů. U regresních modelů je potřeba znát poměr mezi počtem událostí a počtem kandidátních prediktorů, resp. tzv. minimální počet událostí na parametr prediktoru (EPP, Events per Predictor Parametr) [4]. Tento způsob předpokládá, že při definovaném počtu prediktorů je minimální velikost souboru dána počtem událostí, nebo, pokud není možné velikost souboru měnit, je třeba změnit maximální počet kandidátních prediktorů. Podle simulační studie s použitím logistické regrese, lze predikční model bezpečně vytvořit, pokud na 1 proměnnou připadá 10 a více událostí [5]. Přitom událost je definována jako nejméně častá odpověď. Naopak následující dvě simulační studie ukázaly, že tento způsob přesný není [6,7]. Podobná pravidla existují pro různé regresní modely (pro Coxův regresní model proporcionálních rizik se navrhuje zahrnout alespoň 10 selhání pro každý potencionální prediktor a pro model lineární regrese alespoň 2–10 pacientů pro každý potencionální prediktor; u modelů, které obsahují binární prediktory, se navrhuje zahrnout alespoň 20 událostí na proměnnou; ap.) [4,5,8,9]. Není však zaručeno, že při dodržení těchto obecných pravidel nedojde k přesycení modelu.

1.1.1.2. Výběr a úprava prediktorů

Zásadním krokem při tvorbě predikčního nástroje je výběr vhodných prediktorů do výsledného modelu (obr. 2). Pomocí vzájemné asociace jednotlivých proměnných je třeba posoudit, které proměnné jsou v příliš těsném vztahu a predikci by neprospěly. Výběr proměnných je jedním z nejobtížnějších aspektů vytváření modelu a měl by vycházet z klinické znalosti, předchozí literatury a samotné metody statistického výběru [10–13]. Existují různé techniky výběru proměnných, strategie jejich redukce a výběrová kritéria (viz dále) při vytváření predikčního modelu [9,10,13–15]. Správný výběr proměnných přináší řadu výhod, jako je zlepšení výkonu modelu z hlediska predikce, zkrácení doby trénování modelu, usnadnění vizualizace dat a lepší pochopení základního procesu, který generoval data [11,16].

Obecně není vhodné zahrnovat do modelu velkou sadu proměnných. Model je pak více závislý na pozorovaných datech a může docházet k tzv. přesycení modelu (Overfitting). Přesněji řečeno, příliš mnoho proměnných (s ohledem na počet pozorování/ soubor dat) povede k vytvoření vztahu mezi proměnnými a výsledkem, který existuje pouze v tomto souboru dat, ale nikoli ve skutečné populaci. To povede ke snížení přesnosti modelu (pravděpodobnost odhalení efektu, když efekt již je znám/ existuje) [2,13,17]. Navíc konkrétní proměnné nemusí být dostupné pro všechny pacienty nebo jejich sběr může být nákladný. Naopak některé proměnné můžou mít zanedbatelný vliv na výsledek, a proto je lze vyloučit [18]. Nicméně, neexistuje jednotné pravidlo pro stanovení počtu proměnných, které mají být zahrnuty do modelu (více v části 1 Návrh studií pro vývoj predikčního modelu a velikost souboru).

Obr. 2 Kroky při výběru proměnných pro predikční model [12]

1.1.1.3. Strategie redukce proměnných

Jednou ze strategií jak redukovat počty potenciálních prediktorů, je vybrat jen ty, které se zdáli být nejsilnější v dříve publikovaných původních pracích. K identifikaci kandidátních proměnných slouží také systematické přehledové články a meta-analýzy [11,17,19]. Dalším způsobem redukce proměnných je seskupení nebo vyloučení souvisejících proměnných. Odstranění proměnných, které jsou těsně asociované, by nemělo negativně ovlivnit fungování modelu, jelikož reprezentují podobnou informaci. Rozhodujícím faktorem k omezení proměnných může být také způsob jejich distribuce, např. velký počet chybějících hodnot u použité proměnné může snižovat spolehlivost modelu [18,20].

1.1.1.4. Metody selekce proměnných

Jakmile je ze seznamu všech dostupných proměnných v souboru dat identifikován počet potencionálních proměnných, je třeba z nich vybrat ty, které zahrneme do konečného modelu [11]. Jedním ze způsobů je jednorozměrná analýza každé proměnné. Následně ty proměnné, které vykazují prediktabilitu v jednorozměrné analýze by měly být zahrnuty do vícerozměrné analýzy. Nicméně, tento postup ignoruje fakt, že některé proměnné, i když jsou slabě asociované s výsledkem, mohou významně přispět k síle predikce, pokud jsou kombinovány (pozn. to lze částečně vyřešit nastavením hladiny významnosti) [21]. Výhodné může být použití formálních metod:

a) zpětná eliminace (Backward Elimination),
b) dopředný výběr (Forward Selection),
c) postupný výběr (Stepwise Selection),
d) výběr všech možných kombinací (All Possible Subset Selection).

Zpětná eliminace je nejjednodušší metodou výběru proměnných. Začíná úplným modelem, který bere v úvahu všechny proměnné, které mají být zahrnuty do modelu. Proměnné jsou pak na základě testovací statistiky jedna po druhé z modelu odstraňovány tak, aby v modelu zbyly jen ty proměnné, které významně přispívají k predikci [12,22].

Metoda dopředného výběru je opakem předcházejícího přístupu. Začíná bez proměnných v modelu, a poté na základě testovací statistiky je přidávána jedna proměnná za druhou. Tento postup je méně rizikový z hlediska kolinearity (velmi vysoké asociace mezi

proměnnými). Zásadní nevýhodou obou metod je, že přidání/ odstranění proměnné může učinit existující proměnnou nevýznamnou (tzn. proměnná je významná v kombinaci s jinou proměnnou). Tento problém řeší metoda postupného výběru [12,23].

Postupný výběr je v oblasti klinického výzkumu široce používanou technikou variabilního výběru. Tato metoda je kombinací dopředných a zpětných postupů, které umožňují „pohyb" v obou směrech, přidávání a odebírání proměnných v různých krocích. Metoda postupného výběru vyžaduje dvě samostatné hladiny významnosti pro přidávání a redukci proměnných z modelu. Hladiny významnosti pro přidávání proměnných by měly být nižší než pro mazání proměnných, aby se proces nedostal do nekonečné smyčky [2,14,24].

Metodou výběru všech možných kombinací se kontroluje každá možná kombinace proměnných, aby se určila nejlepší podmnožina proměnných pro predikční model. Pomocí tohoto postupu jsou vytvořeny všechny modely s jednou proměnnou, se dvěma proměnnými, se třemi proměnnými atd. Pokud existuje K proměnných, pak existuje 2^K modelů, které lze sestavit [25].

1.1.1.5. Kritéria pro výběr proměnných

U všech metod postupného výběru je potřeba nastavit kritéria pro zahrnutí nebo vyloučení proměnných. Obecně se pro testování hypotéz používá standardní hladina významnosti (p-value), která není vhodná pro všechny typy modelů. S výhodou lze použít statistické metody jako Akaike informační kritérium (Akaike Information Criterion, AIC), Bayesovské informační kritérium (Bayesian Information Criterion, BIC), Mallowova statistika Cp (Constant p) [2,14].

Tradiční volba hladiny významnosti je 0,05 nebo 0,1, nicméně je doporučeno volit vyšší hladinu pro výběr proměnných, aby nedošlo ke ztrátě proměnných relevantních pro výsledek [21,26].

AIC je nástroj porovnávající různé modely. V průběhu vytváření modelu může docházet k určité ztrátě informací. Proto model nemůže přesně reprezentovat skutečný vztah, který existuje v datech. AIC se snaží odhadnout relativní ztrátu informací ve srovnání s jinými potencionálními modely. Předpokládá se, že kvalita modelu je lepší s menší ztrátou informací a cílem je vybrat model, který minimalizuje toto riziko [27–29].

BIC je další kritérium výběru proměnných, které je podobné jako AIC, ale s jinou penalizací za počet proměnných zahrnutých v modelu. Dle AIC je nejlepší model pouze aproximací skutečného modelu a skutečný model, který reprezentuje realitu, neexistuje. Naopak základní teorií BIC je, že data jsou odvozena z jednoduchého modelu a existuje kandidátní model, který představuje skutečný model. Jinými slovy, pokud je našim cílem vybrat nejlepší model, který poskytne maximální přesnost predikce, pak je vhodnější AIC. Pokud je však cílem vybrat správný model, který je konzistentní, použijeme BIC [27,30].

Mallowova statistika Cp se používá jako kritérium pro výběr modelu sestaveného pomocí podmnožiny proměnných [31].

1.1.1.6. Typy predikčních nástrojů a tvorba modelu

Ke generování predikčních modelů v biomedicíně můžeme použít jak vícerozměrné statistické metody, tak techniky strojového učení (Machine Learning, ML). ML je aplikace umělé inteligence (Artificial Intelligence, AI). AI označuje schopnost programovat počítače (nebo obecněji stroje), které jsou schopny řešit komplikované a obvykle velmi časově náročné úkoly [32–35]. Jak statistické metody, tak ML se používají v analýze k dosažení stejného cíle – k získání prediktivního modelu.

Jednou z nejčastějších statistických metod ve vývoji predikčních nástrojů jsou regresní techniky, jejichž výsledkem je regresní vzorec. Cílem predikčního regresního modelu je rozřadit pacienty podle míry pravděpodobnosti dosažení určitého výsledku. Například, pokud má pacient zvýšenou šanci na relaps onemocnění, lékař se může rozhodnout pro agresivnější léčbu, nebo pokud má pacient vysoké riziko vzniku vedlejších účinků, může být indikována mírnější léčba.

Výsledné proměnné můžeme rozlišit na spojité proměnné nebo kategorické. Spojité proměnné jsou popsány číselnými hodnotami a k jejich predikci se často používají lineární modely. Kategorické proměnné jsou omezeny na počet tříd nebo kategorií a pro jejich predikci používáme klasifikační modely. Pokud má výsledek dvě kategorie, označuje se to jako binární klasifikace a typickými technikami jsou rozhodovací stromy a logistická regrese. Pro binární výstupy lze rozlišit dva typy predikčních nástrojů: (A) diagnostický predikční model, k odhadu diagnózy; a (B) prognostický predikční model, k odhadu prognózy pacienta.

Podle předpokládaného vztahu mezi odpovědí a prediktorem se u regresních modelů volí konkrétní typ regresní techniky (modely předpokládají aditivitu a linearitu asociací mezi prediktory a výsledkem (v lineární regresi), mezi prediktory a logaritmickou pravděpodobností výsledku (v logistické regresi) nebo mezi prediktory a log hazard nebo log kumulativním rizikem (v Coxově regresním modelu proporcionálních rizik)). Předpoklad linearity znamená, že sklon regresní křivky (nebo odhadovaného koeficientu) má stejnou hodnotu v celém rozsahu prediktoru a předpoklad aditivity znamená, že změna hodnoty některého z prediktorů bude mít vždy stejný účinek na výsledek, bez ohledu na hodnoty ostatních proměnných. Regresní metody nekladou předpoklady na distribuci prediktorových proměnných. Nicméně některé spojité proměnné (např. hladiny biomarkerů) často fungují lépe po transformaci na zhruba normální distribuci (u proměnných, které mohou nabývat jen kladných hodnot, se používá přirozený logaritmus) [36]. Analýzy vyžadující typ prediktivního modelování pracujícího s číselnými nebo spojitými proměnnými využívají statistické metody vícenásobné regrese. V mnoha klinických situacích je důležité nejen to, zda k jevu či výsledku dojde, ale také za jak dlouho. To se nazývá analýza času do události a typickým příkladem je analýza přežití. Kaplan-Meierovy křivky jsou široce používány pro zkoumání vlivu kategorických proměnných [37], zatímco Coxův regresní model proporcionálních rizik navíc umožňuje zkoumání kvantitativních proměnných [38].

Jak již bylo zmíněno, kromě standardních statistických metod můžeme použít techniky ML a/nebo jejich kombinaci. Tyto metody jsme v našich studiích neaplikovali, nicméně jsou důležitým nástrojem v procesu prediktivního modelování, proto je zde okrajově uvedu. Rozhodovací stromy (Decision Trees) jsou v medicíně oblíbené, protože koncept rozhodovacího stromu odráží postup lékaře, když pacientovi klade řadu otázek, až kým nedojde k určitému závěru/ rozhodnutí [39]. Počáteční bod a body, kde se strom větví se nazývají „kořenový uzel“ a „vnitřní uzly“. Koncové body každé větve se nazývají „listy“. Existuje široká škála algoritmů rozhodovacího stromu, které mohou předpovídat binární, ale i vícenásobné výsledky. Jako každý model bude rozhodovací strom předpovídat výsledek na základě hodnot vstupních atributů. K trénovaní predikčního modelu lze použít další, složitější algoritmy založené na ML. Support Vector Machine neboli SVM je modelovací metoda, která velmi dobře pracuje s vysokorozměrnými daty na principu jejich transformace z nelineárního do lineárního prostoru [40]. Nevýhodou je náročnost a doba provedení výpočtu, a také vyšší riziko přesycení modelu při použití této metody. Přesnost predikce lze zvýšit kombinací předpovědí více modelů pomocí tzv. „meta-learnerů“ neboli Ensebmle metod. Tyto metody pracují tak, že generují základní

klasifikátory ze sady tréninkových dat. Existují dva přístupy: tzv. „posílení“ modelu neboli Boosting, a „balíčkování“ modelu neboli Bagging [41]. Boosting vytváří řadu modelů stejného typu (jako jsou rozhodovací stromy), kde jsou nové modely ovlivněny tím, jak dobře fungovaly předchozí modely [42]. Jde o interaktivní proces, kde se nové modely zaměřují na nápravu nepřesností vzniklých v dřívějších iteracích. Jinými slovy, instance v trénovací sadě, které byly dříve nesprávně klasifikovány, budou mít prioritu v následném vytváření modelu. Bagging se od boostingu liší tím, že staví modely nezávisle na sobě a nenabízí modelům žádné váhy v procesu hlasování [41]. Aby bylo možné předpovědět pravděpodobnost přežití ve zvoleném časovém rozpětí, poskytuje většina metod ML binární výsledek [43,44]. Nicméně lze kombinovat metody ML s Bayesovou větou, která je prostředkem pro výpočet podmíněných pravděpodobností. Kromě toho je možné vytvořit hybridní model, který má strukturu rozhodovacího stromu, ale listy jsou klasifikátory – tzv. naivní Bayesův klasifikátor, které poskytují pravděpodobnosti binárních výsledků [43,44].

1.1.1.7. Software

V současnosti je k dispozici mnoho různých softwarových prostředí pro generování predikčních modelů. Některé z nich, např. Python, R nebo Matlab, vyžadují znalosti programování, nicméně mají k dispozici integrovaná vývojová prostředí, jako je RStudio pro R a Spyder pro Python. Navíc mohou mít bohaté open-source knihovny přizpůsobené speciálně pro ML, například Caret pro R a Scikit-learn pro Python [45,46]. Jiné balíčky mají grafické uživatelské rozhraní a schopnost programovat není povinná, jako Statistical Package for the Social Sciences (SPSS), Statistical Analysis System (SAS) nebo Orange. Zvláštní zmínka je vyhrazena distribuci Anaconda, která na jediné platformě hostí mnoho nejpoužívanějších softwarových balíků pro predikční modelování (RStudio, Spyder, Jupyter Notebook, Orange a další) [https://www.anaconda.com/distribution/]. K tomu, jak správně a efektivně tato prostředí a balíky využívat, slouží online dostupné kurzy (někdy označované jako Massive Open Online Courses, MOOC).

1.1.1.8. Použití klinických predikčních modelů

Steyerberg definoval oblast veřejného zdraví, klinickou praxi a lékařský výzkum jako tři hlavní aplikační odvětví pro klinické predikční modely. Predikce budoucího výskytu onemocnění je jedním z hlavních účelů, který by mohl být použit pro cílení preventivní intervence v oblasti veřejného zdravotnictví [47]. Pro využití v klinické praxi máme několik možností: (I) rozhodnout, zda potřebujeme další testování, na základě predikce pravděpodobnosti základního onemocnění (např. poskytnout invazivní a nákladný test pacientům s vysokým rizikem rozvoje onemocnění, abychom snížili zbytečné poškození; (II) rozhodnout, zda potřebujeme zahájit léčbu/použít intenzivnější léčbu/provést nákladovou efektivitu léčby/odložit léčbu pomocí rozhodovací analýzy (např. léčbu zahájíme po diagnostickém zpracování, pokud je pravděpodobnost diagnózy vyšší než léčebný práh (pravděpodobnost, kdy se očekávaný přínos léčby rovná očekávanému přínosu vyhnutí se léčbě)); (III) rozhodnout, zda potřebujeme operaci, vyvážením krátkodobých rizik (např. 30denní mortalita) a dlouhodobých rizik (např. dlouhodobého přežití). V oblasti medicínskeho výzkumu máme dvě hlavní možnosti využití: (I) v intervenčních studiích pro výběr vhodných účastníků a úpravu kovariát. (II) v observačních studiích pro úpravu zavádějících faktorů [47].

1.2. Zánětlivá střevní onemocnění

Zánětlivá střevní onemocnění (IBD) představují skupinu chronických, polygenně podmíněných onemocnění postihujících primárně gastrointestinální trakt. Mezi tyto nemoci řadíme Crohnovou nemoc (CD), ulcerózní kolitidu (UC) a tzv. neklasifikovatelné IBD (IBD-U) [48]. Nicméně klasifikace IBD je složitá a vyznačuje se mnoha vzácnými fenotypy, které jsou atypické nebo neobvyklé. Vyžaduje rozpoznání typických znaků CD a UC, identifikaci atypických fenotypů, které jsou stále v souladu s diagnózou CD nebo UC, a znalost těch faktorů, které vylučují diagnózu jednoho nebo druhého [49]. Stále aktuálnějším tématem je klasifikace onemocnění ve vztahu k odpovědi na zvolenou terapii [49].

1.2.1. Klinický obraz zánětlivých střevních onemocnění

Přestože příznaky IBD mohou být různorodé, jedná se především o klinické projevy

chronicky probíhajícího zánětlivého postižení gastrointestinálního traktu [50–52]. Krvavý průjem je nejčastějším příznakem UC, zatímco CD se může projevovat neurčitou bolestí břicha, průjmem, nevysvětlitelnou anémií, horečkou, ztrátou hmotnosti nebo zpomalením růstu. Klasická „triáda" bolesti břicha, průjmu a hubnutí se vyskytuje pouze u 25% pacientů s CD [53]. Extraintestinální projevy, mezi které patří např. artritida, kožní projevy (erythema nodosum, pyoderma gangrenosum) nebo uveitida, se mohou projevit při diagnóze u 6 % až 23 % dětí s vyšší frekvencí u dětí starších 6 let [51,54,55].

V průběhu sledování pacientů je typické střídaní klidových období onemocnění (remise) a vzplanutí zánětlivé aktivity (flare). Navození a následné udržení remise má význam ve více rovinách péče o pacienta a jeho prognóze, jelikož časté nebo déletrvající období s vysokou aktivitou onemocnění mohou nepříznivě ovlivnit samotný střevní zánět a jeho průběh [56]. Navíc mohou vést k rozvoji s onemocněním asociovaných komplikací a také významně zasahovat do psychosociální roviny života pacienta [57].

1.2.2. Etiologie a patogeneze zánětlivých střevních onemocnění

Etiologie IBD nebyla dosud spolehlivě popsána. Na rozvoji imunitně podmíněného střevního zánětu se s velkou pravděpodobností podílí kombinace více faktorů u geneticky disponovaného jedince, proto mluvíme o polygenně podmíněných multifaktoriálních onemocněních. Je zřejmé, že zde zásadní význam hrají faktory zevního prostředí [58,59]. Existují také vzácně se vyskytující monogenně podmíněné typy IBD, kde je fenotyp onemocnění definován konkrétním genetickým defektem a zevní prostředí nehraje prakticky žádnou roli [60–62].

Hledání a studium rizikových faktorů ve vztahu k IBD je zájmem mnoha vědeckých skupin. Co se týče faktorů zevního prostředí, jsou zde zásadní limitace v jejich interpretaci, jelikož práce na toto téma byly provedeny jako observační studie. Přináší nám tedy informaci o asociaci mezi IBD a sledovaným rizikovým faktorem, nikoli o kauzalitě. Studie u dospělých popisují asociaci mezi kouřením cigaret a zvýšeným rizikem rozvoje CD, a to až o 90% u aktivních kuřáků ve srovnání s kontrolní skupinou [63]. Naopak u diagnózy UC je kouření asociováno s nižším rizikem vzniku onemocnění a je proto chápáno jako protektivní faktor [64]. Kontroverzní jsou práce posuzující vztah CD a appendektomie považovanou za rizikový faktor

rozvoje onemocnění. Jelikož u sledovaných pacientů dochází k poklesu rizika na populační v průběhu pěti let po chirurgickém výkonu, mohlo by se jednat o nepřesnost v původní diagnóze [65]. Velkým tématem je i vliv stravování ve vztahu k IBD. Několik prací popisuje nepříznivý vliv tzv. „západního" trendu ve stravování na rozvoj onemocnění [66,67]. Recentní studie včetně rozsáhlé meta-analýzy ukazují na asociaci mezi zvýšenou konzumací tzv. průmyslových neboli „ultrazpracovaných" potravin a výskytem CD [68,69]. Ve srovnání s UC by mohla mít strava u CD větší význam a biologický účinek [68]. V jiné studii byl vyšší příjem vlákniny spojený s nižším rizikem vzniku CD [70]. Střevní mirkobiom je u pacientů s IBD velmi podrobně zkoumán a v současnosti přináší vědecká sféra řadu publikací na toto téma týkající se kvantity, diverzity a zastoupení jednotlivých mikrobiálních kmenů a jejich produktů [71,72]. Nicméně, nebylo jednoznačně prokázáno, jestli jsou dané změny mikrobiomu příčinou nebo následkem střevního zánětu. V souvislosti s infekcemi nebo antibiotickou léčbou jsou výsledky ve vztahu ke vzniku IBD kontroverzní bez jednomyslného závěru [73–78]. Nejednoznační výsledky přináší i práce u dětských pacientů sledující možný protektivní vliv kojení na rozvoj IBD a také studie k ověření hygienické hypotézy nebo zabývající se psychickým stresem ve vztahu k IBD [59,74,79,80]. Genetická predispozice pacienta se uplatňuje v patogeneze onemocnění u všech jeho forem s větším podílem u pacientů s CD (nejsilněji asociované varianty jsou například v genech *NOD2*, *IL23R*, *ATG16L1*, *IRGM*, *PTPN22*) [81]. Významnou roli sehrávají genetické defekty u vzniku monogenně podmíněných IBD charakterizované velmi časným nástupem (poruchy signalizace IL10, IPEX syndrom, deficience XIAP, chronická granulomatóza a další) [62].

1.2.3. Epidemiologie zánětlivých střevních onemocnění

Výskyt IBD je charakterizován významnou geografickou rozdílností [82–84]. Nicméně, tyto rozdíly se s narůstající industrializací v oblastech s rychlým socio-ekonomickým rozvojem postupně zmenšují [82,84]. Počet pacientů s diagnózou IBD v zemích „západního světa" se nestabilizoval a i nadále narůstá [84–86]. Severní Amerika a Evropa jsou regiony s nejvýznamnějším výskytem IBD v populaci a vůbec nejvyšší incidence IBD byla zaznamenána na území Faerských ostrovů [87]. Dle nedávné meta-analýzy je celkový počet IBD ve Spojených státech amerických aproximován na 1,5 milionu pacientů [82]. Odhaduje se, že v Evropské unii je IBD postiženo 1,3 milionu obyvatel, a předpokládá se, že do roku 2030 bude prevalence

dosahovat 1 % [88,89].

V dětské populaci je pozorován tzv. severo-jižní gradient výskytu IBD, což opět odkazuje na možný podíl faktorů zevního prostředí na vznik onemocnění. Celkový trend incidencí CD i UC u dětí je nepříznivý a poukazuje na nárůst počtu dětských IBD (v roce 2022 byla v západní Evropě uváděna incidence v rozmezí 5,4-17,4 / 100 000 s odhadovanou prevalencí 58,9-66,3 / 100 000) [90]. V České republice je incidence následující: CD 5,2-6,2 / 100 000 a UC 2,8-3,4 / 100 000 [91].

1.2.4. Diagnostika zánětlivých střevních onemocnění

Diagnóza IBD v dětském věku je dle současných doporučení mezinárodních společností stanovena až po provedení endoskopického vyšetření gastrointestinálního traktu – ezofagogastroduodenoskopie a totální koloskopie do terminálního ilea s histologickým vyšetřením bioptických vzorků ze všech vizualizovaných etáží a zobrazení tenkého střeva (nejčastěji magnetickou rezonanční enterografií, MRE) a jejich zhodnocení podle tzv. Portských kritérií, jež byly aktualizovány [48,92].

Na základě endoskopického vyšetření je možné s velkou pravděpodobností rozhodnout o přítomnosti, resp. nepřítomnosti diagnózy. Jelikož se jedná o invazivní vyšetření u dětí probíhající v celkové anestezii, je na místě použít jiný nástroj k identifikaci pacientů s příznaky, kteří nakonec podstoupí kompletní diagnostický algoritmus. V úvodu by měla být vyšetřena možná infekční příčina klinických příznaků. Dalším krokem je pátrání po laboratorních známkách imunitně podmíněné zánětlivé aktivity ve střevě (CRP (C-reaktivní protein), ESR (sedimentace erytrocytů), hladina hemoglobinu a albuminu, počet trombocytů), které s různou senzitivitou a specificitou samostatně a v kombinacích poukazují na možnou přítomnost IBD. Vyšetření hladiny protilátek ANCA (anti-neutrophil cytoplasmic antibody; protilátky proti cytoplasmě neutrofilů) a ASCA (anti-*Saccharomyces cerevisiae* antibody; protilátky proti *Saccharomyces cerevisiae*) nepřináší z pohledu diagnostiky IBD zásadní informaci ve srovnání z výše uvedenými hematologickými a biochemickými parametry [93]. Naopak klíčový význam pro diagnostický screening mají biomarkery získané ze stolice [94]. Nejvíce studovaným markerem střevního zánětu je v tomto smyslu fekální kalprotetkin (FC), kterému je věnovaná samostatná podkapitola disertační práce [95–97].

Vybraní pacienti dále podstupují kompletní diagnostický algoritmus popsaný v úvodu. Dle Portských kritérií je možné pacientovi přiřadit jeden ze tří fenotypů (CD, UC, IBD-U). K bližšímu určení intenzity a rozsahu střevní zánětu slouží tzv. Montrealská klasifikace a její Pařížská modifikace pro dětské pacienty [98,99].

1.2.5. Terapie zánětlivých střevních onemocnění a její možné nežádoucí účinky

Identifikace terapeutického cíle je jedným z nejdůležitějších kroků v rámci péče o pacienty s IBD. Pro klinickou praxi byl na základě systematického prohledání dostupné literatury a názorů odborníků navržen tzv. „Treat-to-Target" koncept s definicí optimálních léčebných cílů. Záměrem tohoto přístupu je selektovat pacienty z pohledu co nejpřesnější predikce nekomplikovaného průběhu onemocnění [100]. Volba léčebné strategie se odvíjí od základní diagnózy (CD vs. UC), nicméně s narůstajícím významem personalizované terapie pacientů s IBD se liší i v rámci jedné diagnózy [101–103]. V tomto ohledu pak sehrávají důležitou roli informace o rozsahu a chování onemocnění, věku pacienta, přítomnosti poruchy růstu, výskytu možných nežádoucích účinků souvisejících s léčbou a kvalitě života pacienta [101]. Významným aspektem při volbě správného terapeutického postupu je identifikace pacientů ve vysokém riziku komplikovaného průběhu onemocnění. Za možné prediktivní faktory jsou považované mladý věk v době diagnózy, extenzivní forma nemoci, postižení horního gastrointestinálního traktu, zánětlivé postižení ilea, perianální CD, strikturující nebo penetrující forma onemocnění, výskyt hlubokých ulcerací při endoskopickém vyšetření, kouření, nutnost užívání systémových kortikoidů v době diagnózy a některé varianty genu *NOD2* [73,104–111]. Vychází se ze studií na kohortách dospělých pacientů s IBD, jelikož data u dětí nejsou jednotná a faktory predikující komplikovaný průběh nebyly dosud jasně vymezené [112].

Terapeutické přístupy jsou v současnosti mnohem promptnější než v minulých dekádách, což znamená, že pacienti jsou již v počátečních stádiích nemoci (především u CD) stále častěji léčeni intenzivní terapií. I přesto je v léčbě pacientů s nově diagnostikovanou nekomplikovanou formou CD doporučena indukce remise pomocí speciální diety. V případě pacientů s mírnou až středně těžkou aktivitou onemocnění (definované na základě klinického indexu wPCDAI) je k navození remise možné použít tzv. Crohn´s Disease Exclusion Diet (CDED) místo hůře tolerované výhradní enterální výživy (EEN) [101,112]. V publikované

intervenční studii dat oba dietní přístupy vedly k navození remise u dětských pacientů s nově diagnostikovanou CD, nicméně CDED byla pacienty lépe tolerována [113]. V případě intolerance nebo nedostatečného efektu dietních opatření je možné v rámci indukce remise přistoupit k podání kortikoidů nebo zvolit kombinovanou antibiotickou terapii [114]. Současně s indukční léčbou se doporučuje zahájení udržovací terapie [115]. U dětských pacientů byl prokázán poměrně velký protektivní vliv thiopurinů na dobu trvání remise [116]. Dalším preparátem používaným k udržení remise onemocnění je methotrexát. Jeho indikace však vychází zejména z prací na dospělých pacientech [117]. V případě jedinců s rizikem komplikovaného průběhu je indikována intenzivní terapie ihned v úvodu s použitím preparátů inovativní a biologické léčby. V současné době nejčastěji protilátky proti tumor-nekrotizujícímu faktoru alfa (aTNF) infliximab (IFX) nebo adalimumab (ADA) [101]. U části pacientů s lokalizovanou formou CD s přítomností striktury v ileocékální oblasti a prestrikturickou dilatací, penetrující formou onemocnění, těžkou růstovou retardací nebo refrakteritou ke konvenční terapii je možné indikovat chirurgickou léčbu, a to nejčastěji provedení ileocékální resrekce [101]. Následně je potřebné pravidelné endoskopické sledování pacientů a v případě rekurence onemocnění směřovat k eskalaci terapie [118]. U dospělých pacientů byly definovány rizikové faktory související s nutností další léčby, které jsou vzhledem k insuficientním pediatrickým datům aplikovány i u dětí [119].

V léčbě UC má zásadní význam objektivní hodnocení intenzity onemocnění. U dětských pacientů je využíván klinický index aktivity onemocnění PUCAI, který těsně koreluje s endoskopickou aktivitou kolitidy [120,121]. Podle definitivního skóre se volí teraputický postup – u mírné až středně aktivního onemocnění lze zahájit monoterapii 5-aminosalicyláty (5ASA); od středně závažných UC by se mělo zvážit podání systémových kortikoidů. Po navození remise se doporučuje pokračovat v udržovací terapii 5ASA. U pacientů s trvající aktivitou onemocnění nebo s opakovanými relapsy se doporučuje do terapie přidat thiopuriny. Preparáty biologické léčby jsou volbou pro jedince s kortikodependentní nebo chronicky aktivní formou onemocnění. V případě život ohrožující akutní těžké kolitidy je vždy zahájena terapie intravenózně aplikovanými kortikosteroidy a současně přerušena terapie 5ASA. Pokud nedochází k odpovědi na danou terapii, je indikována eskalace ke druhé linii léčby reprezentované IFX, tacrolimem nebo cyclosporinem [122]. Alternativou dané situace je použití nových léčebných modalit (inhibitory Janus kinázy (JAK)), ev. v případě pacientů neodpovídajících na medikamentózní léčbu se přistupuje k provedení kolektomie [122,123].

Od roku 1999, s příchodem preparátů biologické léčby pro IBD (aTNF; IFX), se cíle

léčby posunuly od dosažení klinické remise ke kombinaci kontroly symptomů a slizničního hojení (mucosal healing (MH)) [124]. Mezitím se terapeutické armamentarium podstatně rozšířilo zavedením několika dalších aTNF protilátek (včetně ADA; golimumabu (GLM) a subkutání formy IFX), anti-integrinové léčby (např. vedolizumab (VDZ)) a protilátkám tlumícím anti-IL12 /23 (např. ustekinumab (UST), rizankizumab (RZB), mirikizumab) [125–128]. Postupně se do klinické praxe dostávají tzv. malé molekuly především ze skupiny JAK inhibitorů (např. tofacitinib, upadacitinib), a také modulátor receptoru pro sfingosin-1-fosát (ozanimod) [126,129]. Přibližně jedna třetina pacientů s IBD nereaguje na léčbu aTNF (primární nonrespondéři) a dalších 30 % pacientů ztrácí v průběhu času odpověď [130–132]. Bez primární odpovědi na VDZ a UST je přibližně 40 % a 35 % pacientů, nicméně se jedná o skupinu pacientů refrakterních ke konvenční terapii [133–136]. Studie VARSITY je head-to-head RCT zaměřená na rozdíly v účinnosti konkrétních preparátů biologické léčby srovnávající VDZ s ADA u pacientů s UC. V 52. týdnu terapie byla v klinické a endoskopické remisi významnější proporce pacientů léčených VDZ (31 % versus 23 %, P = 0,006) [137]. Tyto výsledky podporují další studie u pacientů s UC, kdežto u jedinců s CD nebyly nalezeny zásadní rozdíly mezi VDZ a léčbou aTNF preparáty [138,139]. U pacientů s CD, u kterých selhala aTNF terapie, byla prokázána vyšší míra endoskopické remise v případě preparátu RZB ve srovnání s UST [140,141]. Další práce SEAVUE neprokázala lepší účinnost UST oproti ADA u pacientů se středně těžkou až těžkou CD [142]. Z network meta-analýzy vyplývá, že účinnost aTNF preparátů je podobná jako u VDZ. Nicméně, u IFX je popisována vyšší úspěšnost v rámci indukční léčby a u UST naopak nižší míra odpovědi a remise na konci indukční fáze terapie ve srovnání s VDZ [143]. Jak naznačuje další network meta-analýza u pacientů se středně těžkou až těžkou CD by měl být v první linii preferován IFX v kombinaci s AZA nebo ADA v monoterapii, následován RZB nebo ADA ve druhé linii indukční léčby [125]. Data z reální klinické praxe poukazují na srovnatelnou účinnost UST a aTNF preparáty v navození remise u pacientů s CD [144].

Jednotlivé preparáty biologické léčby se liší charakterem i mírou rizika rozvoje nežádoucích účinků. U aTNF je nejčastějším projevem kožní léze psoriasiformního, méně frekventně atopického charakteru, což popisuje i retrospektivní studie u dětských pacientů léčených IFX (48% mělo kožní projev v průběhu 2 let sledování) [145]. Významnou akutní komplikací je infuzní reakce, která je důvodem k ukončení podávání dané biologické léčby. Na jejím vzniku se mohou podílet tvořící se protilátky proti aTNF, které jsou v určitých případech zodpovědné i za ztrátu terapeutické odpovědi [146]. aTNF léčba je také spojena s vyšším rizikem

závažně probíhajících oportunních infekcí [147].

Ve vztahu k nežádoucím účinkům terapie IBD je důležité zmínit thiopuriny. Mezi nejčastější negativní projevy léčby patří gastrointestinální intolerance, reakce alergického typu, myalgie a artralgie, suprese kostní dřeně a rozvoj akutní pankreatitidy [148,149]. Nejobávanějším, i když vzácným, rizikem je rozvoj některých maligních diagnóz [150]. Jsou popisovány lymfoproliferativní onemocnění, u kterých kromě léčby sehrává velmi pravděpodobně svou roli chronicky probíhající zánět [151–153]. Častěji je také uváděn výskyt dvou typů lymfomů, na jejichž vzniku se významně podílí EBV infekce, proto bývá vyšetřován EBV sérologický profil pacienta před zahájením terapie thiopuriny [101].

1.3. Fekální kalprotektin

Fekální kalprotektin (FC) je kalcium a zinek vázající protein patřící do rodiny S100 a představuje přibližně 5 % celkové buněčné bílkoviny a 60 % bílkoviny obsažené v cytosolu lidských neutrofilů [154]. Uvolňuje se především z neutrofilů, v menší míře také z monocytů a aktivovaných makrofágů [155]. FC je odolný vůči bakteriální degradaci a jeho plasmatická hladina se může v průběhu zánětu zvýšit pět až čtyřicetinásobně [95]. Stavy vedoucí ke zvýšenému průniku neutrofilů přes střevní stěnu do lumen střeva způsobují elevaci FC ve stolici, FC lze tedy považovat za specifický k detekci zánětu, nikoli specifický ke konkrétní nemoci[156–159]. Jak u dospělých, tak u dětí s IBD se ukázalo, že hladiny FC korelují s aktivitou onemocnění definovanou klinickými parametry, endoskopickým nálezem a histologií [157,160–163]. FC je i dle aktuálních doporučení považován za vhodný marker pro monitoraci remise i relapsu IBD [100]. Jeho velká variabilita však přináší mnohá úskalí. Na ní se mohou podílet rozdíly v pre-analytické (odběr) i analytické fázi zpracování vzorků (metodika extrakce, odlišné testovací soupravy, apod.). Limitací FC je i definice referenčních hodnot vzhledem k výrazným rozdílům v pediatrickém věkovém rozmezí a vliv individuálních faktorů, jako je strava, léky, apod. [164]. Ve všech těchto oblastech je třeba ještě mnoho objasnit. Lze předpokládat, že bude třeba dle jednotlivých modalit léčby nastavit nejen různá cut-off, ale také různé časové limity [165–167].

1.3.1. Role fekálního kalprotektinu v procesu klinického rozhodování v oblasti zánětlivých střevních onemocnění

V následujících podkapitolách se zaměřuji na využití FC v procesu klinického rozhodování – od konkrétní hodnoty přes skórovací systémy (resp. indexy), až po složité predikční modely - a to v pěti klinických situacích, kterými jsou diagnostika a fenotypizace onemocnění, monitorace aktivity onemocnění, predikce efektu terapie, remise a relapsu onemocnění (obr. 3).

Obr. 3 Klinické situace u pacientů s IBD

1.3.2. Fekální kalprotektin jako samostatný prediktor

1.3.2.1. Fekální kalprotektin v diagnostice zánětlivých střevních onemocnění

Dle studií je FC lepší screeningový nástroj na přítomnost IBD u nediagnostikovaných pacientů než další laboratorní vyšetření, jako je CRP, ESR, počet trombocytů, hladina hemoglobinu, nebo jejich kombinace [48]. V meta-analýze zaměřené na výběr pacientů k endoskopickému vyšetření pro podezření na IBD pomocí FC byla senzitivita 97,8 % (95% CI 94,7-99,6), avšak specificita byla jen 68,2 % (95% CI 50,2-86,3) při použití cut-off hladiny FC 50 µg/g [97,168]. V jiné meta-analýze zahrňující i práce u dětí, která studovala použití FC k odlišení IBD od funkčních onemocnění, byla při použití cut-off hladiny FC 50 µg/g zjištěna senzitivita 93 % (95% CI 85-97) a specificita 96 % (95% CI 79-99) u dospělých, a 92 % (95% CI 84-96) a 76 % (95% CI 62-86) u dětí a adolescentů [169]. Počet indikovaných endoskopických vyšetření by se dle analyzovaných studií v daném období snížil o 67 % u dospělých a o 35 % u dětí [169].

Diferenciální diagnostika zvýšených hladin FC je široká (např. bakteriální nebo virová gastroenteritida, juvenilní polyp, celiakie, cystická fibróza, ap.) [168,170–173]. Nejlepší mezní hodnota ve screeningu IBD je 212 µg/g, která odpovídá senzitivitě 90 % (95% CI 0,87-0,93) a specificitě 87 % (95% CI 0,81-0,88), navíc predikční model založený na koncentraci FC a věku předpověděl individuální riziko IBD (AUC 92 % (95% CI 89-94 %)) [174].

Hodnoty FC jsou v obecné populaci vyšší u dětí než u dospělých. Přestože byla zjištěna tendence k nižším hodnotám s rostoucím věkem, neexistují referenční hodnoty pro konkrétní věková rozmezí [175]. Dle publikovaných studií bylo prokázáno, že nejvýznamnější rozdíl v hodnotách FC je dán věkovou hranicí 4 roky [176]. Největší interindividuální variabilita byla pozorována u kojenců do 1 roku [177].

Dle publikovaných studií nebyly nalezeny velké rozdíly v hladině FC mezi jednotlivými fenotypy IBD [167,178]. U pacientů s CD bez postižení tlustého střeva mohou zvýšené hladiny FC znamenat aktivní onemocnění v tenkém střevě [179,180]. Také mohou být hladiny FC nízké u pacientů s CD izolovanou na ileocekální chlopeň [118,181–184]. Izolovaná CD horní části gastrointestinálního traktu je vzácná a data o hodnotách FC v této skupině pacientů chybí. U UC byla nalezena nízká míra asociace mezi rozsahem onemocnění a hladinami FC. Pouze na základě hodnot FC nelze rozlišit, zda se jedná o pankolitidu nebo levostrannou kolitidu . U izolované proktitidy mohou být hladiny dokonce v normálním rozmezí [185].

1.3.2.2.Fekální kalprotektin v hodnocení aktivity zánětlivých střevních onemocnění Hladiny

FC jak u dospělých, tak u dětí, korelují s aktivitou onemocnění definovanou klinickými parametry, endoskopickým nálezem a histologií [162,186–188], a proto je FC vhodným markerem k monitorování aktivity střevního zánětu u IBD pacientů.

Z aktuálních ESPGHAN doporučení pro CD vyplývá, že čím více se hodnota FC blíží ≤ 50 µg/g, tím vyšší je pravděpodobnost úplného endoskopického zhojení [101,166]. Dle meta-analýzy byla senzitivita FC 82 % a specificita FC 72 % bez ohledu na použité cut-off v průkazu endoskopické aktivity onemocnění CD [189]. Systematický přehled dokládá cut-off pro FC okolo 150 µg/g pro endoskopickou remisi [100].

Nicméně vzhledem k nízké spolehlivosti FC je rozmezí 100-250 µg/g považováno za šedou zónu. Pediatrická studie ukázala, že FC < 500 µg/g nebo pokles o > 50 % má prediktivní

hodnotu pro makroskopicky inaktivní onemocnění [190]. Běžně akceptovanou a dobře testovanou hodnotou pro přítomnost aktivního onemocnění u UC i CD je cut-off FC 250 μg/g [191]. Nicméně není překvapením, že některé studie prokázaly slabší korelaci mezi FC a endoskopickou aktivitou u CD s izolovaným postižením ilea ve srovnání s onemocněním tlustého střeva [157,158,160,192]. Podobně Jones a kolektiv ukázali, že hladina FC > 145 μg/g predikovala těžké onemocnění při zobrazení magnetickou rezonancí se senzitivitou 69,3 % (95% CI 57,6-79,5 a specificitou 71,4 % (95% CI 53,7-85,4) [193]. Další scénáře, kde se limity pro cut-off FC v souvislosti s aktivitou onemocnění mohou lišit, jsou v kontextu pooperační CD. V *post hoc* analýze studie POCER (Post-operative Crohn´s Endoscopic Reccurence Study) hodnota FC > 100 μg/g nejlépe předpověděla endoskopickou rekurenci po operaci [194]. Přísnější hodnoty pro cut-off FC byly také spojeny se slizničním a transmurálním hojením [192,195–198].

U dětí i dospělých s UC byla pospána těsná korelace FC s klinickou aktivitou onemocnění, endoskopickými i histologickými indexy aktivity [199]. Pro predikci endoskopické aktivity je FC citlivější než jiné laboratorní markery (CRP, ESR) [199].

1.3.2.3. Fekální kalprotektin v predikci odpovědi na zvolenou léčbu a v predikci navození remise

Současná data naznačují, že pokles FC stejně jako dosažení hodnoty FC pod určitou stanovenou hodnotu, má jasný prognostický význam. Dle několika studií FC předpovídal dlouhodobé klinické výsledky ve 12. týdnu od zahájení terapie [189,200,201]. U dětí s nově diagnostikovaným IBD bez ohledu na zvolenou terapii bylo dosaženo normalizace FC při cut-off 250 μg/g v průměru za 11 týdnů u UC a za 37 týdnů u CD [200]. Četné prospektivní a randomizované kontrolované studie (RCT) prokázaly významné snížení za 4-12 týdnů [202–208]. Doporučení STRIDE-II nabídla určité vodítko ohledně doby potřebné k dosažení cílů léčby (např. definované i jako pokles/ normalizace FC) u UC a CD na základě různých léčebných modalit [100]. Podle dostupný dat je tedy možné FC použít jako marker dosažení cíle léčby. U pacientů s CD léčených v indukčním režimu výhradní enterální výživou (EEN) může být pokles FC méně zřetelný a může se objevit za delší dobu 6 až 8 týdnů terapie [48,209–212]; a po opětovném zavedení běžné stravy se tento pokles opět rychle ztrácí [209,213]. Malá studie u dětí s endoskopicky aktivním IBD ukázala, že FC se snížil v prvních 2 měsících léčby glukokortikoidy. Pouze

menšina pacientů dosáhla hladin pod 100 μg/g, i když byla klinická odpověď považována za dobrou a většina pacientů zaznamenala vzestup FC po ukončení léčby glukokortikoidy [214]. Eskalace léčby u pacientů s CD založené pouze na výsledcích FC se v současnosti nedoporučuje. Studie u dospělých i dětí prokázaly, že kombinace FC s CRP zvyšuje přesnost detekce i predikce aktivního onemocnění než samotný FC [215–218].

Několik studií prokázalo souvislost mezi postindukčními hodnotami FC a výsledky aTNF terapie v jednom roce této léčby. U CD studie uvádí, že hodnota FC < 300 μg/g nebo jeho pokles o 50 % ve 12. týdnu aTNF léčby byl spojen s remisí bez kortikosteroidů v prvním roce léčby [219]. Podobně u obou diagnóz bylo prokázáno, že postindukční hodnoty FC 168 μg/g u CD se senzitivitou 83 % a specificitou 76 % a FC 121 μg/g u UC se senzitivitou 79 % a specificitou 57 %, predikují setrvalou klinickou odpověď po prvním roce léčby aTNF [220]. Molander et al. uvádí, že normalizace FC ve druhém týdnu aTNF terapie předpovídá endoskopickou remisi v 10. týdnu u CD i UC (senzitivita 87 %, specificita 65 %; AUC 0,77 [0,65-0,89], P < 0,001, společně pro všechny IBD) [221]. U pacientů s mírnou a středně aktivní UC léčených IFX se ukázalo, že zvýšené hodnoty FC před léčbou byly spojeny s endoskopickou odpovědí v 52. týdnu (senzitivita 75 % a specificita 58 % pro FC cut-off 817 μg/g, P = 0,0001) [222]. Další práce sledovala FC v souvislosti s trendem odpovědi na terapii kortikosteroidy (nonrespondeři 1100 μg/g vs. respondeři 864 μg/g, P = 0,08) a srovnávala hladiny FC u pacientů, kteří podstoupili kolektomii (1200 μg/g vs. 887 μg/g, P = 0,04) [223]. Dat na využití FC u akutní těžké kolitidy je málo a tato studie naznačuje, že FC by mohl předpovídat odpověď na první nebo druhou linii terapie v této skupině pacientů.

1.3.2.4. Fekální kalprotektin v predikci histologické remise

U části pacientů, kteří kompletně dosáhli endoskopického zhojení bez ohledu na danou terapii, zůstávají hodnoty FC poněkud zvýšené, což ukazuje na probíhající zánětlivou aktivitu na úrovni tkáně (histologická aktivita onemocnění) [197,224,225]. V tomto ohledu se vede diskuze, zda existuje dostatek důkazů, že navození histologické remise vede k déletrvající remisi pacientů. Rozdílná je situace u pacientů s CD a UC. U UC byl prokázán jednoznačný benefit histologické remise nad endoskopickou v predikci dlouhodobé remise a prevence onkologických komplikací [226–232]. Nicméně tohoto cíle je obtížné dosáhnout. Pouze u jedné

třetiny pacientů s UC s makroskopickou remisí bylo současně dosaženo histologické remise [233]. U CD jsou výsledky ještě nepříznivější. Navíc chybí data týkající se jednotlivých léčebných modalit v efektu navození histologické remise a spolehlivý validovaný nástroj k jejímu hodnocení [234]. I když se zdá, že FC je asociován těsněji s histologickým než s endoskopickým nálezem a mohl by být vhodným markerem k hodnocení histologické remise [96], další studie sledující přímo FC toto prokazují pouze v terénu UC [196,235,236]. V retrospektivní studii u dětí s UC dosáhla hodnota FC 275 μg/g senzitivitu 94 % a specificitu 95 % v hodnocení histologické aktivity [237]. Důvodem, který se podílí na rozporuplných výsledcích u CD, může být vlastní povaha onemocnění – segmentální transmurální distribuce zánětu a komplikace jako jsou striktury, které vedou k nereprezentativnímu odběru vzorků [226,230,238]. Navíc doposud nebylo dosaženo konsensu pro definování histologické remise a ze 14 číselných indexů byly pouze dva parciálně validovány [239,240].

1.3.2.5. Fekální kalprotektin v predikci klinického relapsu onemocnění

V současnosti je FC nejdůležitějším nástrojem k detekci relapsu u pacientů s IBD, a to i z důvodu jeho schopnosti předpovídat vzplanutí onemocnění u asymptomatických pacientů [241,242]. Hladiny FC začnou stoupat před klinickým nebo endoskopickým relapsem, jak bylo ukázáno u skupiny dospělých pacientů s IBD s laboratorní remisí potvrzenou endoskopicky [243]. Dle publikovaných dat lze FC použít k identifikaci pacientů vyžadujících pečlivé sledování v klinické praxi [221,244]. Studie zkoumající mezní hodnoty FC v predikci relapsu onemocnění (u konzervativně léčeného onemocnění) se pohybují mezi 400 a 800 μg/g u pediatrické CD a UC, a okolo 300 μg/g u dospělých pacientů s CD a UC [236,245–248]. Rozdíl v hodnotách u dětí a dospělých by mohl odpovídat odlišným fenotypům onemocnění typickým pro danou věkovou skupinu. Dle recentní meta-analýzy na toto téma byla hodnota FC 152 μg/g optimální hranicí pro detekci pacientů s rizikem relapsu, a to se senzitivitou 72 % (95% CI 53-87 %) a specificitou 74 % (95% CI 62-83) [249]. V prospektivní multicentrické studii byly u pacientů s UC v klinické remisi na terapii IFX hladiny FC stanovovány v čtyřtýdenních intervalech. Nejsilnějším prediktorem relapsu onemocnění byly dvě po sobě zjištěné hodnoty FC nad 300 μg/g v odstupu měsíce se specificitou 100 %, nicméně nízkou senzitivitou 62 % [241]. Systematický přehled 6 studií ukázal, že u asymptomatických pacientů s IBD měla po sobě se opakující měření FC

souhrnnou senzitivitu a specificitu pro predikci relapsu během 2-3 měsíců 78 % (95% CI 72-83) a 73 % (95% CI 68-77), a AUC 0,83 [242]. Evidence tedy podporuje roli proaktivního průběžného monitorování FC pro časnou detekci relapsu onemocnění [215].

1.3.3. Fekální kalprotektin jako součást indexů a skórovacích systémů

Několik prací poukazuje na benefit kombinace FC s dalšími proměnnými ve vztahu k aktivitě onemocnění v predikci výstupů léčby. Přelomová studie CALM zaměřená na strategii léčby u dospělých s CD ukázala, že kombinaci Indexu aktivity Crohnovy choroby (CDAI) s laboratorními markery (FC <250 μg/g a CRP <5 mg/l) lze použít jako léčebný cíl [215]. *Post hoc* analýza studie zjistila, že přidání CRP k cílové hodnotě FC významně zlepšilo výstupy v prvním roce léčby [250]. Vývoj indexu MINI (Mucosal Inflammation Noninvasive Index) u pediatrických pacientů a podobná studie u dospělých, která vytvořila Utrechtský index aktivity onemocnění, skutečně ukázaly, že přidání CRP k FC dosahuje přesnější výsledky v detekci endoskopické remise než samotný FC [251,252]. Díky své neinvazivní povaze usnadňuje MINI posouzení odpovědi na léčbu a výběr pacientů indikovaných k endoskopickému vyšetření [251]. V další studii u dětí kombinace FC, CRP a PCDAI také velmi přesně predikovala endoskopicky inaktivní onemocnění (negative likelihood ratio (NLR) 0,2) [253]. Stejný cíl měly i další práce, ve kterých přidání CDAI k FC zlepšilo AUC z 0,88 na 0,96 a přidání CRP k FC zvýšilo specificitu z 88 % na 100 % v detekci endoskopické remise [254,255]. I řada jiných prací naznačuje výhodu kombinace více potencionálních prediktorů vedoucí ke zpřesnění předpovědi pro vedení další terapie u pacientů s CD [192,217,256,257].

FC není součástí indexů hodnotících aktivitu onemocnění u dětských pacientů s UC. V několika studiích bylo totiž ukázáno, že samotný klinický index (PUCAI) koreluje lépe s endoskopickým nálezem než FC a byl použitý ve studii PROTECT jako vodítko pro volbu počáteční léčby při nástupu onemocnění [120,121,185,258,259]. Nízké PUCAI při diagnóze se zdá také dobrým prediktorem dlouhodobé remise a dobře koreluje s endoskopickým nálezem na sliznici tlustého střeva [121].

1.3.4. Prediktivní modely a fekální kalprotektin

V oblasti IBD bylo publikovaných několik prací zaměřených na tvorbu modelů k predikci komplikací onemocnění, odpovědi na terapii imunomodulátory a preparáty biologické léčby, potřeby podávání kortikosteroidů a míru hospitalizace [260–264]. FC byl použitý jako součást několika z nich [264–267].

Ve snaze předpovědět endoskopickou remisi pacientů s UC léčených VDZ po roce léčby bylo testováno několik variant prediktivních modelů. Do některých z nich byl přidán také FC: 1) základní model, s FC jako prediktorem před zahájením terapie VDZ (AUC 0,58 [95% CI: 0,52– 0,63]); 2) složený model z dat v 0. a v 6. týdnu (AUC 0,73 [95% CI: 0,65–0,82]); 3) zjednodušený model s poměrem hladiny FC a VDZ v 6. týdnu léčby s cut-off 12 μg/g (AUC 0,58 [95% CI: 0,52– 0,63]); 4) zjednodušený model 6. týdne s cut-off FC < 234 μg/g pro predikci složeného výsledku (AUC 0,71 [95% CI: 0,66–0,76]). Za nejlepší model byl vybrán model 2), který zahrnoval změnu FC v průběhu času, hladiny VDZ a laboratorní výsledky v 6. týdnu. Studie byla posouzena dle protokolu PROBAST jako studie s nízkým rizikem zkreslení (Risk of Bias (ROB)) [267].

V další studii u IBD pacientů léčených thiopuriny autoři vyvinuli modely k predikci přítomnosti slizničního zánětu, kdy byla remise definována hodnotou FC, CRP a ESR (AUC 0,79 [95% CI 0,78–0,81]) [263]. Studie byla hodnocena jako studie s nízkou ROB; autoři provedli také hodnocení predikčního modelu dle doporučení (Out of Bag (OOB)), aby minimalizovali riziko „přesycení“ modelu.

Jiná studie byla zaměřena na predikci rizika hospitalizace a užívání kortikosteroidů při léčbě imunomodulátory a/nebo aTNF preparáty [268]. Autoři vytvořili dva typy modelů 1) bez longitudinálních dat; a 2) s longitudinálními daty. Ukázalo se, že začlenění průběžných dat s předchozími relapsy onemocnění zlepšilo přesnost jejich modelu (AUC 0,87 [95% CI 0,87–0,88]) ve srovnání se spoléháním se pouze na vstupní data (AUC 0,85 [95% CI 0,84–0,85]). Také tato studie byla posouzena jako studie s nízkým ROB.

FC se v kombinaci s dalšími laboratorními a klinickými daty stal součástí spolehlivých prediktivních modelů u IBD, nicméně většina z nich byla validizována jenom interně. Pokud by se modely podařilo externě validovat, mohly by se stát součástí běžné klinické praxe. To by mohlo být levnější a současně méně pracné ve srovnání s tradičním laboratorním monitorováním [263,266]. Tyto modely by mohly být přínosné v předpovědi odpovědi na léčbu

thiopuriny a preparáty biologické léčby, včetně novějších preparátů, jako je VDZ a UST [263,267]. Dle recentního přehledu srovnávajícího metody ML se standardními statistickými metodami v oblasti IBD, se ML zdají být efektivnější [269].

2. Cíle práce

V současné klinické praxi má lékař k dispozici poměrně velké množství informací o pacientovi, podle kterých se snaží intuitivně odhadnout nejlepší postup. Překážkouje schopnost zhodnotit relevanci informací a dále omezený čas k vytvoření závěru a volbě doporučení. Z toho důvodu je snahou identifikovat konkrétní znaky pro jednotlivé klinické situace, které by umožnily standardizovat běžný rozhodovací proces ve chvíli, kdy je možné ovlivnit budoucí výsledek. Cílem tohoto přístupu je naplňování principů precizní medicíny umožňující minimalizaci rizika nepříznivého průběhu onemocnění a rozvoji s onemocněním a/nebo léčbou asociovaných komplikací na úrovni konkrétního pacienta.

Tématem disertační práce je využití metod biomedicínské informatiky v oblasti pediatrických IBD. Soustředili jsme se především na FC v predikci aktivity onemocnění a odpovědi na léčbu. Dále také na hledání dalších potencionálních prediktivních faktorů a tvorbu predikčních modelů pro konkrétní klinické situace u dětí s IBD.

Původní hlavní podprojekt disertační práce nebylo možné realizovat v plném rozsahu. Ve spolupráci s Fakultou aplikovaných věd Západočeské Univerzity v Plzni se podařilo připravit databázi pro systematický prospektivní pseudonymizovaný sběr a kategorizaci klinických, laboratorních a dalších údajů o pacientech s IBD. Nicméně, management Fakultní nemocnice v Motole, a zejména vedení Oddělení informačních systému, zavedení databáze i přes opakované konzultace s právníky nakonec po sedmi letech neschválilo. Z výše uvedených příčin se nepodařilo uskutečnit její implementaci a naplnit tak další cíle práce s rozšířením vlastností a funkcí databáze o kontrolu úplnosti dat a jejich statistické zpracování s možností automatizovaného vytváření predikčních modelů na základě vložených dat. Z tohoto důvodu jsme se tedy věnovali více studiu potencionálních prediktivních faktorů u dětských pacientů s IBD.

3. Metody

3.1. Definice primárního outcome jako cíle predikce

Pro úspěšnou identifikaci prediktorů je nutné určení vhodně definovaného cíle studie. Do dizertační práce je zařazeno třináct původních prací zabývajících se problematikou predikce aktivity onemocnění, předpovědí udržitelnosti a na druhé straně selhání zvoleného terapeutického přístupu; rizikovými faktory rozvoje nežádoucích účinků konkrétních léčebných modalit a predikcí výskytu extraintestinálních komplikací onemocnění.

3.2. Způsoby sběru a ukládání dat

Jak již bylo zmíněno, než je možné vyvinout jakýkoli nový predikční nástroj, je třeba retrospektivně nebo prospektivně shromáždit data o jednotlivých pacientech stran prediktorů a odpovědi. Do dizertační práce je zařazeno celkově třináct původních prací, dvě v retrospektivním designu [270,271], zbytek prospektivních [115,272–280]. Zdrojem dat pro retrospektivní studie byla lékařská dokumentace reprezentovaná elektronickým zdravotním záznamem (EZZ). Základním problémem stávající EZZ je omezená schopnost zpětného použití. Objemná data získána při odběru anamnézy či v nemocničním provozu nejsou rutinně systematicky zaznamenávána, kategorizována ani následně statisticky zpracována. Aktuálně využívané informační systémy zdravotnických zařízení nejsou navrženy k implementaci modulů pro konkrétní diagnózy a integraci vědomostních modulů. Další nevýhodou v souvislosti s retrospektivním přístupem je riziko zkreslení a v případě velkých datových souborů časová náročnost.

V prospektivním designu byla provedena většina zde uvedených prací, pro které byly vytvářeny datové soubory většinou prostřednictvím standardizovaného formátu Microsoft Excel [211,278,279,281–286]. Jedna ze studií pracovala s daty uloženými cestou zabezpečené webové aplikace pro vytváření a správu online databází REDCap (Research Electronic Data Capture) [277]. Důležitým zdrojem dat pro jednu ze studií byl prospektivní národní registr IBD pacientů léčených preparáty biologické terapie (CREdIT) [280]. Dlouhodobé prospektivní, optimálně multicentrické registry jsou v současnosti považovány za nejvhodnější způsob získavání dat pro identifikaci a studium potencionálních prediktivních faktorů. Všechny uvedené práce byly realizované na Pediatrické klinice 2. lékařské fakulty Univerzity Karlovy a Fakultní nemocnice Motol a data byla získána od dětských pacientů s diagnózou IBD.

3.3. Statistické vyhodnocení dat

Statistické zpracování dat probíhalo ve vývojovém prostředí R, které poskytuje širokou škálu statistických a grafických technik, včetně lineárního a nelineárního modelování, klasických statistických testů, analýzy časových řad, shlukování a dalších. R je snadno rozšiřitelný pomocí balíčků (packages) vytvořených samotnými uživateli obsahující konkrétní funkce a také pro možnost využití lexikálních pravidel. Navíc má silnější objektově orientované programování než většina ostatních statistických programovacích jazyků. Další pozitivní stránkou R je statická grafika umožňující generování grafů vhodných do vědeckých publikací. Dynamická a interaktivní grafika je dostupná prostřednictvím dalších balíčků.

V rámci našich prací byly použity následující metody statistické analýzy: v případě sledování pacientů v průběhu času jsme aplikovali na data survival analýzy a Cox regrese; v souvislosti s opakovaným měřením jednoho pacienta jsme použili mix model; pro kategorické výsledky zobecněný lineární smíšený model a pro lineární výsledky lineární smíšený model; v rámci některých studií bylo možné na základě klinického výběru prediktorů zkonstruovat konečný predikční model a když to bylo vhodné přidali jsme do modelu efekty interakce; k vyhodnocení prediktorů udržitelnosti léčby byly aplikovány Coxovy modely se smíšenými efekty a analýzy propensity skóre; pomocí DeLong testu ROC (receiver operating characteristic) jsme srovnávali přesnost diagnostických metod.

3.4. Interní spolupráce

Studie zabývající se postvakcinačním a imunizačním profilem u dětských pacientů s IBD očkovaných proti těžkému akutnímu respiračnímu syndromu vyvolaného koronavirem 2 (SARS-CoV-2) byla realizována ve spolupráci s Ústavem imunologie 2. lékařské fakulty Univerzity Karlovy a Fakultní nemocnice Motol. Každému pacientovi byly odebrány 2 ml krve pro měření postinfekčních a postvakcinačních protilátek, oddělené sérum bylo zmraženo při -20 °C až do analýzy. Protilátky byly měřeny u všech pacientů pomocí Microblot-Array COVID-19 IgG/IgA (TestLine Clinical Diagnostics, Brno, Česká republika) podle pokynů výrobce. Měřili jsme anti-RBD IgG a IgA, anti-spike S2 IgG a IgA a anti-nukleokapsidové IgG a IgA. Titry protilátek byly kvantifikovány v U/ml na základě kalibrační křivky v rámci testu. Titry >210 U/ml byly považovány za pozitivní a titry 185–210 U/ml byly považovány za hraničně pozitivní. Platnost testu byla ověřena pomocí interního testu a kontrol konjugátu [278].

Dvě práce zaměřené na studium svalově-kostní jednotky u dětských pacientů s IBD byly uskutečněny společně s endokrinologickou skupinou a ambulancí klinické antropologie Pediatrické kliniky 2. lékařské fakulty Univerzity Karlovy a Fakultní nemocnice Motol. Kvalita kostní hmoty byla objektivizována pomocí periferní kvantitativní výpočetní tomografie (pQCT) a svalové funkce skákací mechanografií [282,283].

Spolupracovníci z Ústavu lékařské mikrobiologie 2. lékařské fakulty Univerzity Karlovy a Fakultní nemocnice Motol stanovovali serologický profil EBV a jeho nálož pomocí PCR pro potřeby studie zabývající se prevalencí EBV v pediatrické populaci pacientů s IBD léčených imunomodulátory [271].

3.5. Externí spolupráce

Ve spolupráci s Fakultou aplikovaných věd Západočeské Univerzity v Plzni jsme tvořili databázi pro prospektivní pseudonymizovaný systematický sběr a kategorizaci klinických, laboratorních a dalších údajů o pacientech s IBD. Protože se nám nakonec nepodařilo získat svolení vedení nemocnice, nebylo možno ji implementovat a naplnit tak další cíle s rozšířením vlastností a funkcí databáze o kontrolu úplnosti dat a jejich statistické zpracování s možností vytváření predikčních modelů pro široké spektrum klinických situací.

Projekt CREdIT, který byl zahájen v roce 2016, je neintervenční multicentrická retrospektivně prospektivní longitudinální studie, v rámci níž jsou sbírána data o pacientech s IBD léčených léčivými přípravky biologické a inovativní léčby. Projekt vznikl z iniciativy České gastroenterologické společnosti České lékařské společnosti Jana Evangelisty Purkyně. Naše centrum přispívá daty do pediatrického ramene registru a účastní se jejich statistického vyhodnocení v rámci společných vědecko-výzkumných projektů [280].

Výrobce a distributor testu vyvinutého pro kvantitativní stanovení hladiny FC v domácím prostředí (IBDoc, Buhlmann) dodával v rámci grantového projektu pro účely studie komerční experimetální sety. Set obsahuje extrakční nástroj k odběru přesného množství stolice (Calex Valve), zkumavku s médiem, do nějž je vzorek bezprostředně po odběru aplikován a destičku určenou pro následné zpracování takto vzniklé směsi. Koncentrace FC je stanovena metodou imunochromatografické analýzy. Pomocí aplikace chytrého mobilního telefonu je odečten výsledek, který je současně po zpracování měření odeslán do webového úložistě IBDoc Portal [279,285].

Stanovení hladin metabolitů AZA v rámci studie zabývající se predikcí úspěšnosti

kombinované terapie aTNF a AZA u dětských pacientů s CD probíhalo v Laboratoři růstových regulátorů Přírodovědecké fakulty Univerzity Palackého v Olomouci. Za tímto účelem byla pacientům odebrána plná krev do jedné zkumavky s EDTA (kyselina etylendiamintetraoctová). Zpracování vzorku bylo provedeno dle protokolu Dervieux a Boulieu. Po odběru byly odděleny leukocyty a plasma v procesu centrifugace. Zbylé erytrocyty byly dvakrát promyty a naředěny vodou v poměru 1:1 s následným zmražením na -20°C. Definitivní odečtení hladin metabolitů AZA probíhalo najdenou u více vzorků metodou vysoce účinné kapalinové chromatografie s více vlnovým detektorem Agilent 1000 [279].

4. Výsledky

4.1. Fekální kalprotektin jako prediktivní faktor

4.1.1. Fekální kalprotektin jako prediktivní faktor endoskopické aktivity onemocnění

Cílem prospektivní observační studie bylo srovnat domácí test k detekci hladin FC se standardní laboratorní metodou ELISA (enymed-linked immuno sorbet assay) v určení endoskopické aktivity onemocnění. Právě endoskopická aktivita zánětu přesně definovaná použitím endoskopických skórovacích systémů byla zvolena za referenční standard, ke kterému se vztahovalo srovnání dvou různých metod ke stanovení koncentrace FC ve stolici pacientů s IBD. Studie měla přinést informaci o možnostech bezpečného a efektivního využití nové rychlejší metody pro testování u lůžka nebo pro „samotestování" pacientů s IBD v rámci tzv. self-monitoringu onemocnění a přispět tak k dřívější detekci narůstající aktivity zánětu s následným časnějším klinickým řešením. U obou použitých metod byla nalezena statisticky významná asociace s makroskopickou aktivitou střevního zánětu. Standardní metoda ELISA přinesla při srovnání obou testů signifikantně spolehlivější výsledky hladin FC v predikci slizničního hojení. Na základě výsledků práce je domácí test možné použít k orientačnímu měření FC, např. v rámci výše popsaného self-monitoringu, neměl by ale nahradit rutinní měření FC standardními laboratorními metodami [272].

4.1.2. Fekální kalprotektin jako prediktivní faktor trvajícího efektu zvoleného léčebného postupu

Výhradní enterální výživa je dle aktuálních mezinárodních doporučení terapií volby v indukční léčbě dětí s nově stanovenou diagnózou CD. Nicméně, část pacientů nedosáhne remise CD ani po 6-8 týdnech léčby pomocí EEN. Z klinického pohledu by byla výhodná časná identifikace „nonrespondérů" spojena s úpravou terapie. Práce se zaměřuje na možnost využití FC k předpovědi trvajícího efektu konkrétního léčebného postupu v kohortě dětských pacientů s nově diagnostikovanou CD, u kterých byla zahájena indukce remise pomocí EEN. Studie neprokázala, že by změna hladiny FC mezi počátkem léčby (týden 0) a druhým týdnem mohla vést k predikci dosažení remise onemocnění v šestém týdnu terapie EEN. Výsledky práce

ukazují, že FC není vhodným markerem k časné detekci pacientů, kteří nedosáhnou remise onemocnění při indukční léčbě EEN [211].

4.2. Prediktivní faktory rizika selhání zvoleného terapeutického postupu

4.2.1. Prediktivní faktory eskalace aTNF terapie u dětských pacientů s Crohnovou nemocí

Cílem prospektivní observační studie bylo srovnání dvou preparátů aTNF (ADA a IFX) v terapii dětských pacientů s CD naivních k biologické léčbě pomocí propensity-skóre analýzy a pátrání po potencionálních prediktorech nutnosti eskalace terapie. Sub-analýzou celé studované kohorty ani srovnáním použitím propensity-skóre nebyl nalezen rozdíl v celkovém čase do nutnosti eskalace terapie mezi pacienty léčenými ADA nebo IFX, a to ani po adjustaci s ohledem na současné užívání preparátu ze skupiny imunomodulátorů. Nicméně, pokud existuje menší rozdíl v efektivitě nemuselo se jej podařit nalézt z důvodu menšího počtu pacientů zařazených do studie. Pomocí regresní analýzy byla identifikováná kombinace serologických markerů - seropozitivita pANCA a seronegativita ASCA – jako jediný a silný nezávislý prediktor v předpovědi nutnosti eskalace terapie aTNF. Další sledované parametry, včetně fenotypu onemocnění, konkomitatní imunomodulační léčby a/nebo typu aTNF preparátu, neměly vliv na sílu predikce po přídání do modelu [277].

4.2.2. Prediktivní faktory udržitelnosti biologické léčby u dětských pacientů s Crohnovou nemocí

Byla provedena analýza dětského ramene prospektivního národního registru IBD pacientů léčených preparáty biologické terapie (CREdIT). Studie byla zaměřena na hledání potencionálních prediktorů udržitelnosti biologické léčby u dětských pacientů s CD. Výstup byl definován ukončením terapie, jelikož léčba biologickými preparáty není v dětském věku úmyslně přerušována, lze toto považovat za selhání nebo intoleranci terapie. Asociace doby do vysazení biologické léčby a potencionálním prediktorem byla testována pomocí Coxova regresního modelu se smíšeným efektem. Ve studii se podařilo identifikovat následující

prediktory udržitelnosti biologické léčby u dětí s CD: terapie ADA (verzus IFX), časné zahájení biologické léčby a normální výchozí hladina hemoglobinu. Terapie IFX byla spojena s dřívější potřebou intenzifikace léčby, vyšší expozicí léku a častějším současným podávaním imunomodulátoru. Práce přináší informace o výhodách a nevýhodách jednotlivých preparátů biologické léčby, zejména ze skupiny aTNF. Pacienti a jejich zákonní zástupci by měli být během rozhodovacího procesu plně oboznámeni s vlastnostmi jednotlivých léčebných modalit a také o důležitosti časného zahájení biologické léčby [280].

4.2.3. Prediktivní faktory efektu kombinované léčby imunomodulátorem a preparátem aTNF u dětských pacientů s Crohnovou nemocí

Výstupem multicentrické prospektivní observační studie bylo posouzení přínosu monitorace hladin metabolitů AZA u pacientů na kombinované terapii (AZA a aTNF). Na základě výsledků lze říci, že dostatečné hladiny 6-thioguanin nukleotidu (6-TGN) v erytrocytu jsou asociovány se sérovými hladinami aTNF. U pacientů s nedetekovatelnými hladinami 6-TGN byla nalezena vyšší míra ztráty odpovědi k aTNF. Práce přináší důležitý poznatek o možnostech optimalizace terapie u pacientů s CD léčených kombinací AZA a aTNF [279].

4.2.4. Prediktivní faktory selhání terapie azathioprinem u dětských pacientů s nově diagnostikovanou Crohnovou chorobou léčených kortikosteroidy nebo výhradní enterální výživou

Jak již bylo popsáno, EEN je dle současných doporučení nejvhodnější indukční léčbou dětí s nově diagnostikovanou nekomplikovanou formou CD. Na našem pracovišti jsme postupně u dané skupiny pacientů přecházeli z indukce remise onemocnění kortikosteroidy na léčbu pomocí EEN. Následně jsme ve studii případů a kontrol srovnávali délku trvání remise zmíněných dvou terapeutických přístupů u pacientů s diagnózou IBD současně léčených AZA a hledali prediktory selhání léčby. Oproti výsledkům předchozích prací nebyl v naší IBD kohortě nalezen rozdíl mezi případy a kontrolami. Nicméně v těchto studiích nebyla u pacientů podávána konkomitantní terapie thiopuriny. Je možné předpokládat, že indukční léčba nehraje zásadní roli v délce trvání remise onemocnění u pacientů současně užívajících AZA. Podařilo se nám identifikovat potencionální prediktory selhání terapie AZA: nízký věk v době diagnózy,

postižení horního gastrointestinálního traktu, zvýšený počet trombocytů v době dosažení remise a růstová retardace v době diagnózy IBD. Vzhledem k nedostatečné síle jednoltivých prediktorů, nebylo možné vytvořit predikční model k selekci pacientů již v době stanovení diagnózy [115].

4.2.5. Prediktivní faktory selhání terapie infliximabem u dětských pacientů s Crohnovou nemocí

Cílem práce bylo na dětské skupině s CD ověřit hypotézu vycházející ze studií u dospělých pacientů s IBD léčených IFX, zda je možné na základě monitorace farmakokinetického/ dynamického profilu IFX (hladina IFX a protilátky proti IFX (ATIs)) předpovědět selhání terapie a identifikovat další potencionální prediktory. U pacientů s významnější aktivitou onemocnění definovanou pomocí zánětlivých markerů (CRP, FC) byly detekovány nižší hladiny IFX. Nejoptimálnější kombinací senzitivity a specificity pro určení remise (definované jako CRP pod 5 mg/l) na základě hladin IFX byla hodnota 1,1 μg/ml. Na rozdíl od předchozích studií jsme neprokázali, že by průkaz přítomnosti ATIs a jejich kvantifikace přinášely klinicky významnou informaci [275].

4.2.6. Prediktivní faktory endoskopické rekurence onemocnění u dětských pacientů s Crohnovou nemocí podstupujících ileocékální resekci

V prospektivní studii na kohortě pediatrických pacientů s CD indikovaných k ileocékální resekci (ICR) jsme hledali prediktory endoskopické aktivity onemocnění v šestém měsíci po chirurgickém výkonu. Právě endoskopická aktivita zánětu přesně definovaná použitím endoskopického skórovacího systému (Rutgeerts) byla zvolena za zlatý standard, ke kterému se vztahovalo testování potencionálních rizikových faktorů rekurence onemocnění [118]. Pacienti zařazeni do studie byli rozděleni do dvou skupin na základě údajů o aTNF léčbě před ICR a v dalším průběhu byl každý pacient léčen AZA v monoterapii. Ve studii jsme neprokázali rozdíl v aktivitě onemocnění po ICR ve vztahu k předchozí léčbě aTNF preparáty. Současně nebyly nalezeny klinicky významné faktory asociované s endoskopickou rekurencí v šestém měsíci po ICR [274].

4.3. Prediktivní faktory rozvoje nežádoucích účinků zvoleného terapeutického postupu

4.3.1. Prediktivní faktory rozvoje kožních nežádoucích účinků u dětských pacientů s Crohnovou nemocí léčených různými preparáty aTNF

Ve studii na kohortě dětských pacientů s diagnózou CD léčených aTNF preparáty (IFX a ADA) bylo cílem identifikovat prediktivní faktory rozvoje kožních nežádoucích účinků terapie. Na základě výsledků práce lze konstatovat, že IFX je ve srovnání s ADA prediktorem pro vznik neinfekční kožní léze, včetně atopické dermatitidy, pro kterou je riziko až šestinásobně vyšší. Dále byla zjištěna asociace mezi kožními projevy a nízkou laboratorní zánětlivou aktivitou onemocnění (CRP) ukazující na dobře kompenzovanou CD. Na základě daných zjištění bude v nejbližší době možné selektovat rizikové pacienty pro vhodnější preparát biologické léčby [270].

4.3.2. Prediktivní faktory serologického profilu a virové nálože EBV, CMV a dalších potencionálně rizikových virů u dětských pacientů s Crohnovou nemocí léčených imunospuresivní terapií

Dle současných doporučení je u nekomplikované formy CD zahajována v době diagnózy imunosupresivní léčba, převážně preparáty ze skupiny thiopurinů, současně s indukční léčbou, aby bylo dosaženo optimálních výsledků v udržení remise onemocnění. Nejobávanějším, i když vzácným, rizikem terapie je rozvoj některých maligních diagnóz. Jsou popisovány lymfoproliferativní onemocnění, u kterých kromě léčby sehrává velmi pravděpodobně svou roli chronicky probíhající zánět. Častěji je také uváděn výskyt dvou typů lymfomů, na jejichž vzniku se významně podílí EBV infekce, proto je důležité pečlivě zjišťovat EBV sérologický profil pacienta před zahájením terapie thiopuriny. V doporučeních pro dospělé pacienty se začaly objevovat názory, zda je vhodné podávat tuto léčbu mladým EBV séronegativním jedincům s IBD. Cílem průřezové studie bylo objektivizovat sérologickou prevalenci EBV a dalších potencionálně rizikových virů (CMV, JC a BK polyomavirus) a současně prevalenci uvedených virů detekovanou metodou real-time PCR a najít možné prediktory ve vztahu k EBV séropozitivitě a virové nálože u dětských pacientů s IBD léčených thiopuriny a/nebo aTNF

preparáty. Léčba AZA byla asociována s kratší dobou do dosažení séropozitivity EBV ve sledované skupině pacientů. S virovou náloží EBV byla asociována aTNF terapie [287].

4.4. Prediktivní faktory extraintestinálních komplikací u dětských pacientů se zánětlivými střevními onemocněními

4.4.1. Prediktivní faktory insuficience svalově-kostní jednotky u dětských pacientů se zánětlivými střevními onemocněními

Snížená kostní denzita a zvýšená incidence fraktur patří mezi komplikace asociované s IBD [288]. V prospektivní observační studii jsme se zaměřili na vyhodnocení muskuloskeletálních parametrů ve vztahu k výskytu vertebrálních fraktur u dětí a dospívajících s IBD. Zjistili jsme, že námi sledovaní pacienti měli změněnou kostní denzitu a geometrii, ale normální dynamické svalové funkce. Dalším zajímavým výstupem je zjištění, že kostní změny jsou nezávislé na hladině 25-hydroxyvitamin D (25-OHD) v séru. Na základě výsledků je možné dále konstatovat, že rentgenové zobrazení torakolumbální páteře by nemělo být rutinně doporučováno u dětí s IBD a indikace k provedení by měla vycházet z individuálních nálezů u konkrétních pacientů [283].

4.4.2. Prediktivní faktory úspěšné substitučné léčby cholekalciferolem u dětských pacientů se zánětlivými střevními onemocněními a současnou hypovitaminózou D

V další prospektivní observační studii jsme sledovali změny parametrů svalově-kostní jednotky v průběhu substituční léčby cholekaciferolem v kohortě dětí s IBD a hledali prediktory ve vztahu k týmto změnám. Kvalita kostní hmoty byla objektivizována pomocí periferní kvantitativní výpočetní tomografie (pQCT) a svalové funkce skákací mechanografií. V našem souboru pacientů jsme pozorovali významné zlepšení hodnoty maximálního svalového výkonu a trabekulární kostní denzity při pravidelné substituci 2000 jednotkami cholekalciferolu. Nebylo možné vytvořit vhodný model k selekci pacientů profitujících ze substituční léčby z důvodu nedostatečné síly asociace potencionálních prediktorů. Nicméně vzhledem k vysoké prevalenci hypovitaminózy D v terénu IBD a k významnému procentu pacientů dosahujících optimalizaci

alespoň jedného z uvedených parametrů svalově-kostní jednotky je na místě suplementaci 25-OHD zvážit u všech pediatrických pacientů s IBD [276].

4.5. Prediktivní faktory postvakcinační odpovědi v kohortě adolescentů se zánětlivým střevním onemocněním očkovaných proti těžkému akutnímu respiračnímu syndromu vyvolaného koronavirem 2

Prospektivně jsme porovnali protilátky po očkování vakcínou BNT162b2 proti těžkému akutnímu respiračnímu syndromu vyvolaného koronavirem 2 (SARS-CoV-2) ve skupině dětských pacientů s IBD a zdravých kontrol. Současně jsme hledali prediktory míry imunitní odpovědi po očkování. Vyšší hladiny anti-spike S2 protilátek byly detekovány u IBD-pacientů. U dětí jsme potvrdili, že léčba aTNF je asociována s nižší produkcí postvakcinačních protilátek, a naopak, že předchozí infekce SARS-CoV-2 je spojena s jejich vyšší hladinou [278].

5. Diskuze

Vzhledem k různorodosti zvoleného primárního outcome jako cíle predikce u uvedených studií je diskuze zpracována právě s ohledem na tematické zaměření jednotlivých původních prací. Fekální kalprotektin je uváděn samostatně jako prediktivní faktor, který ověřujeme pro konkrétní klinickou otázku, a nikoli hledáme, jak je to u zbylých studií.

5.1. Fekální kalprotektin jako prediktivní faktor

V prospektivní studii jsme zjistili, že prediktivní hodnota FC během časné fáze léčby pomocí EEN u dětí s nově diagnostikovanou luminální CD není dostatečná pro výběr pacientů, kteří pravděpodobně nedosáhnou remise onemocnění na konci této indukční léčby. Jednalo se o první studii, která se snažila předpovědět efekt indukční léčby EEN na základě vývoje parametrů v její časné fázi. U většiny pacientů došlo k poklesu hladiny FC, nicméně, individuální koncentrace FC dosahovaly nadále vysokých hodnot u několika klinických respondérů a to i na konci indukční léčby. V tomto ohledu je důležité zjisťovat nejen aktuální hodnoty FC, nýbrž jejich vývoj v čase nebo přesněji procentuální změnu FC v logaritmické škále. Navíc u části pacientů došlo k přechodnému dalšímu vzestupu hladiny FC v úvodu EEN, které nevylučovalo její pokles v době ukončení terapie [211].

Na základě našich zjištění vyvstává otázka, kdy je nejvhodnější čas pro vyhodnocení odpovědi na indukční terapii EEN pomocí FC. Podobná studie, která v průběhu léčby EEN sledovala vývoj biochemických zánětlivých parametrů a FC poukazuje na předpoklad, že námi zvolený interval je pravděpodobně příliš krátký pro změnu tak senzitivního parametru [209,210]. Podstatně delší dobu k obdobně fromulované predikci si zvolila další studie, která hodnotila ve třicetidenním intervalu potencionální markery ve vztahu k trvající odpovědi na konci léčby EEN [289]. Nicméně, z klinického pohledu je tento postup nelogický a vhodnější je vyčkat efektu indukční léčby ve standardním trvání šesti týdnu než odhadovat navození remise ve čtvrtém týdnu.

V nasledující práci jsme jako první porovnali domácí test určený k vyhodnocení FC se standardní ELISA metodou vůči referenčnímu standardu. Dalším přínosem této studie oproti dříve publikovaným pracím bylo nalezení optimálního cut-off pro domácí test určený k vyhodnocení FC počítaného ve vztahu k endoskopické remisi (FC 48 µg/g). Předchozí studie uváděly cut-off pro domácí testy v intervalu od 50 do 150 µg/g [290–292]. Pro standardní laboratorní

metodu ELISA byl meta-analýzou zjištěn nejlepší cut-off FC předpovídající slizniční hojení 250 µg/g [293], v naší studii to byla hodnota 136 µg/g. U obou použitých metod byla nalezena statisticky významná asociace s makroskopickou aktivitou střevního zánětu. Nicméně, standardní metoda ELISA přinesla při srovnání obou testů signifikantně spolehlivější výsledky v predikci slizničního hojení. Na základě výsledků práce je domácí test možné použít k orientačnímu měření FC, např. v rámci tzv. self-monitoringu, neměl by ale nahradit rutinní měření FC standardními laboratorními metodami [272].

5.2. Prediktivní faktory ve vztahu k léčbě dětských pacientů se zánětlivými střevními onemocněními

Námi realizovaná práce byla první prospektivní observační studií srovnávající efektivitu a bezpečnost dvou preparátů aTNF (ADA a IFX) u dětských pacientů s CD naivních k biologické léčbě. Vzhledem k nepřítomnosti head-to-head intervenčních studií porovnávající IFX a ADA je třeba se orientovat podle network meta-analýz a studií z realné praxe. Zatímco network-metanalýza ukazuje, že při standardní dávkování obou léků je ADA efektivnější, výsledky studií z reální praxe jsou rozporuplné [294–300]. Jedna dříve publikovaná meta-analýza zjistila, že i přes srovnatelnou efektivitu obou preparátů, je vyšší pravděpodobnost úspěšnosti indukční léčby při volbě IFX (86 %) a udržení remise v případě terapie ADA (48 %) [125]. Výsledky studií u dětí přináší obdobné výsledky, včetně RCT prokazující bezpečnost a účinnost obou preparátu v pediatrické populaci [301,302].

Proporce pacientů vyžadujících eskalaci terapie v průběhu tří let sledování byla v naší kohortě přibližně polovina, na rozdíl od recentně publikovaného systematického přehledu pediatrických prací uvádějícího vyšší procento pacientů pokračujících v nastaveném režimu léčby IFX (61-85 %) [303]. Příčinou této diskrepance by mohla být definice eskalace léčby, která v našem souboru zahrnovala také potřebu úpravy dávky a intervalu [277].

Identifikace prediktivních faktorů dlouhodobé odpovědi na terapii aTNF byla důležitou součástí naší práce [277]. V dosud publikovaných studiích u dětí bylo nalezených několik rizikových faktorů pro nepříznivý průběh onemocnění, nicméně bez vztahu k aTNF terapii [304,305]. Námi nalezená kombinace pANCA pozitivity a ASCA negativity v predikci eskalace léčby aTNF preparáty nebyla dosud pospána a je vhodná její prospektivní validace v nezávislé kohortě. Jelikož pozitivita pANCA protilátek je typická pro fenotyp UC a účinnost aTNF terapie je prokazatelně nižší u pacientů s diagnózou UC, je otázkou, zda by zjištěna serologická

kombinace protilátek mohla být potencionálním proxy markerem odlišného fenotypu CD s nižší citlivostí na léčbu aTNF [306,307].

Konkomitantní terapie imunomodulátorem nebyla v naší studii identifikována jako prediktor relapsu onemocnění, což by mohlo být důsledkem vysoké míry souběžné terapie v obou sledovaných skupinách [277].

Je třeba přiznat, že zařazený počet pacientů do této analýzy neumožňoval objevit menší rozdíli mezi preparáty. V další práci jsme proto využili celonárodní populační databáze CREdIT a zjišťovali jsme udržitelnost jednotlivých preparátů biologické léčby. V sub-analýze jsme nyní na větším počtu pacientů zjistili, že udržitelnost IFX je o 40 % nižší než při léčbě ADA. Studie u dospělých pacientů s IBD přinášejí v oblasti udržitelnosti ADA verzus IFX protichůdné výsledky [294,296,300,308]. V populaci dětských pacientů jsou data omezena [277,309]. Naše výsledky tedy ukazují rozdíly v dlouhodobé udržitelnosti mezi těmito dvěma sledovanými preparáty aTNF navzdory jejich srovnatelné účinnosti v provedených RCT [301,302]. Důvodem tohoto rozdílu může být způsob jejich aplikace (intravenózní podání je spojeno s větší variabilitou sérových koncentrací léčiva) [310–312]. V souvislosti s dosažením co nejstabilnějších hladin biologické léčby se v rámci udržovací léčby v současnosti prosazují subkutánní preparáty [313].

Pravděpodobnost udržitelnosti terapie v naší kohortě pacientů se zvyšovala s časnějším zahájením biologické léčby a volbou konkrétního preparátu (ADA). V tomto ohledu je důležité zdůraznit, že prodleva v zahájení aTNF terapie může ovlivnit pravděpodobnost její budoucí udržitelnosti. Při rozhodování o volbě preparátu je podstatné popsat pacientovi a jeho zákonným zástupcům možné nevýhody intravenózního IFX oproti subkutánnímu ADA [280].

Proaktivní monitorování hladin aTNF léků je stále kontroverzní otázkou. Zatímco dvě RCT u dospělých pacientů léčených IFX neprokázali její přínos, studie PILOT sledující hladiny ADA naopak ukázala vyšší míru remise u pacientů proaktivně sledovaných [314–318]. V naší další práci jsme hledali asociaci mezi hladinami IFX, eventuelně ATIs a zánětlivou aktivitou onemocnění v pediatrické skupině pacientů s CD. Nejoptimálnější kombinací senzitivity a specificity pro určení remise (definované jako CRP pod 5 mg/l) na základě hladin IFX byla hodnota 1,1 µg/ml. U dospělých pacientů jsou ve spojení s remisí CD uváděny hodnoty koncentrace IFX nad 2,79 µg/ml [319]. Na rozdíl od předchozích studií jsme neprokázali, že by průkaz přítomnosti ATIs a jejich kvantifikace přinášely klinicky významnou informaci [275,314]. Kombinovaná léčba thiopuriny, nejčastěji AZA vede k větší míře remise než monoterapie [320,321].

Předpokládá se, že spíše než aditivním efektem, je to dáno sníženou tvorbou neutralizačních protilátek proti biologiku [322].

Stanovení hladin metabolitů AZA a jejich vhodného cut-off predikující dosažení dostatečné hladiny IFX u dětských pacientů s CD na kombinované terapii (AZA a aTNF) bylo výstupem jedné z multicentrických studií [279]. Ve srovnání s jedinou prací realizované na dospělé kohortě byl námi nalezen cut-off 6-TGN významně vyšší (125 pmol/ 8 x 10^8 6-TGN v erytrocytu verzus 278 pmol/ 8 x 10^8 6-TGN v erytrocytu) [323]. U pacientů s nedetekovatelnými hladinami 6-TGN byla nalezena vyšší míra ztráty odpovědi k aTNF, což je v souladu s výsledky publikovanými jinými autori [324]. Z toho vyplývá, že kombinovaná léčba je úspěšnější v udržení remise než monoterapie IFX [303].

Ve studii případů a kontrol jsme ve srovnání s předchozími publikacemi nenalezli rozdíl v délce trvání remise u dětských pacientů s CD indukovaných pomocí EEN nebo kortikosteroidů [325]. Oproti uvedeným studiím byl našim pacientům od počátku současně podáván AZA [325,326]. Lze tedy předpokládat, že pokud jsou pacienti současně léčeni AZA, indukční léčba nehraje zásadní roli v délce trvání remise [327]. Námi identifikované nezávislé prediktivní faktory časného selhání terapie AZA byly nízký věk a snížené Z-skóre výšky v době diagnózy CD, postižení horního gastrointetsinálního traktu a zvýšený počet trombocytů v krvi v čase navození remise onemocnění. Jiná studie u dětí popisovala normální hladinu CRP a dosažení remise onemocnění bez použití kortikosteroidů za prediktivní faktory udržení remise v 52. týdnu [328]. Již výše uvedená práce vyhodnotila možnost použití poklesu koncentrace FC pod 200 μg/g ve čtvrtém týdnu indukční léčby pomocí EEN v predikci udržení remise v pediatrické kohortě pacientů s CD [210]. V naší studii jsme neprokázali asociaci CRP ani FC ve vztahu k trvání remise CD indukované EEN u dětských pacientů [115].

Jako první prospektivní studie u dětí s CD po ICR byla realizovaná naše práce s cílem objasnit rizikové faktory rekurence onemocnění u pacientů léčených po výkonu AZA. Recidiva onemocnění definovaná pomocí endoskopického skóre byla nalezena u 38 % jedinců šest měsíců po proběhlé ICR. Ve studiích u dospělých pacientů je udávaná četnost recidivy onemocnění vyšší [118,329]. Na základě výsledků se nezdá, že by terapie aTNF nebo pomocí imunomodulátoru před ICR byla rizikovým faktorem rekurence onemocnění. Použití FC jako markeru aktivity onemocnění v terénu ICR není dle našich výsledků dostatečně přesné. To je

v rozporu s publikovanou meta-analýzou u dospělých pacientů a také studií u dětí, ve kterých byl úspěšně detekován jako prediktivní faktor pooperační rekurence u CD [330,331]. Námi identifikovaným potencionálním prediktorem recidivy onemocnění v šestém měsíci po ICR byla hladina sérového albuminu v době chirugického výkonu, nicméně je potřeba dalších studií k jeho ověření a definování vhodného cut-off [274]. Limitací pro detekci potencionálních prediktorů a hledání rozdílů mezi dvěma různými terapeutickými přístupy před ICR byla velikost souboru.

5.3. Prediktivní faktory ve vztahu k rozvoji nežádoucích účinků zvoleného terapeutického postupu

V retrospektivní práci jsme prokázali, že IFX je ve srovnání s ADA rizikovým faktorem rozvoje neinfekčních kožních komplikací. Naše výsledky jsou v souladu s jedinou realizovanou studií v rámci dětské populace pacientů s IBD léčených preparáty první linie biologické léčby [332]. Incidence kožních komplikací asociovaných s aTNF léčbou se zdá být nižší ve skupině dospělých pacientů s IBD [333,334].

Naše práce navíc prokázala zvýšenou pravděpodobnost rozvoje dermatologických nežádoucích účinků při pozitivní anamnéze atopické dermatitidy. Vzhledem k velikosti sledovaného souboru a dalším limitacím studie není realné učinit definitivní závěr. Nicméně v rámci personalizace terapeutických přístupů, lze tato zjištění zohlednit v péči o dětské pacienty s IBD v procesu výběru preparátu aTNF a u jedinců se zvýšeným rizikem rozvoje uvedených komplikací preferovat terapii ADA se srovnatelnou efektivitou a bezpečnostným profilem [270].

Doporučení Evropské organizace pro Crohnovou chorobu a kolitidu (European Crohn´s and Colitis Organisation, ECCO) naznačují, že u EBV séronegativních pacientů mužského pohlaví mladších 35 let je vhodné se vyhnout podávání thiopurinů [150]. Dostupných údajů o séroprevalnci a virové náloži EBV v dětské populaci s IBD je nedostatek [335]. Ve srovnání s dřívější publikací byla prevalence EBV séropozitivních pacientů s nově diagnostikovaným IBD v naší kohortě vyšší (40 % vs. 64 %), navíc naše data naznačují zvyšující se proporci séropozitivních výsledků v průběhu dětství a adolescence [335]. Na základě daných zjištění bychom se mohli domnívat, že tento podstatný rozdíl v séropozitivitě EBV by mohol souviset

s terapií používané u naší skupiny pacientů, u kterých byl identifikován AZA jako prediktivní faktor časných pozitivních sérologických nálezů EBV. Dalším vysvětlením by mohla být regionální epidemiologická situace a faktory spojené s příslušností k určitému etniku [336–338].

Prevalence EBV DNA pozitivity u dětí s IBD byla v naší kohortě pacientů nižší ve srovnání s publikovanými údaji z dospělé populace (14 % vs. 35 %) [339]. Stejná studie uvádí, že léčba IFX v monoterapii nebo v kombinaci s AZA byla spojena s vyšší prevalencí EBV DNA séropozitivity. Naše výsledky ukazují srovnatelnou prevalenci v kontrolní skupině a prevalenci pozorované v jiné studii u pacientů léčených IFX (15 % a 12 %) [340]. Navíc jsme prokázali, že počet kopií EBV DNA a dávka IFX (na kg a 8 týdnů) byly významně asociovány. Dle dostupných dat je známo, že virová nálož je prediktorem rozvoje lymfomu v potransplantačních podmínkách [341]. Nicméně se nám nepodařilo potvrdit předchozí pozorování, že věk při stanovení by byl rizikovým faktorem pro přítomnost a virovou nálož EBV [271,339].

5.4. Prediktivní faktory extraintestinálních komplikací u dětských pacientů se zánětlivými střevními onemocněními

V prospektivní observační studii jsme se zaměřili na vyhodnocení muskuloskeletálních parametrů ve vztahu k výskytu vertebrálních fraktur u dětí a dospívajících s IBD. Zjistili jsme, že námi sledovaní pacienti měli mírně sníženou trabekulární kostní denzitu, ale normální dynamické svalové funkce. Na rozdíl od našich výsledků, dřívě publikované práce popisovaly, signifikantně sníženou svalovou sílu u dětí a adolescentů s IBD ve srovnání se zdravou populací [342–344]. Důvodem rozdílných výsledků by mohol být odlišný metodologický přístup (denzitometrie (DXA) vesus pQCT) [345,346]. Na rozdíl od pQCT, DXA nezohledňuje velikost kosti měřeného pacienta. Proto údaje získané touto metodou musí být interpretována ve vztahu k výšce pacienta. Kromě toho je DXA omezena v tom, že nedokáže odlišit jednotlivé kosti a posoudit geometrii kosti. Kostní parametry vyšetřené na kohortě našich pacientů byly srovnatelné s výstupy studií, ve kterých bylo měření provedeno pomocí pQCT s nálezem signifikantně snížené trabekulární kostní denzity a normální nebo zvýšené kortikální kostní denzity [344,347–349].

V souladu s výsledky u dospělých pacientů s IBD jsme nenalezli žádnou korelaci mezi snížením výšky obratle a trabekulární denzitou kosti [350]. Z toho vyplývá, že tento parametr kostní jednotky není jediným rizikovým faktorem zvýšeného výskytu vertebrálních fraktur u

jedinců s IBD. Na základě těchto zjištění je možné konstatovat, že rentgenové zobrazení torakolumbální páteře by nemělo být rutinně doporučováno u asymptomatických dětí s IBD a indikace k provedení by měla vycházet z individuálních nálezů u konkrétních pacientů [283].

Dalším zajímavým výstupem je zjištění, že kostní změny jsou nezávislé na hladině 25-hydroxyvitamin D (25-OHD) v séru. S tím souvisí i naše další práce, která se zaměřila na otázku, zda by pacienti s IBD a nedostatečnými hladinami 25-OHD měli být suplementováni a pokud ano, jaké by mělo být optimální dávkování. Zjistili jsme, že pravidelné podávání 2000 jednotek cholekalciferolu dětem a adolescentům s IBD bylo spojeno se zvýšením trabekulární kostní denzity a zlepšením svalové síly. Protože efekt substituce byl nezávislý na koncentraci 25-OHD v úvodu studie, rutinní suplementace vitaminem D se zdá být rozumným přístupem ke zlepšení pevnosti kostí u této skupiny pacientů, a to bez ohledu na zjištěnou hladinu 25-OHD [276].

5.5. Prediktivní faktory postvakcinační odpovědi v kohortě adolescentů se zánětlivým střevním onemocněním

Pacienti s IBD dětského a adolescentního věku vykazovali v naší studii vynikající postvakcinační odpověď, a to i přes terapii aTNF preparáty, u kterých by bylo možné předpokládat insuficientní reakci [278]. V souvislosti s negativní asociací mezi aTNF a postvakcinačními protilátkami je sporné, zda by pacienti léčeni aTNF preparáty měli být upřednostňováni v indikaci k aplikaci posilovacích vakcín. Na základě dalších studií se zdá, že IBD pacienti jako celek nemají oproti běžné populaci zvýšené riziko těžkého průběhu infekce vyvovalné SARS-CoV-2 [351]. Na druhou stranu jiné práce ukazují, že některé preparáty jsou asociovány s vyšším výskytem postvakcinačně se objevujících infekcí. To předpokládáme u kortikosteroidů a dále, ve shodě s nižšími titry v naší studii také ve skupině pacientů léčených aTNF [351].

6. Závěr

Se vzestupem důrazu na používání principů personalizované medicíny došlo k obrovskému nárůstu popularity nástrojů pro predikci výsledků pro jednotlivé pacienty. Tyto algoritmy jsou schopny pomáhat v diagnostické a terapeutické části rozhodování. Zánětlivá střevní onemocnění představují oblast medicíny, kde se intenzivně uplatňují jak klasické statistické metody k hledání prediktorů a tvorbě prediktivních modelů pro různé klinické situace, tak metody ML pracující s velkými objemy dat.

Nedílnou součástí prediktivního modelování je uplatnění vhodných proměnných do konečného modelu, tak aby model dokázal zachytit skutečný vztah, který existuje v datech mezi výsledkem a vybranými proměnnými. Ve své práci se zabývám hledáním a studiem prediktorů IBD, včetně slibného markeru, FC.

V jednotlivých studiích jsme uplatnili klasické biostatistické metody k identifikaci prediktivních faktorů. V některých jsme se pokusili vytvořit predikční modely. Statistické zpracování dat probíhalo ve vývojovém prostředí R, které poskytuje širokou škálu statistických a grafických technik, včetně lineárního a nelineárního modelování. V rámci našich prací byly použity následující metody statistické analýzy: v případě sledování pacientů v průběhu času jsme aplikovali na data survival analýzy a Cox regrese; v souvislosti s opakovaným měřením jednoho pacienta jsme použili mix model; pro kategorické výsledky zobecněný lineární smíšený model a pro lineární výsledky lineární smíšený model; v rámci některých studií bylo možné na základě klinického výběru prediktorů zkonstruovat konečný predikční model a když to bylo vhodné přidali jsme do modelu efekty interakce; k vyhodnocení prediktorů udržitelnosti léčby byly aplikovány Coxovy modely se smíšenými efekty a analýzy propensity skóre.

Na základě našich zjištění lze uzavřít, že FC lze využít pro účely domácího testování, nicméně zlatým standardem ke stanovení jeho koncentrace ve stolici zůstává přesnější klasická laboratorní metoda ELISA. Nebyl potvrzen potencionální přínos FC k časné identifikaci pacientů, kteří nedosáhnou odpovědi na konkrétní typ indukční léčby.

S velkým přispěním našich dat se zdá, že terapie ADA je u pediatrických pacientů udržitelnější než terapie IFX. Zda je to efektivitou však zatím nelze na základě dostupných dat zodpovědět. Částečně je tuto vyšší udržitelnost možno vysvětlit častějším výskytem kožních nežádoucích účinků oproti ADA. Identifikovali jsme četné prediktivní faktory zachování remise onemocnění či udržitelnosti jednotlivých aTNF preparátů. Ve skupině pacientů léčených aTNF se zdá nejsilnějším prediktorem kombinace serologických markerů - séropozitivita pANCA a

séronegativita ASCA. Naopak nejsilnějším a asi nejlépe potvrzeným prediktorem časného selhání je delší doba do zahájení aTNF terapie. Ve shodě s publikovanými daty, se zdá, že hladiny aTNF i hladiny thiopurinů jsou slabými prediktivními faktory remise onemocnění. Ve skupině pacientů léčených monoterapií AZA není typ indukční léčby zásadním faktorem. Přestože jsme v této skupině identifikovali několik rizikových faktorů, jejich síla neumožňovala vytvořit klinicky relevantní predikční model. Seropozitivita EBV je asociovaná s terapií AZA a naopak virová nálož s dávkou aTNF. Ať již je pacient před ICR léčen AZA či aTNF terapií riziko rekurence je relativně vysoké.

Naše studie ukazují, že a rentgenové zobrazení torakolumbální páteře není vhodné paušálně vyšetřovat u všech pediatrických IBD pacientů. Naopak substituci vitaminem D lze zvážit u všech, bez ohledu na jeho hladinu v séru.

Disertační práce přináší v podobě výsledků jednotlivých studií a jejich interpretace pro konkrétní klinické otázky spektrum cenných informací v oblasti péče o pediatrickou populaci s IBD. Identifikace prediktivních faktorů ve vztahu k přesně definovaným jevům umožňuje spřesňovat a více personalizovat rozhodovací proces a vedení vyšetřovacích postupů v konkrétních klinických situacích. Nicméně, samotné nalézání prediktivních faktorů je insuficientní a je potřeba jejich ověření na nezávislých kohortách. Ve většině našich prací nebylo možné sestavit predikční model zejména z důvodu nedostatečného množství asociovaných proměnných a/nebo nevyhovující síly identifikovaných prediktorů.

Významným přínosem pro vědecko-výzkumné projekty v oblasti dětských IBD by byl původně plánovaný hlavní projekt postgraduálního studia - software pro prospektivní pseudonymizovaný systematický sběr a kategorizaci klinických, laboratorních a dalších údajů o pacientech s IBD s možností jejich následného statistického zpracování a automatizovaného vytváření predikčních modelů pro konkrétní klinické situace. Pozitivní je, že v současnosti existují dlouhodobé prospektivní multicentrické registry, které jsou považovány za nejvhodnější způsob získavání dat pro identifikaci a studium potencionálních prediktivních faktorů. V rámci spolupráce se tak daří získavat kvalitní výstupy opírající se o dostatečně objemné a konzistentní datové soubory.

Věřím, že se nám v navazujících studiích bude dařit naplňovat primární myšlenku naší práce a přispět tím ke zlepšení péče o dětské pacienty s IBD díky nově identifikovaným možnostem predikce konkrétního klinického jevu.

Souhrn

Zánětlivá střevní onemocnění (IBD) představují skupinu chronických, polygenně podmíněných onemocnění postihujících primárně gastrointestinální trakt a jejichž incidence v dospělé i dětské populaci globálně narůstá. Mezi tyto nemoci řadíme Crohnovou nemoc (CD), ulcerózní kolitidu (UC) a tzv. neklasifikovatelné IBD (IBD-U). Fekální kalprotektin (FC) je markerem zánětu u IBD a jeho hladiny korelují s aktivitou onemocnění definovanou klinickými parametry, endoskopickým nálezem a histologií. Současná lékařská praxe je spojena s dostupností velkého množství klinických dat a snahou jejich efektivního uplatnění v procesu medicínskeho rozhodování takovým způsobem, aby bylo dosaženo maximální možné redukce rizika nepříznivého průběhu onemocnění a výskytu s onemocněním a/nebo léčbou asociovaných komplikací.

Primárním cílem disertační práce bylo použít metody biomedicínské informatiky v procesu ověření FC v predikci aktivity onemocnění a odpovědi na léčbu u dětských pacietnů s IBD. Zjistili jsme, že na základě vývoje hladin FC v časné fazi indukční léčby pomocí výhradní enterální výživy nelze rozhodovat o dalším vedení terapie. Ověřením domácího testu na stanovení koncentrace FC ve stolici jsme poukázali na jeho možné benefity pro urychlení procesu rozhodování, nicméně s nutností konfirmace výsledku klasickými laboratorními metodami.

Další práce byly zaměřené na hledání potencionálních prediktorů pro přesně definovaný outcome. Stanovením vyváženého cut-off metabolitů azathioprinu k předpovědi dosažení efektivních hladin infliximabu jsme získali vhodný nástroj pro optimalizaci kombinované terapie u pacientů s CD. Srovnáním dvou preparátů první linie biologické léčby jsme upozornili na potřebu zohlednění nalezených rizikových faktorů při volbě terapie. Zjistili jsme, že indukční léčba nehraje zásadní roli v délce trvání remise onemocnění u pacientů s nekomplikovanou CD současně užívajících azathioprin. Zdůvodnili jsme vhodnost suplementace vitaminu D u dětských pacientů s IBD a poukázali na možnost redukce dřívě indikovaných vyšetření u asymptomatických jedinců.

Nové poznatky vyplývající z realizovaných studií s sebou přináší možnost exaktnějšího a individuálně zaměřeného přístupu v procesu klinického rozhodování. Nicméně, samotná identifikace prediktivních faktorů je insuficientní a je potřeba jejich ověření na nezávislých kohortách.

Summary

Inflammatory bowel diseases (IBD) are a group of chronic, polygenic diseases primarily affecting the gastrointestinal tract, and their incidence is increasing globally in both adult and paediatric populations. These diseases include Crohn's disease (CD), ulcerative colitis (UC) and so-called IBD unclassified (IBD-U). Fecal calprotectin (FC) is a marker of inflammation in IBD and its levels correlate with disease activity as defined by clinical parameters, endoscopic findings and histology. Current medical practice is associated with the availability of a large amount of clinical data and the desire to apply it effectively in the medical decision-making process in such a way as to achieve the maximum possible reduction in the risk of adverse disease course and the occurrence of disease- and/or treatment-associated complications.

The primary aim of this dissertation was to apply biomedical informatics methods in the process of validating FC in predicting disease activity and response to treatment in paediatric patients with IBD. We found that the evolution of FC levels in the early phase of induction therapy using exclusive enteral nutrition cannot be used to make decisions about further management. By validating a home test for the determination of FC concentration in stool, we highlighted its potential benefits for speeding up the decision-making process, however, with the need to confirm the result by conventional laboratory methods.

Other work focused on finding potential predictors for a well-defined outcome. By establishing a balanced cut-off of azathioprine metabolites to predict the achievement of effective infliximab levels, we have obtained a suitable tool for optimizing combination therapy in CD patients. By comparing two first-line biologic therapy agents, we highlighted the need to consider the identified risk factors in the choice of therapy. We found that induction therapy does not play a major role in the duration of disease remission in patients with uncomplicated CD concomitantly taking azathioprine. We justified the appropriateness of vitamin D supplementation in paediatric patients with IBD and highlighted the possibility of reducing previously indicated testing in asymptomatic individuals. The new findings from the conducted studies bring with them the possibility of a more exact and individualized approach in the clinical decision-making process. However, the identification of predictive factors alone is insufficient and their validation in independent cohort.

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The Accuracy of a Home-performed Faecal Calprotectin Test in Paediatric Patients With Inflammatory Bowel Disease

Tereza Lerchova, Ondrej Hradsky, Ivana Copova, Kristyna Potuznikova, Lucie Gonsorcikova, and Jiri Bronsky

ABSTRACT

Objectives: Owing to the invasiveness of endoscopy, the use of biomarkers, especially faecal calprotectin (FC), has become standard for remission assessment. This study aimed to compare the accuracy for detection of endoscopic activity using recently developed FC home test using smartphone application (FC-IBDoc) against standard enzyme-linked immunosorbent assay (ELISA).

Methods: In all, 102 consecutive observations (89 participants) were included in prospective observational study. FC-IBDoc was performed parallelly with FC-ELISA in paediatric patients with inflammatory bowel disease indicated for endoscopy. Both tests were performed by trained staff. Mucosal healing was defined using Simple Endoscopic Score for Crohn disease (CD) <2 in patients with CD (n ¼ 44), ulcerative colitis (UC) Endoscopic Index of Severity <4 in patients with UC (n ¼ 27) and Rutgeerts score i0 and i1 without colon involvement in patients with CD after ileocaecal resection (n ¼ 19).

Results: Out of 102 endoscopic findings 23 were assessed as mucosal healing. We found an association of the mucosal healing scores of the entire group both with FC-ELISA (P ¼ 0.002) and FC-IBDoc (P ¼ 0.001). The area under the receiver operating characteristic curve for FC-ELISA was 0.883 (95% confidence interval 0.807–0.960), with optimal cut-off at 136.5 mg/g. The area under the receiver operating characteristic curve for FC-IBDoc was 0.792 (95% confidence interval 0.688–0.895) with optimal cut-off at 48 mg/g. The FC-ELISA was more accurate than FC-IBDoc when tested by a Delong test (P ¼ 0.023).

Conclusions: Standard FC-ELISA for FC evaluation is more reliable predictor of mucosal healing than the FC-IBDoc in paediatric patients with inflammatory bowel disease. The cut-off values for both tests were incongruous.

Key Words: bedside, biomarkers, endoscopy, point-of-care, smartphones

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What Is Known

- The utility of monitoring faecal calprotectin levels in assessment of inflammatory bowel disease activity was clearly demonstrated.
- The concentration of faecal calprotectin is usually measured by quantitative enzyme-linked immunosorbent assay in laboratory.
- The main disadvantages of enzyme-linked immunosorbent assay are the requirement of a well-equipped laboratory to analyse the samples, high financial cost, and slow evaluation time.

What Is New

- Enzyme-linked immunosorbent assay for faecal calprotectin evaluation is more reliable predictor of mucosal healing than the device for faecal calprotectin home testing in paediatric patients with inflammatory bowel disease.
- The cut-off values for enzyme-linked immunosorbent assay and home faecal calprotectin test were highly incongruous.

Proper diagnosis and precise monitoring play an essential role in treatment of patients diagnosed with inflammatory bowel disease (IBD) (1,2). When assessing disease activity, the results of the whole set of examinations, monitoring various aspects of the disease, must be included. Mucosal healing should, however, be the

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From the Department of Paediatrics, 2nd Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic.

Address correspondence and reprint requests to Tereza Lerchova, MD, University Hospital Motol, Second Faculty of Medicine, V Uvalu 84, Prague 5, 150 06, Czech Republic (e-mail: terezadrskova@gmail.com).

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main goal in the treatment of paediatric patients with IBD (3,4). Even though endoscopic examination is the criterion standard in evaluation of mucosal healing (1,3,5,6), noninvasive methods (eg, evaluation of complex clinical status using scales of disease activity (6), radiological examinations (7,8), and laboratory parameters (6,9,10)) have recently gained importance because of disadvantages related to endoscopy, such as invasiveness, financial cost, or limited extent of examination (1,6,11,12). In addition, many of the listed methods allow for assessment of other parameters apart from mucosal healing, such as transmural healing, deep remission, or general clinical conditions of the patient. According to recent data, mentioned parameters are becoming an important part of disease activity monitoring (13,14).

Based on previous data, biomarkers in the stool are considered to be the most sensitive laboratory parameters when assessing the endoscopic and histological activity of inflammation (1,6). It has been shown that the concentration of the neutrophil-derived marker, faecal calprotectin (FC), is associated with endoscopic activity scales and reflects mucosal healing (1,15– 19). Moreover, a correlation was demonstrated in the comparison of FC levels and transmural or deep remission (13). The utility of monitoring FC levels in assessment of IBD activity was clearly demonstrated in a randomized controlled trial in 2017 performed in adult patients with Crohn disease (CD) (Cochran-Mantel-Haenszel test adjusted risk difference between the tight control group and the clinical management group of 16.1% in remission rate [95% confidence interval (CI) 3.9–28.3; P ¼ 0.010]) (20).

The concentration of FC in stool samples is usually measured by quantitative enzyme-linked immunosorbent assay (FC-ELISA) (6,21). The main disadvantages of this method are the requirement of a well-equipped laboratory to analyse the samples, high financial cost, and slow evaluation time (6,21). To be readily applicable, the test should be simple and the results rapidly available to the physician. A new FC home test based on smartphone application, (FC-IBDoc, Buhlmann Laboratories AG, Switzerland) produces an immediate result and allows FC level monitoring on-site or at home.

The primary aim of the study was to compare the accuracy of detection of endoscopically assessed inflammation using the recently developed FC-IBDoc to a standard FC-ELISA. A secondary objective was to compare the variability of the results from multiple measurements of 1 sample.

METHODS

Participants

The study was designed as a prospective observational study focused on comparing of 2 different FC tests taking endoscopy as a criterion standard. Data from 103 paediatric patients were consecutively collected between October 2016 and March 2018. The stool samples were collected from paediatric patients diagnosed with IBD or with suspicion of IBD indicated for endoscopic examination and hospitalized at the Department of Paediatrics, Motol University Hospital, Prague.

The inclusion criteria were age between 3 and 19 years, diagnosis of IBD based on Porto criteria (22), and indication for endoscopy. Based on study protocol, patients who were unable to provide a stool sample before starting the bowel preparation for endoscopic examination, were taking nonsteroidal anti-inflammatory drugs or anticoagulants, or were diagnosed with IBD unclassified, and patients with diverting stoma, or infectious gastrointestinal disease were excluded. Eligibility of patients is shown in Figure 1. In total, 103 patients, who provided stool sample, were screened to

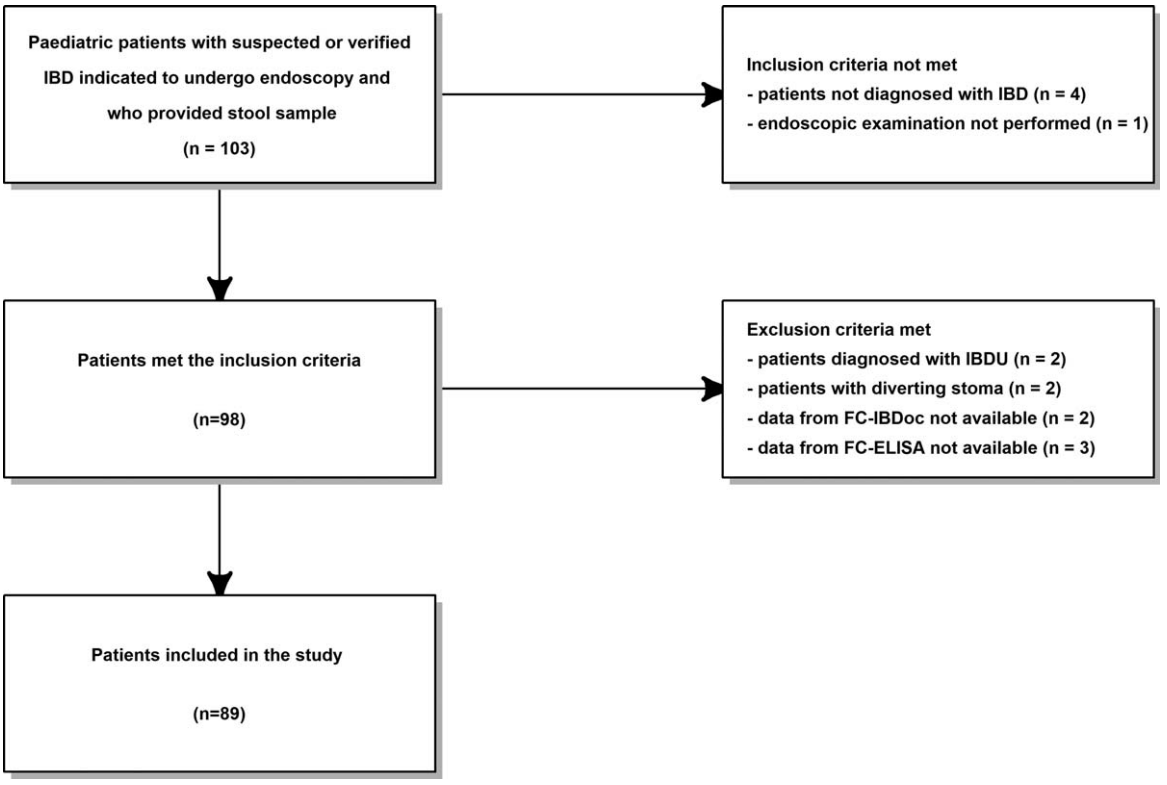

FIGURE 1. Participants selection. Flowchart showing selection of patients based on inclusion and exclusion criteria. FC-ELISA ¼ faecal calprotectin quantitative enzyme-linked immunosorbent assay; FC-IBDoc ¼ faecal calprotectin home test based on smartphone application; IBD ¼ inflammatory bowel disease.

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be enrolled in the study, 5 did not meet inclusion criteria and had to be excluded. Out of the remaining 98 patients, 9 were excluded based on exclusion criteria. Finally, 89 subjects were included in the study, a total of 102 measurements were performed.

Detection of Faecal Calprotectin Concentration

The same sample of stool taken before starting bowel preparation for colonoscopy was analysed using an FC-IBDoc in parallel with a standard FC-ELISA.

A recently developed home test for quantitative determination of FC levels in stool samples is based on an image evaluation from a Lateral Flow device designed for sample processing. Extraction tube called CALEX Valve is used to collect a precise amount of stool sample. In the same tube the stool sample is mixed with an extraction solution and subsequently applied on the lateral flow device. The mixture is then detected by red and gold particles as rising through the test cassette and reaching testing and control strip. The FC levels are measured based on immunochromatographic method. The image is taken by a smartphone and sent for evaluation using an Internet application. The result is displayed within 15 seconds and simultaneously sent to the FC-IBDoc Portal. According to manufacturer's data (23), the test has a range of 30 to 1000 mg/g, results above 1000 mg/g are less accurate. According to the manufacturer's instructions (23), all samples were collected in screw-capped plastic containers and stored in a refrigerator. The time from sample acquisition to processing was no longer than 7 days. All FC-IBDoc analyses were performed by a trained physician, and each sample was tested twice, with the mean of the 2 obtained values used for statistical analysis. In addition, stool samples from 14 patients were measured 5 times to perform intra-assay reliability analysis.

FC-ELISA is a standard laboratory method using a fluorescence immunoassay. Stool samples were collected in the same screw-capped plastic containers and sent to the laboratory to be prepared and analysed using the EliA Calprotectin 2 test (Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden). Based on the manufacturer's data, results >6000 mg/g are less precise.

Endoscopic Evaluation

We chose the categorical variable of mucosal healing as a primary goal because it corresponds to the main target of the FC-IBDoc test. Results from both tests were compared with mucosal healing defined by endoscopic scores, in patients with CD, by simple endoscopic score for CD (SES-CD) <2 (n ¼ 44) (24,25), in patients with ulcerative colitis (UC), by UC endoscopic index of severity (UCEIS) <4 (n ¼ 27) (26,27), and in patients with CD after ileocaecal resection (ICR), by Rutgeerts score i0 and i1 without signs of colon involvement (n ¼ 19) (28).

No information regarding other tests results were available to readers of a particular test.

Statistical Analysis

All data were analysed using R statistical software (version 3.4.4). Continuous variables were described as median and interquartile range (IQR). Categorical variables were described as absolute frequencies and percentages. Values of FC were analysed on a logarithmic scale. Because of multiple observations from 1 subject, the associations between mucosal healing and FC were assessed using a generalized linear mixed model and a linear mixed model (R package ''lme4'' and ''lmerTest''). The accuracy of FC-IBDoc and FC-ELISA for respective scores was analysed using receiver operating characteristic (ROC) curve analysis with cross-validated area under the ROC curve (AUC) measurements. The AUCs were compared using a DeLong test. Among patients with multiple observations of IBDoc at 1 time, we performed a 2-way mixed-effects model to assess intraclass correlation and, using a bootstrap method, a CI was constructed. To compute Fleiss' kappa, R package ''irr'' was used. P values <0.05 were considered statistically significant. All P values were 2 sided.

Ethical Considerations

The study was approved by the Ethics Committee of the authors' institution (EK-1491/16). The patients' guardians received written information on the study and signed a consent form. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

RESULTS

Patients Characteristics

We included 102 observations from 89 patients (62 CD and 27 UC, 48 boys [54%], median age 15.36 years [IQR 11.14–17.28]). The patient's demographic and clinical characteristics according to Paris classification (29) are listed in Table 1.

Prediction of Mucosal Healing Based on Faecal Calprotectin Levels

Among 102 performed endoscopic examinations from 89 patients included in the study, 79 did not fulfil the criteria for mucosal healing, 46 SES-CD 22 in patients with CD, 22 UCEIS 24 in UC, and 11 Rutgeerts score 2i0 and i1 without signs of colon involvement in CD after ICR (Supplemental Table 1, Supplemental Digital Content, *http://links.lww.com/MPG/B621*). The median measured concentration of FC in all 102 samples was 690 mg/g (IQR 117.5–1924.5) when measured with FC-ELISA and 78.0 mg/g (IQR 30.0–324.0) in FC-IBDoc. Medians measured FC concentration in endoscopically active and inactive patients differ applying both, FC-ELISA and FC-IBDoc, tests (Fig. 2). We found a correlation between the results of both tests (Pearson correlation coefficient of 0.70, 95% Cl 0.58–0.78, $P < 0.0001$).

We found an association of the mucosal healing of the entire IBD group both with FC-ELISA (P ¼ 0.002) and FC-IBDoc (P ¼ 0.001). To determine the best cut-off value of each test to predict mucosal healing, ROC curves were constructed (Fig. 3). The crossvalidated AUC for FC-ELISA was 0.883 (95% CI 0.807–0.960) with optimal cut-off at 136.5 mg/g. The AUC for FC-IBDoc was 0.788 (95% CI 0.674– 0.902) with optimal cut-off at 48 mg/g. When a Delong test was applied, the FC-ELISA was more accurate than FC-IBDoc (P ¼ 0.023). Similarly, the regression mix-model constructed using FC-ELISA as a predictor, was significantly better than the model using FC-IBDoc. We did not find an association with FC levels, measured with FC-ELISA or with FC-IBDoc, and mucosal healing in subset of patients assessed by SES-CD, UCEIS, or Rutgeerts score.

Prediction of Intensity of Inflammation Based on Faecal Calprotectin Levels

When assessing intensity of inflammation using a linear mixed model for subsets of observations evaluated by SES-CD as the continuous variable, we found an association with FC-ELISA (b ¼ 2.35, 95% CI 0.87–3.91), but not with FC-IBDoc. We found

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TABLE 1. Patient's demographic and clinical characteristics according to Paris classification (29)

	All	CD	UC
Number of patients	89	62	27
Sex, male (%)	48 (0.54)	33 (0.53)	15 (0.56)
Age at diagnosis, y, median (IQR)	12.5 (9.5–14.9)	12.7 (9.9–14.9)	11.5 (7.9–14.8)
Ileal involvement, L1 (%)	20 (0.22)	20 (0.32)	
Colonic involvement, L2 (%)	10 (0.11)	10 (0.16)	
Ileocolonic involvement, L3 (%)	31 (0.35)	31 (0.5)	
L4a (%)[y]	25 (0.28)	25 (0.4)	
L4b (%)[z]	3 (0.03)	3 (0.05)	
L4ab (%)[§]	0 (0.00)	0 (0.00)	
Nonstricturing and nonpenetrating disease, B1 (%)	39 (0.44)	39 (0.63)	
Stricturing disease, B2 (%)	22 (0.25)	22 (0.35)	
Penetrating disease, B3 (%)	0 (0.00)	0 (0.00)	
B2B3 (%)[jj]	1 (0.01)	1 (0.02)	
Perianal disease (%)	7 (0.08)	7 (0.11)	
Proctitis, E1 (%)	2 (0.02)		2 (0.07)
Left-side colitis, E2 (%)	5 (0.06)		5 (0.19)
Extensive colitis, E3 (%)	1 (0.01)		1 (0.04)
Pancolitis, E4 (%)	19 (0.21)		19 (0.7)
Never acute severe colitis, S0 (%)	24 (0.27)		24 (0.89)
Ever acute severe colitis, S1 (%)	3 (0.03)		3 (0.11)

The Paris classification applies to the time of diagnosis.
CD ¼ Crohn disease; IQR ¼ interquartile range; UC ¼ ulcerative colitis.
Out of 62 following patients with CD 19 underwent ileocaecal resection since the time of diagnosis.
[y]Upper gastrointestinal (GI) involvement up to Treitz ligament correspond to L4a, according to Paris classification.
[z]Upper GI involvement distal to Treitz ligament correspond to L4b, according to Paris classification.
[§]Upper GI involvement up and distal to Treitz ligament correspond to L4ab, according to Paris classification.
[jj]Stricturing and penetrating disease correspond to B2B3, according to Paris classification.

an association of UCEIS with both FC-ELISA and FC-IBDoc (b ¼ 0.62, 95% CI 0.37–0.86, respectively; b ¼ 0.85 ,95% CI 0.44–1.26). Rutgeerts score in a subgroup of patients with CD after ICR was associated with both FC-ELISA and FC-IBDoc (b ¼ 0.46, 95% CI 0.20–0.69, respectively; b ¼ 0.57, 95% CI 0.02–0.16).

Intraclass Variability of Faecal Calprotectin Levels Measured by FC-IBDoc

Based on obtained Intraclass correlation coefficient (ICC) in a subset of 70 repeated measurements from 14 patients, computed by a 2-way mixed-effects model, the level of reliability was concluded as good (ICC ¼ 0.79, 95% CI 0.53– 0.89). When using Fleiss kappa, the level of reliability was rated as fair to good (Fleiss kappa coefficient ¼ 0.68).

DISCUSSION

To the best of our knowledge this was the first study simultaneously evaluating FC levels measured with the use of a smartphone application, standard ELISA, and endoscopy as a criterion standard. We found that the standard ELISA for FC evaluation is a more reliable predictor of mucosal healing than the FC-IBDoc in paediatric patients with IBD.

Along with the development of a new field of healthcare, e-Health (30), home testing is becoming more accessible. This new concept, the main purpose of which is to use information and communication technology to promote prevention, diagnosis, and treatment of various diseases, intervenes in all branches of medicine including the care of patients with IBD. Home monitoring of FC levels using a smartphone application is now available.

To establish the value of home monitoring of FC concentration in management of care of patients with IBD, it was necessary to compare the results of the home test and standard tests. Several studies comparing the accuracy of FC home testing with the standard test have been recently published. Vinding et al (31) reported in 2016 a moderate agreement between FC levels obtained using ELISA and the smartphone-based home test (r ¼ 0.685). The study was conducted on large number of adult patients who performed the test themselves. Elkjaer et al (32) compared 3 methods of FC level measurement including ELISA, the smartphone-based home test, and a lateral flow device–based test linked to a laptop computer. Agreement was found both when comparing FC concentration measured by ELISA with the 2 rapid tests (r ¼ 0.954, respectively, r ¼ 0.939), and when comparing the smartphone-based home test and lateral flow device–based test to each other (r ¼ 0.961). Similarly, a smaller study in 2012 (33) performed in patients with suspicion of IBD (n ¼ 40) or IBD relapse (n ¼ 45) showed a correlation between the quantitative method, time-resolved fluorescence immunoassay (34), and 2 rapid tests, namely Quantum Blue Calprotectin (k 0.77; 95% CI 0.64–0.90) and Prevent ID CalDetect (k 0.46; 95% CI 0.32–0.60). Bello et al (11) confirmed a good correlation between the home-based and central ELISA measurements of FC levels in an adult IBD population (ICC ¼ 0.88 with inferior limit of the coefficient 0.82). Similarly, Heida et al (35) has shown that measurements of FC concentrations done with a home-based lateral flow method using a smartphone application were in agreement with measurements made by a hospital-based reader using the Quantum Blue method (Spearman rank correlation coefficient r ¼ 0.94, $P <$ 0.001) and central ELISA (Spearman rank correlation coefficient r ¼ 0.85, $P < 0.001$). In this study, performed in a combined adult (n ¼ 82) and paediatric (n ¼ 19) population, agreement between the home-based lateral flow test and ELISA was found when FC concentrations were <500 mg/g

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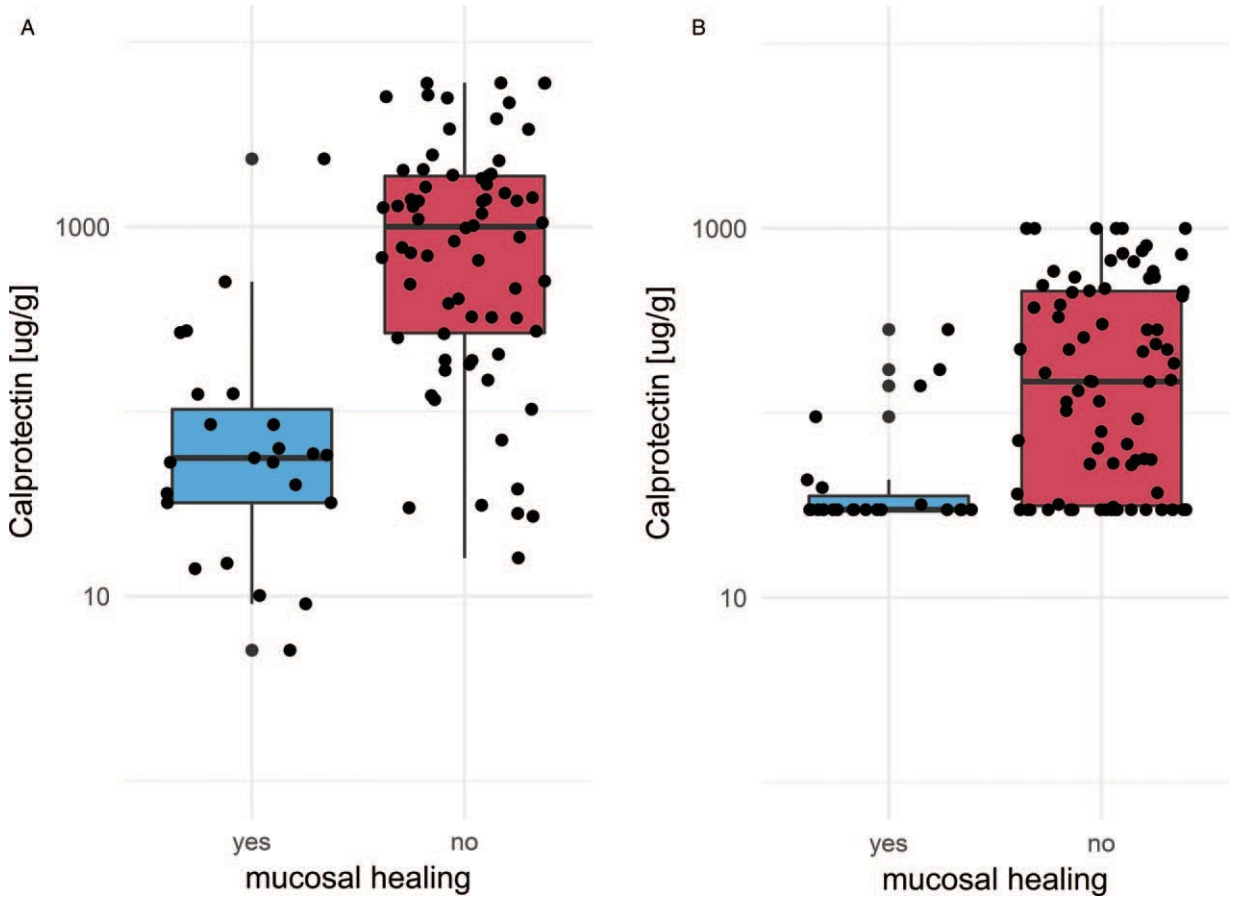

FIGURE 2. Box plots comparing medians FC values measured with FC-ELISA (A) and FC-IBDoc (B) in patients with CD (n ¼ 44), UC (n ¼ 27), and with CD after ileocaecal resection (n ¼ 19) with and without mucosal healing. A, The median value of FC concentration in the stool samples measured by FC-ELISA in endoscopically active (n ¼ 79) and inactive patients (n ¼ 23). B, The median value of FC concentration in the stool sample measured by FC-IBDoc in endoscopically active (n ¼ 79) and inactive patients (n ¼ 23). Mucosal healing was defined as SES-CD <2 in patients with CD (n ¼ 44), UCEIS <4 in patients with UC (n ¼ 27) and Rutgeerts score i0 and i1 without colon involvement in patients with CD after ileocaecal resection (n ¼ 19). CD ¼ Crohn disease; FC ¼ faecal calprotectin; FC-ELISA ¼ faecal calprotectin quantitative enzyme-linked immunosorbent assay; FC-IBDoc ¼ faecal calprotectin home test based on smartphone application; UC ¼ ulcerative colitis; UCEIS ¼ ulcerative colitis Endoscopic Index of Severity.

(71% of IBDoc results were with agreement with ELISA results). In the latter 2 studies (11,35), the home test was performed by the patients themselves. In these studies, different levels of agreement between ELISA and the home-based method of FC evaluation have been described. None of the studies mentioned above took into account clinical data, but only focused on comparison of measured levels of FC, which were not directly compared with the endoscopic findings, so it is difficult to evaluate which of the 2 methods is more accurate.

Although it is known that FC remains relatively stable in stool samples for at least 3 days at a room temperature (36,37), higher accuracy due to quick and immediate processing with no need for time for transportation, can be assumed. Because FC-ELISA, however, proved to be more accurate than the FC-IBDoc, this speculation was not confirmed in the present study.

It should be noted that results from FC-IBDoc are available in $2^{1}/_{2}$ hours, whereas it takes several days to obtain results from FC-ELISA, which is however approximately 2 times cheaper than FC-IBDoc.

Several point-of-care testing methods for FC detection were available before home-based tests were developed. A good accuracy of these rapid methods has been confirmed in multiple studies (38–42), but only a few compared measured FC concentration with endoscopic findings. Positive correlation between endoscopically assessed activity of inflammation and FC levels measured with point-of-care testing methods was confirmed in several studies performed in adult populations (6,12,43,44). The excellent accuracy of a bedside faecal calprotectin enzyme-linked immunosorbent assay in prediction of histological inflammation was shown in a study performed in paediatric treatment-naive patients (45). The same study confirmed a good accuracy of the method when assessing endoscopic inflammation.

Since the cut-off values for both the FC-ELISA and FC-IBDoc tests were highly incongruous, we have shown that it is not possible to freely compare their results. As none of the previously performed studies considered clinical data, the optimal cut-off values of FC home-based test were calculated with respect to FC levels measured with ELISA. The range of calculated cut-off points was from 50 to 150 mg/g, which tightly meets the optimal cut-off indicated in this study (31,32,35). The optimal cut-off point predicting mucosal healing of ELISA was 250 mg/g in a large meta-analysis conducted in patients with CD and UC (46). The cut-off

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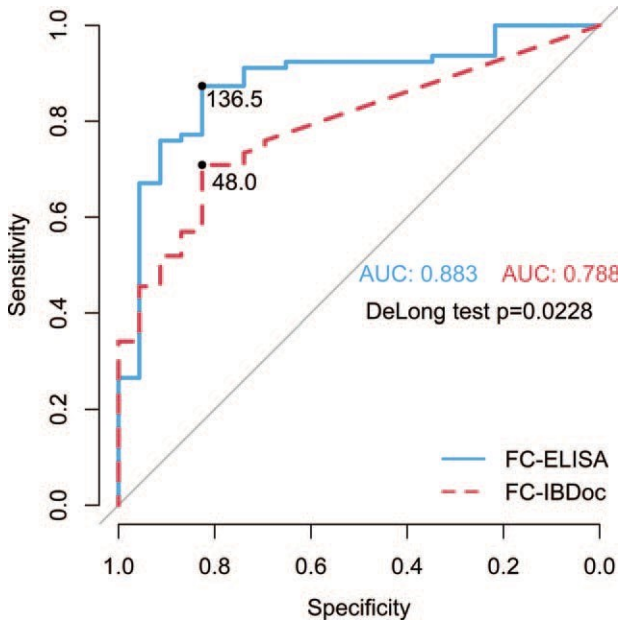

FIGURE 3. Comparison of accuracy in prediction of mucosal healing using FC levels measured by FC-ELISA and FC-IBDoc. FC-ELISA is a more reliable predictor of mucosal healing, defined as SES-CD <2 in patients with CD (n ¼ 44), UCEIS <4 in patients with UC (n ¼ 27) and Rutgeerts score i0 and i1 without colon involvement in patients with CD after ileocaecal resection (n ¼ 19), than the FC-IBDoc in paediatric patients with IBD based on AUC (Delong test *P* ¼ 0.023). In total, 102 observations were analysed. AUC ¼ area under the curve; CD ¼ Crohn disease; FC-ELISA ¼ faecal calprotectin quantitative enzyme-linked immunosorbent assay; FC-IBDoc ¼ faecal calprotectin home test based on smartphone application; ROC ¼ receiver operating characteristic; SES-CD ¼ simple endoscopic score for Crohn disease; UC ¼ ulcerative colitis; UCEIS ¼ ulcerative colitis Endoscopic Index of Severity.

indicated in the present study can be considered as verging to the value proposed in the meta-analysis.

Comparison of multiple measurements using FC-IBDoc of each sample was performed to reveal the reliability of the test, which was indicated as good. We avoided the potential influence of intraindividual variability of FC concentrations, described in many previously published studies (47,48), by multiple analyses of 1 stool sample. The intra-assay variability was slightly higher in this study than the variability of the FC home test described in recently published studies. Kristensen et al (48) referred the mean coefficient of variation of the duplicated FC level measurements 3.4% (95% CI 2.5%–4.5%). The test of reliability performed in relatively large Scottish study (47) was rated as moderately good (k 59% [95% CI: 0.27– 0.90] for FC >50 mg/g).

The present study has certain limitations to note. First, although mucosal healing is usually defined using endoscopic scoring systems, there is still no clear consensus on the exact value allowing establishment of mucosal healing. We defined mucosal healing as SES-CD <2 in patients with CD, UCEIS <4 in patients with UC, and Rutgeerts score i0 and i1 without signs of colon involvement for patients after ICR in agreement with several recently published data (24– 27). Second, the sample size was relatively small. Assuming a larger sample size, it is possible that the association between inflammation intensity defined by SES-CD and FC concentration measured with FC-ELISA could be supported. In addition, the FC-IBDoc is designed to be done at home; however, the test was performed in a hospital with participation of trained staff. We chose this approach to eliminate possible measurement failures and errors, whose relatively high number has been described at least in 1 study. Piekkala et al (49) indicated approximately 15% of failures were caused by inappropriate use of the test at home.

CONCLUSIONS

In conclusion, this was the first study comparing the accuracy of FC home test, namely IBDoc, to the standard FC-ELISA using endoscopic scores as a criterion standard. We have shown that in paediatric patients with IBD, the standard FC-ELISA is a more reliable test in predicting endoscopically defined mucosal healing than the FC-IBDoc. Based on our data, the FC-IBDoc should not replace the FC-ELISA in monitoring disease activity. The test may be particularly suited for obtaining an approximate result for monitoring disease activity at home setting. The caution that sensitivity of the FC-IBDoc is lower than the one of ELISA method must be bear in mind.

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ORIGINAL ARTICLE

Fecal calprotectin is not a clinically useful marker for the prediction of the early nonresponse to exclusive enteral nutrition in pediatric patients with Crohn disease

Ivana Copova[1] & Ondrej Hradsky[1] & Kristyna Zarubova[1] & Lucie Gonsorcikova[1] & Kristyna Potuznikova[1] & Tereza Lerchova[1] & Jiri Nevoral[1] & Jiri Bronsky[1]

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Abstract

Exclusive enteral nutrition (EEN) has been recommended as the first-line therapy in children with active Crohn disease (CD). The primary aim of our study was to determine whether it is possible to use the difference between basal fecal calprotectin (F-CPT) and the value at week 2 of EEN to predict clinical response at week 6. We prospectively collected stool samples for F-CPT analysis and clinical and laboratory parameters during EEN from 38 pediatric patients (28 boys, median age 12.8 years) with newly diagnosed active luminal CD. The difference between F-CPT concentrations before EEN and at week 2 did not predict clinical non-response at week 6 (OR 0.9996 95% CI 0.9989–1.0002, $p = 0.18$); however, it predicted patients who did not achieve clinical remission at week6 (OR 0.9993, 95% CI 00.9985–0.9998, $p = 0.006$) with sensitivity of 58%, and specificity of 92% for cut-off of F-CPT increase by 486 μg/g.

Conclusions: An early decrease in F-CPT levels in children with newly diagnosed active luminal CD did not predict clinical response at week 6 of EEN induction therapy, and clinical remission was predicted with low accuracy. Therefore, F-CPT cannot be used as a predictor to select the patients in whom EEN should be terminated.

What is Known:

- *The fecal calprotectin (F-CPT) is an important marker of intestinal inflammation.*
- *Approximately 25% of pediatric patients with Crohn disease (CD) do not achieve clinical remission, and there is still no sufficient predictor of response to exclusive enteral nutrition (EEN) treatment.*

What is New:

- *The difference between the F-CPT concentrations before EEN treatment and at week 2 did not predict clinical response to treatment at week 6, even if it predicted clinical remission, however, with low accuracy. F-CPT is not a suitable predictor to select the patients for discontinuing of EEN induction therapy.*

Keywords Calprotectin · Inflammatory bowel disease · Prediction · Remission · Response

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✶ Ivana Copova
ivana.copova@fnmotol.cz

Ondrej Hradsky
ondrej.hradsky@lfmotol.cuni.cz

Kristyna Zarubova
zarubova.kristyna@gmail.com

Lucie Gonsorcikova
gonsorcikova@email.cz

Kristyna Potuznikova
potuznikovakristyna@seznam.cz

Tereza Lerchova
terezadrskova@gmail.com

Jiri Nevoral
jiri.nevoral@seznam.cz

Jiri Bronsky
jiri.bronsky@gmail.com

[1] Department of Pediatrics, University Hospital Motol and Second Faculty of Medicine, Charles University in Prague, V Uvalu 84, 150 06 Prague 5, Czech Republic

Abbreviations

AUC	Area under the curve
AZA	Azathioprine
CD	Crohn disease
CI	Confidence interval
CRP	C-reactive protein
ECCO	European Crohn and Colitis Organization
EEN	Exclusive enteral nutrition
ELIA	Fluorescence immunoassay
ESR	Erythrocyte sedimentation rate
F-CPT	Fecal calprotectin
IQR	Interquartile ranges
OR	Odds ratio
ROC	Receiver operating characteristic
wPCDAI	Weighted Pediatric Crohn Disease Activity Index

Introduction

Crohn disease (CD) is a chronic inflammatory condition characterized by a relapsing and remitting course. Exclusive enteral nutrition (EEN) has been shown to be as effective as corticosteroids [17], without the side effects, in achieving clinical remission, and superior in achieving mucosal healing in pediatric patients with luminal CD [14]. In addition, EEN promotes optimal nutritional supply to improve malnutrition, linear growth, muscle mass, and bone strength [2]. Based on its multiple benefits, the European guidelines recommend EEN as the first-line therapy in children with active CD [31]. However, approximately 25% of patients do not achieve clinical remission after an EEN course, and the European Crohn and Colitis Organization (ECCO) suggested that an alternative treatment should be considered if EEN does not induce clinical response within 2 weeks [24]. There is still no sufficient predictor of the successful induction of remission in children with CD who are treated with EEN [7, 10].

Over the past few years, fecal calprotectin (F-CPT) has been gaining recognition as an important marker of intestinal inflammation, and data pertaining to its usefulness in monitoring disease activity is becoming evident [13, 15, 20]. The F-CPT value correlates closely with clinical, endoscopic, and most tightly with histological findings in adult and pediatric CD patients, as well as with disease severity and extent [5, 9, 18, 19, 26].

As described above, EEN has many benefits for patients, but the question is how to identify patients in whom the continuation of EEN will probably be ineffective and another induction treatment should be used instead. We hypothesize that early change in F-CPT levels could be used to select the patients in whom continuing in EEN treatment will probably be ineffective. Therefore, the primary aim of our study was to determine whether it is possible to use the difference in the F-CPT value before treatment and in the early phase of treatment with EEN (F-CPTΔ02) to predict the clinical non-response at week 6, as defined by the weighted Pediatric Crohn Disease Activity Index (wPCDAI). The secondary aim was to identify whether F-CPTΔ02 can be used to predict patients who did not achieve clinical remission (as defined by the wPCDAI, F-CPT, or C-reactive protein (CRP)).

Methods

Participants

The study was designed as a prospective-observational study. Between 2015 and 2017, we screened all the pediatric patients with CD followed at our tertiary referral center for eligibility (Fig. 1) to be included in this study. Patients were diagnosed according to the revised Porto criteria [23]. In addition, they had to have a clinically active disease (wPCDAI > 12.5). Enrolled children had to receive EEN for the first time and had a follow-up of at least 6 weeks. Patients could choose a single or combinations of any polymeric enteral formula(s) available at our department (ʙFresubin^, Fresenius; ʙNutridrink^, Nutricia; ʙEnsure^, Abbott; ʙModulen^, Nestlé) according to their preferences. Individuals who could not complete feeds orally were fed via a nasogastric tube. The goal intake for the EEN treatment corresponded to approximately 120% of the daily caloric needs of the patient. The resting energy expenditure was calculated according to the Schofield equation [16] using a web application. All the patients had to be treated with azathioprine (AZA) concomitantly (AZA therapy was initiated along with EEN and continued after EEN withdrawal) [24]. Patients with a history of systemic or local steroid treatment 3 months before the initiation of EEN, and those with a history of receiving anti-TNF-α therapy or having active perianal disease were not included. A responder was defined as a patient with a wPCDAI lower than or equal to 12.5, or a decrease in the wPCDAI by more than 17.5 points at week 6. Remission was defined as wPCDAI ≤ 12.5 points at week 6, as previously published [30]. The remission by CRP (CRP remission) was defined as CRP < 5 mg/kg, and the remission by F-CPT (F-CPT remission) was defined as F-CPT < 150 mg/g [21, 29]. As predictors we used the difference between the baseline level of F-CPT and week 2 (F-CPTΔ02) and percentage change of the F-CPT until week 2 (F-CPT%02). The patients' demographic and clinical characteristics are listed in Table 1.

Detection of F-CPT concentrations

F-CPT was analyzed using fluorescence immunoassay (ELIA) in three consecutive stool samples collected at the time of diagnosis (samples were taken before the start of bowel preparation for colonoscopy) and during treatment with EEN (at

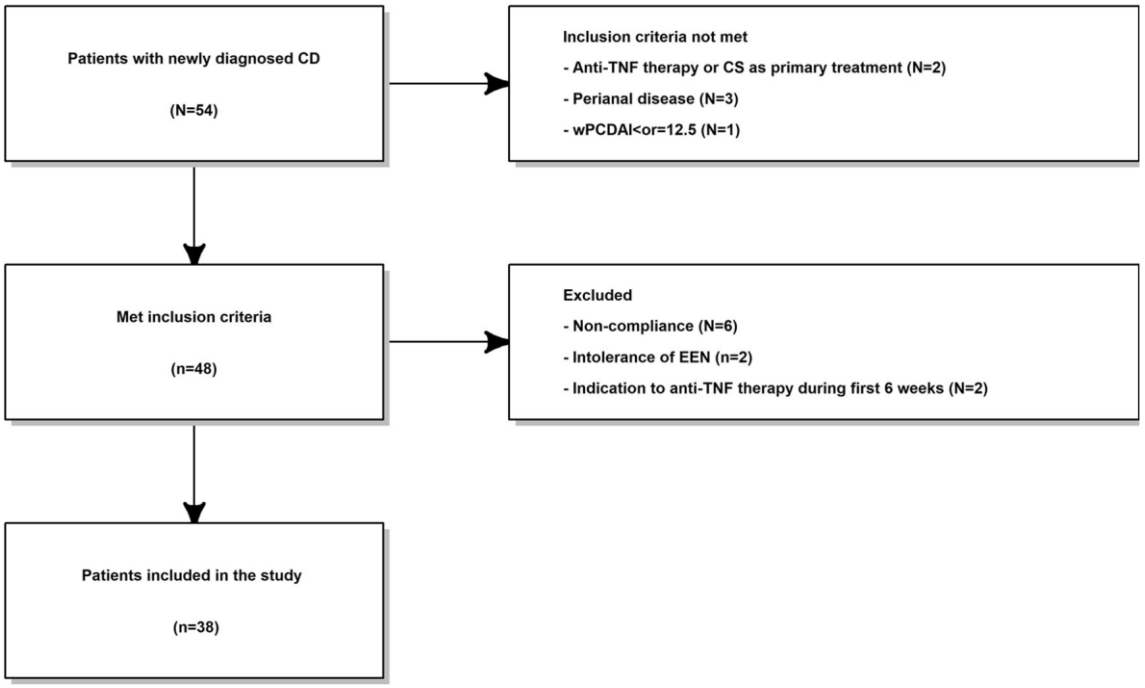

Fig. 1 Participants' selection. Fifty-four pediatric patients, newly diagnosed with Crohn disease (CD), underwent exclusive enteral nutrition (EEN) treatment at our center. In the case of 48 patients, signed consent forms were obtained and EEN treatment was started. Eligible children received EEN treatment for the first time and had a follow-up of at least 6 weeks. During the follow-up, we excluded another ten cases

weeks 0, 2, and 6). Stool samples were collected in screw-capped plastic containers and immediately prepared and analyzed using the EliA Calprotectin 2 test (Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden) according to the manufacturer's instructions. Based on the manufacturer's data, the measuring assay with results above 6000 μg of calprotectin per gram of stool (μg/g) was less exact.

Data collection

We prospectively collected F-CPT samples at the time of diagnosis, at week 2 and at week 6 of EEN treatment and clinical, anthropometric, and laboratory data at the time of diagnosis and at week 6 of EEN treatment.

Statistical analysis

All data analysis was performed using the R statistical software (version 3.3.2). Continuous variables were described as medians and interquartile ranges (IQR). Categorical variables were described as absolute frequencies and percentages. Changes in the clinical and systemic markers of disease activity during EEN were assessed with a paired *t* test. To test the association between two numerical variables, a correlation test was used. Differences between the groups of patients were tested using the Welch two sample *t* test. Linear regression and a generalized logistic regression model were used to determine the association between numeric outcomes and numeric predictors and binary outcome numeric or categorical variables, respectively. We used receiver operating characteristic (ROC) curves to define the optimal cut-off values of the inflammatory markers (R package ʙpROC^). For plot construction, we used R's ggplot2. A *p* value less than 0.05 was considered statistically significant.

Results

Patients′ characteristics

Fifty-four pediatric patients, newly diagnosed with CD, underwent EEN treatment at our center during the study period. Signed consent forms were obtained from 48 patients and these were enrolled in the study. During the study, we excluded another ten children due to non-compliance (stool samples were not provided) of patient or family ($n = 6$), intolerance of EEN (also via nasogastric tube) ($n = 2$) and need for initiation of anti-TNF-α therapy for the development of perianal disease (n = 2). Finally, we included 38 pediatric patients (28 boys, median age 12.8 years (IQR 9.7–15.5)) from whom three consecutive stool samples were prospectively collected and tested for F-CPT concentrations at the time of diagnosis and during EEN treatment (at weeks 0, 2, and 6) (Fig. 1). All the patients completed at least 6 weeks of EEN treatment, and all but one child tolerated EEN orally. This child was fed via a nasogastric tube, introduced on the second day of therapy.

Table 1 Characteristics of patients according to the Paris classification and laboratory parameters at the time of diagnosis

	Patients with Crohn disease
Number of patients	38
Gender, female, (%)	10 (26)
Age at diagnosis, years, (median [IQR])	12.8 (9.7–15.5)
Disease location	
L1, (%)	17 (45)
L2, (%)	1 (3)
L3, (%)	20 (53)
Upper GI involvement, (%)[a]	25 (66)
Disease behaviour	
B1, (%)	30 (79)
B2, (%)	8 (21)
B3, (%)	0 (0)
p, (%)[b]	1 (3)
Growth retardation, (%)	2 (1)
F-CPT [μg/g], (median [IQR])[c]	1096 [559, 3814]
CRP [mg/l], (median [IQR])[d]	20.50 [8.00, 34.50]
ESR [mm/h], (median [IQR])[e]	31.00 [19.25, 40.00]
wPCDAI, (median [IQR])[f]	41.00 [28.25, 49.50]
wPCDAI >12.5, (%)[g]	38 (100)

[a] Upper gastrointestinal (GI) involvement corresponded to L4a, L4b or L4ab

[b] p = perianal disease (the Paris classification)

[c] F-CPT = fecal calprotectin

[d] CRP = C-reactive protein

[e] ESR = erythrocyte sedimentation rate

[f] wPCDAI = weighted Pediatric Crohn Disease Activity Index

[g] Active disease based on the wPCDAI

Development of the wPCDAI, systemic inflammatory markers, and F-CPT levels during EEN treatment

At the beginning of the treatment, 19 (50%) patients had mild disease (wPCDAI > 12.5 < 42.5), 13 (34%) had moderate disease (wPCDAI ≥ 42.5 < 57.5), and 6 (16%) had severe disease (wPCDAI ≥ 57.5) (median wPCDAI 41.0 (IQR: 28.25, 49.5)). At week 6, based on the wPCDAI, 87% responded (assigned as responders), 68% of the patients achieved clinical remission, and the median wPCDAI decreased to 7.0 (IQR 0.0–17.0), Fig. 2a. The development of the laboratory parameters is shown in Fig. 2 b, c.

The basal F-CPT levels in all the patients (100%) were elevated above 150 μg/g (median 1096 μg/g, IQR: 559.2–3814.2) at the time of diagnosis. Until week 2, there was no significant improvement in the F-CPT concentrations, and the median F-CPT dropped to 1055 μg/g (IQR: 535–2003). In addition, in 16 children (42%) the F-CPT levels increased. From the start of treatment to week 6 of EEN treatment, the median F-CPT concentrations decreased by 510 μg/g (IQR: 115–1545) ($p = 0.004$). In only four patients, F-CPT levels ≤ 150 μg/g at week 6 of EEN treatment were achieved, and during the course of the treatment, we found interindividual changes in the F-CPT concentrations, which varied considerably. In summary, while four children (11%) had F-CPT ≤ 150 μg/g, and seven (18%) had concentrations > 150/≤ 300 μg/g, most of the participants ($n = 27$, 71%) continued to have high levels, above 300 μg/g, after 6 weeks of treatment (Fig. 2d). The correlation between the change in F-CPT levels from the time of EEN initiation until weeks 2 and 6 was significant ($r = 0{,}71$) ($p < 0.001$).

Prediction of patients who did not respond to EEN treatment

The F-CPTΔ02 did not predict patients who did not respond to EEN at week 6 (OR 0.9996013, 95% CI 0.9988623–1.000162, $p = 0.18$), even after adjusting for logarithms of base-line F-CPT (OR 0.9991118, 95% CI 0.9979461–1.000076, $p = 0.073$). We did not find an association between F-CPT%02 (OR 1.021935, 95% CI 0.7072691–1.23026, $p = 0.85$) and clinical non-response to EEN treatment at week 6 (Table 2).

Prediction of patients who did not achieve clinical remission

The F-CPTΔ02 predicted patients who did not achieve clinical remission at week 6 (OR 0.9993066, 95% CI 0.9985436–0.9998284, $p = 0.006$). The non-remission was best predicted using the increase of F-CPT by 486 μg/g; this had a sensitivity of 58% and specificity of 92% (AUC 0.753) (Fig. 3a — supplementary material). We found border-line association between F-CPT%02 and non-remission ($p = 0.048$). The association of non-remission expressed as CRP > 5 mg/l or as CPT > 150 μg/g and with F-CPTΔ02 was also found. The CRP > 5 mg/l was not predicted by F-CPT%02. Remission defined as F-CPT > 150 μg/g was predicted by F-CPT%02, please see Table 2.

Using a cut-off of 1.47%, specificity was 79% and sensitivity was 80% (AUC 0.788) to predict patients who did not achieve clinical remission at week 6 of EEN treatment (Fig. 3b — supplementary material). The accuracy of predictors was described in Table 3.

Discussion

This prospective study has shown that the predictive value of F-CPT during the early phase of EEN treatment in children with newly diagnosed luminal CD is not sufficient to select patients who will probably not respond to EEN at the end of treatment.

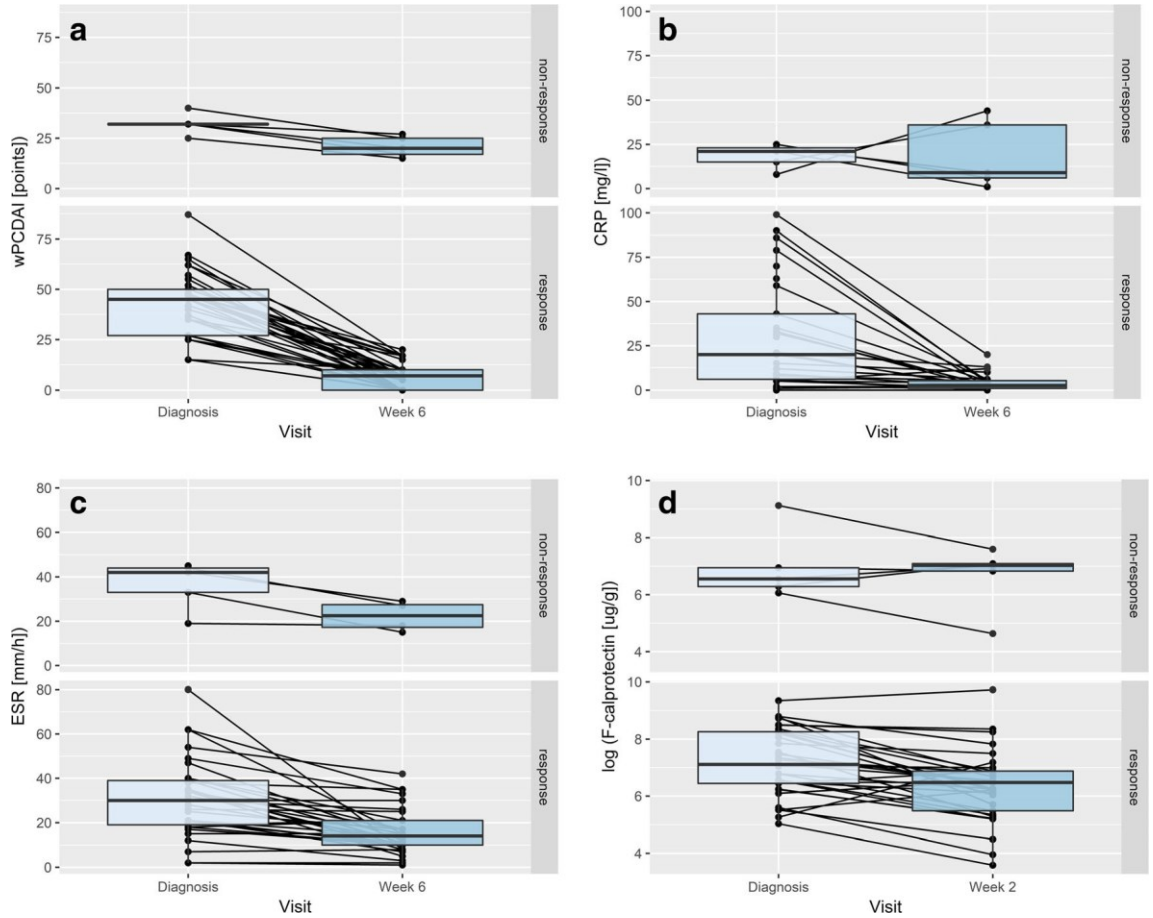

Fig. 2 The development of disease activity from the beginning of EEN treatment till week 6. Expressed as: a Weighted Pediatric Crohn Disease Activity Index (wPCDAI): at the beginning of exclusive enteral nutrition (EEN) treatment, all the included children had clinically active disease (wPCDAI > 12.5), and at week 6, 68% of the patients achieved clinical remission and the median wPCDAI decreased to 7.0 (interquartile range (IQR) 0.0, 17.0). b, c The erythrocyte sedimentation (ESR) and C-reactive protein (CRP) were abnormal in most of the participants (58 and 82%). At week 6 of EEN treatment, the levels of the laboratory markers improved, reaching the normal reference range (median ESR/CRP: 15.0 (IQR: 10.0, 22.0)/4.0 (IQR: 1.0, 6.0)) in majority of the children. d The fecal calprotectin (F-CPT) levels in all the patients were elevated above 150 μg/g (median 1096 μg/g, IQR: 559.2, 3814.2) at the time of diagnosis. Till week 2, the median F-CPT dropped to 1055 μg/g (IQR: 535, 2003) and till week 6 it decreased by 510 μg/g (IQR: 115–1545) ($p = 0.004$). In only four patients, F-CPT levels ≤ 150 μg/g at week 6 were achieved

To the best of our knowledge this was the first study trying to predict non-response during the early phase of EEN. Gerasimidis et al. [12] used the F-CPT value at day 30 for the prediction of remission. In this small pilot study, the reduction in baseline F-CPT levels had a sensitivity of 100% (95% CI: 54–100) and specificity of 89% (52 to 100) to predict induction of clinical remission at the end of treatment. However, we also found predictability of remission using F-CPTΔ02 and F-CPT%02; the study differs from the current one in important parameters. The authors chose remission defined by the original version of PCDAI as a main outcome, they used day 30 as the predictor, the duration of EEN was 8 weeks and only patients with colonic involvement were included. We believe that for routine practice the estimate of the patients who should discontinue EEN treatment earlier (non-responders) is an important outcome and that prediction needs to be done in the early stage of induction therapy. For response and remission, we used the wPCDAI to define disease activity based on a modified version of the original PCDAI, which assigns weight for the items of clinical presentation and laboratory parameters and excludes three items (height velocity, abdominal examination, and hematocrit) with insignificant discriminatory power [30]. Although using CPTΔ02, we could predict non-remission assessed by wPCDAI or CRP. The predictive ability was weak (AUC 0.739, 0.787 respectively), and thus clinically not useful. Endoscopic assessment at the end of induction therapy was not part of the protocol and would be ethically problematic; therefore, we chose F-CPT < 150 as approximation of mucosal healing and we were able to predict this using both F-CPTΔ02 and F-CPT%02. However, same as for remission, the predictive parameters were not accurate enough to be useful for clinical practice. Moreover, we found that a transient moderate increase in the F-CPT level in the early phase of

Table 2 Prediction of non-response, and non-remission at week 6 of EEN treatment based on absolute and percentage change of F-CPT till week 2

	F-C PTΔ02[a]	*p*	F-CPT%02[b]	*p*
[d]Non-response	0.9991, (0.9979–1.0001)[c]	0.073	1.0219, (0.7073–1.2303)	0.845
[e]Non-remission	0.9975, (0.9952–0.9989)[c]	<0.001	1.3337, (0.9984–2.2975)	0.048
[f]CRP > 5 mg/l	0.9985, (0.9969–0.9995)[c]	<0.001	1.1541, (0.9297–2.1174)	0.237
[g]F-CPT > 150 μg/g	1.0009, (1.0001–1.002)	0.023	0.7956, (0.5006–0.9838)	0.033

The associations were expressed as odds ratio (OR) with 95% confidence interval (CI). wPCDAI = weighted Pediatric Crohn Disease Activity Index

[a] F-CPTΔ02, difference between baseline level of F-CPT and week 2

[b] F-CPT%02, percentage change of the F-CPT

[c] Adjusted for logarithm of basal F-CPT level

[d] Non-response defined as wPCDAI > 12.5 points, or there is not a decrease in the wPCDAI by more the 17.5 points

[e] Non-remission defined as wPCDAI > 12.5 points

[f] CRP = C-reactive protein

[g] F-CPT = fecal calprotectin, EEN = exclusive enteral nutrition

EEN induction therapy did not exclude its decrease after the completion of treatment. The effect of EEN treatment on mucosal healing and, hence, on F-CPT levels seemed to be gradual, confirming the necessity of a given length of treatment. In a clinical CD trial in which F-CPT and serological inflammatory markers were measured during induction therapy with EEN, F-CPT was the slowest to return to a normal level, implicating its limited responsiveness to change [13]. We measured F-CPT concentration at week 2, which seemed to be too short a period to monitor a significant change in F-CPT levels.

We sought to address the question: when is the best time to evaluate the response to EEN induction therapy using F-CPT? We found an improvement in the levels of F-CPT in a majority of patients; however, the individual concentrations of F-CPT reached high levels in several clinical responders, even after the EEN course. Therefore, both F-CPT values and the trend of the levels (or more accurately, the percentage change of F-CPT in the log scale) during treatment are important. According to our results, the decrease in the F-CPT levels to values of the normal reference range seems not to be necessary in a short time period in patients receiving EEN. As already mentioned above, children with CD achieve clinical remission after the EEN course in approximately 75% [7, 10]; therefore, EEN is still the first choice for induction of remission in children with active CD regardless of the F-CPT levels. In many children with clinical remission/response, F-CPT levels were still high at the end of EEN and we know that not all children with clinical response have mucosal healing already at the end of 6 weeks [14]. For most children, the ability to tolerate EEN is already obvious within the first few days of treatment. In addition, F-CPT has been demonstrated to continue to fall up to 8 weeks of EEN and all the studies that have demonstrated mucosal healing from EEN have been after 8 weeks or more of EEN [3, 12, 28].

Other authors tried to use the predictive value of F-CPT during EEN in a different way. Frivolt et al. did not find changes in the F-CPT level between the baseline and week 4 or 12 of EEN treatment to be predictive of remission duration

Table 3 Prediction of non-response (defined as wPCDAI or CRP or F-CPT level), and non-remission at week 6 of EEN treatment based on absolute and percentage change of F-CPT till week 2

	[e]AUC	Specificity	Sensitivity	Cut-off (μg/g)	AUC	Specificity	Sensitivity	Cut-off (%)
[a]Non-response	0.739	0.848	0.800	− 486.0	0.788	0.788	0.800	1.468
[b]Non-remission	0.753	0.923	0.583	− 486.0	0.801	0.615	1.000	0.643
[c]CRP > 5 mg/l	0.787	1.000	0.588	246.5	0.721	0.750	0.853	1.821
[d]F-CPT > 150 μg/g	0.787	1.000	0.588	246.5	0.721	0.750	0.853	1.821

wPCDAI = weighted Pediatric Crohn Disease Activity Index

[a] Non-response defined as wPCDAI > 12.5 points, or there is not a decrease in the wPCDAI by more the 17.5 points

[b] Non-remission defined as wPCDAI > 12.5 points

[c] CRP = C-reactive protein

[d] F-CPT = fecal calprotectin

[e] AUC = area under the curve, EEN = exclusive enteral nutrition

[11]. Another study confirmed that a reduction in the F-CPT levels in children with CD undergoing EEN treatment was associated with the induction of clinical remission after EEN completion [12]. The members of the pediatric committee of the ECCO suggested that F-CPT be used only as a secondary outcome measure due to large variability in the results, leading to low precision and ill-defined cut-off levels in the assessment of disease activity [25].

Despite the fact that other clinical and laboratory parameters were previously studied in patients treated with EEN [1, 3, 8], the results in purpose for the prediction of response to EEN treatment are scarce. Day et al. [8] showed an improvement in inflammatory markers (erythrocyte sedimentation rate (ESR), CRP, serum albumin and platelets) after 8 weeks of EEN in patients with newly diagnosed CD who achieved remission. All the improvements in laboratory markers paralleled changes in PCDAI, both seen as early as 4 weeks. Moreover, there are studies which found improvement in ESR and CRP over a much shorter period (1 week) [1, 3]. According to the study by Borrelli et al. [3], the most significant improvement in weight and inflammatory markers is seen in the first 4 weeks of EEN; however, most studies have shown that weight continues to improve up to 8 weeks of treatment [6]. Another study by Gerasimidis et al. [12] performed development of inflammatory markers (serum albumin, CRP, and ESR) in responders and non-responders to EEN treatment. In the responders, systemic markers improved at the end of the EEN course and in the majority of patients they reached values in the normal reference range, contrary to non-responders, in whom none of those markers of disease activity changed significantly.

Another unsolved question regarding EEN treatment is the effect of disease location on the treatment outcome. Based on several studies [6, 27], EEN is not as effective for isolated colonic disease as it is for ileal or ileocolonic disease; however, more recent studies did not find an effect of disease location on EEN treatment outcome [4, 28]. It is very difficult to find a definite conclusion about the predictive role of disease location on treatment outcome according to several differences in published studies, even though it is probably not a strong predictor.

A limitation of the study was the relatively small sample size. However, if a decrease in the F-CPT concentration by week 2 can act as a strong predictor, this can also be proven in a small group of patients. We could not exclude the non-compliance of patients to EEN treatment. However, the patients and their caregivers did not know the results of the F-CPT at week 2, and, thus, this probably did not influence the results of the study. We allowed the patients to choose between different types and tastes of formula in order to decrease non-compliance due to low palatability. Nevertheless, two patients were not able to continue EEN and were excluded. We believe that exclusion of these patients did not fundamentally affect the results. Patients collected the stool samples by themselves, at home; therefore, the impact of this on the F-CPT concentrations is unknown. The less-than-accurate results in the case of high F-CPT levels, as obtained by the assay, is also a potential limitation. According to published studies, the F-CPT concentrations are highest in samples collected when there is a long interval between the defecations, and there is also correlation between stool consistency and the level of the F-CPT [22]. Therefore, the changes in gastrointestinal transit time could affect the F-CPT outcomes in our patients. On the other hand, all mentioned limitations of using F-CPT could be the explanation for its variation; however, it should not affect the main results. The study was not designed to identify patients who will achieve remission or even mucosal healing in the long-term follow-up.

The effect of AZA treatment on induction of remission by EEN is improbable. Based on the published data [25], AZA in monotherapy did not induce remission in active CD; therefore, we did not expect that AZA influenced the induction of remission in our patients treated with EEN.

In conclusion, we found that the difference between the F-CPT concentrations before EEN treatment and at week 2 or the percentage of the baseline value cloud not be used as a single predictor for non-response at the end of induction treatment. Another marker in the early phase should be considered in future studies as a potential predictor since the F-CPT seems to return to a normal level too slowly.

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Authors' contributions Ivana Copova M.D. worked on the conception and design of the study as well as on background research, data collection, and its control and completion of the missing data, statistical analysis of data, and writing of this original article. She was responsible for financial planning and work efficiency within the budget of the study.

Ondrej Hradsky M.D., Assoc. Prof., Ph.D. worked on the conception and design of the study as well as on data collection and its control, statistical analysis of data, and revision of the original article. He critically revised the work for important intellectual content.

Kristyna Zarubova M.D. actively participated in data collection and its control in the context of the research, and revision of the original article.

Lucie Gonsorcikova M.D., Ph.D. participated in data collection and its control.

Kristyna Potuznikova M.D. participated in data collection and its control. She obtained the necessary documents from patients and their guardians, and revised the work.

Tereza Lerchova M.D. participated in data collection and its control. She obtained the necessary documents from patients and their guardians, and revised the work.

Jiri Nevoral M.D., Prof., Ph.D. worked on the conception and design of the study as well as on revision of the original article.

Jiri Bronsky M.D., Assoc. Prof., Ph.D. was responsible for leading the project team, he was involved in study scheduling, obtaining the necessary documents, highlighting potential problems, and proposing solutions. He critically revised the work critically for important intellectual content.

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Compliance with ethical standards

The study was approved by the Ethics Committee of the authors' institution (the Ethics Committee Reference number is 1491/16).

Conflict of interest The authors alone are responsible for the content and writing of the paper. There is no direct conflict of interest in relation to the topic of this study in any of the authors. Jiri Bronsky received honoraria/speaker's fees/coverage of travel expenses from AbbVie, MSD, Nutricia, Nestle, Biocodex, Walmark, and Ferring. Ondrej Hradsky received honoraria/speaker's fees/coverage of travel expenses from AbbVie, MSD, Nutricia, Nestle, Biocodex, Falk, and Ferring.

Informed consent The patients' guardians received written information on the study and signed a consent form.

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Adalimumab vs Infliximab in Pediatric Patients With Crohn's Disease: A Propensity Score Analysis and Predictors of Treatment Escalation

Jiri Bronsky, MD, PhD[1], Ivana Copova, MD[1], Denis Kazeka, MD[1], Tereza Lerchova, MD, PhD[1], Katarina Mitrova, MD, PhD[1,2], Kristyna Pospisilova, MD[1], Miroslava Sulovcova, MD[1], Kristyna Zarubova, MD[1] and Ondrej Hradsky, MD, PhD[1]

INTRODUCTION: Two antitumor necrosis factor therapies (infliximab [IFX] and adalimumab [ADA]) have been approved for the treatment of pediatric Crohn's disease (CD) but have not been compared in head-to-head trials. The aim of this study was to compare the efficacy and safety of ADA and IFX by propensity score matching in a prospective cohort of pediatric patients with luminal CD and at least a 24-month follow-up.

METHODS: Among 100 patients, 75 met the inclusion criteria, and 62 were matched by propensity score. We evaluated time to treatment escalation as the primary outcome and primary nonresponse, predictors of treatment escalation and relapse, serious adverse events, pharmacokinetics, and effect of concomitant immunomodulators as secondary outcomes.

RESULTS: There was no difference between ADA and IFX in time to treatment escalation (HR 5 0.63 [95% CI 0.31–1.28] P 5 0.20), primary nonresponse (P 5 0.95), or serious adverse events. The median (interquartile range) trough levels at the primary outcome were 14.05 (10.88–15.40) and 6.15 (2.08–6.58) mg/mL in the ADA and IFX groups, respectively. On a multivariate analysis, the combination of anti-*Saccharomyces cerevisiae* antibody negativity and antineutrophil cytoplasmic antibody positivity was a strong independent predictor of treatment escalation (HR 5.19, [95% CI 2.41–11.18], $P < 0.0001$). The simple endoscopic score for CD, L3 disease phenotype, and use of concomitant immunomodulators for at least the first 6 months revealed a trend toward significance on a univariate analysis.

DISCUSSION: Propensity score matching did not reveal substantial differences in efficacy or safety between ADA and IFX. The anti-*S. cerevisiae* antibody negativity and antineutrophil cytoplasmic antibody positivity combination is a strong predictor of treatment escalation.

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/A798, http://links.lww.com/CTG/A799, http://links.lww.com/CTG/A800

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INTRODUCTION

To date, 2 anti-tumor necrosis factor (TNF) agents have been approved for the treatment of pediatric Crohn's disease (CD): infliximab (IFX) and adalimumab (ADA). Both agents have been proven to be effective and safe in randomized controlled trials (RCTs) (1,2). However, these RCTs differed in some aspects of methodology. In the REACH trial, only patients who responded to induction IFX therapy were randomized, and in the IMAgINE trial, patients who previously failed on anti-TNF therapy were enrolled. Moreover, cessation of immunomodulator (IMM) therapy was permitted from week 26. Age at enrollment and disease activity based on the Pediatric Crohn's Disease Activity Index (PCDAI) were similar in both studies. However, no direct head-to-head comparison of both anti-TNF agents has been performed in pediatric or adult patients. Several indirect comparisons, including network meta-analyses, have been published, but these rarely consider pediatric populations (3–9). Owing to the low number of pediatric patients per center, it is difficult to perform RCTs that can demonstrate differences between these drugs. In particular, a noninferiority design would require a high

[1]Gastroenterology and Nutrition Unit, Department of Paediatrics, 2nd Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic; [2]IBD Clinical and Research Centre, ISCARE, Prague, Czech Republic. Correspondence: Jiri Bronsky, MD, PhD. E-mail: jiri.bronsky@gmail.com.
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number of patients. Therefore, we aimed to perform a propensity score analysis of our cohorts of prospectively followed up patients.

Study aims

The primary aim of this study was to compare the time to treatment escalation between patients treated with ADA and those treated with IFX. Secondary aims were to evaluate primary nonresponse to anti-TNF, predictors of treatment escalation and relapse, safety, pharmacokinetics (PK), and effect of concomitant IMM treatment.

METHODS

Study design and ethical considerations

This prospective observational cohort study was performed using propensity score matching. The study was approved by the local ethics committee, and all participants and/or parents signed written informed consent.

Study subjects and dosage of anti-TNF

Patients naive to biologic therapy, newly started on anti-TNF treatment between 2013 and 2017 (Motol PIBD cohort), were recruited into the study and prospectively followed up according to the standard protocol reflecting usual clinical practice (see Supplementary Figure 1, http://links.lww.com/CTG/A798). Patients were initiated on an anti-TNF agent based on a detailed discussion between the family and the treating physician. The minimal follow-up period required for evaluation of study outcomes was 24 months. Inclusion and exclusion criteria are listed in Supplementary Digital Content (see Supplementary Table 1, http://links.lww.com/CTG/A799). Patients were initiated on a standard dose of anti-TNF: ADA (Humira) 160-80-40 mg s.c. every other week, followed by 40 mg s.c. every other week, and IFX (Remicade) 5 mg/kg i.v. at weeks 0, 2, and 6 and every 8 weeks. No biosimilars were used in this study. In patients weighing less than 40 kg, the dose of ADA was calculated according to the body surface area. When applicable, a decision on therapy intensification (ADA up to 40 mg weekly and IFX up to 10 mg/kg every 4 weeks) was made by the treating physician, based primarily on clinical and laboratory data, and secondarily on trough levels and anti-drug antibodies (ATI) to the respective anti-TNF (reactive therapeutic drug monitoring [TDM]). No proactive TDM was applied during the study period. All patients, except for 3, received IMM (97% azathioprine [AZA], 3% methotrexate [MTX]) from diagnosis until the start of anti-TNF treatment (Table 1).

Primary outcome

The primary outcome of the study was the time to treatment escalation on anti-TNF therapy evaluated by survival analysis after propensity score matching.

Secondary outcomes

The following secondary outcomes were considered: (i) Proportion of patients with a primary nonresponse to ADA or IFX, (ii) identification of predictors of treatment escalation and relapse, (iii) rate of serious adverse events (SAEs) occurring on-treatment, (iv) PK of both drugs, and (v) effect of concomitant IMM treatment.

Definition of treatment escalation, relapse and primary nonresponse

Treatment escalation was defined as dose escalation or interval shortening due to a lack of drug efficacy (not due to adjustment for body weight) or bowel surgery due to disease activity, development of abscess, perianal or intra-abdominal fistula, change of anti-TNF therapy (due to side effects or ineffectivity), need for reinduction (corticosteroids, exclusive enteral nutrition, or antibiotics), or change of IMM treatment (AZA to MTX or vice versa), not dose adjustment for body weight (see Supplementary Figure 1, http://links.lww.com/CTG/A798). For the purpose of secondary subanalysis, dose escalation or interval shortening due to a lack of drug efficacy (not due to adjustment for body weight) was omitted from the abovementioned definition. This situation was marked as relapse.

Primary nonresponse was defined as the need for treatment change (switch to another anti-TNF therapy, treatment interruption, bowel surgery, or persisting need for induction therapy corticosteroids, exclusive enteral nutrition, antibiotics) until week 14 (12–16) due to clinical symptoms (weighted pediatric Crohn's disease activity index [wPCDAI], fistula, and stricture), laboratory signs of disease activity (erythrocyte sedimentation rate, C-reactive protein [CRP], fecal calprotectin [F-CPT]), endoscopic disease activity, need for bowel surgery, drug intolerance (side effects), or noncompliance.

Clinical and laboratory data

At the onset of anti-TNF therapy, we recorded general patient characteristics, factors that may influence the outcome or allocation of patients to the respective treatment group (ADA and IFX), and factors considered as potential predictors of treatment efficacy (Table 1). The data underlying this article will be shared on reasonable request to the corresponding author.

During follow-up, we prospectively recorded the following every 3 months: body height; weight; wPCDAI; CRP; F-CPT; perianal fistulas; extraintestinal manifestations; SAEs; dose and interval of anti-TNF; need for treatment escalation, cessation, or switch, including the reason; concomitant medication; trough levels and ATI to anti-TNF if applicable; and occurrence of primary and secondary outcomes.

Regarding clinical indication, the following checks were performed: bowel ultrasound, magnetic resonance enterography, or endoscopy. Endoscopy, including biopsies and evaluation of simple endoscopic score for CD (SES-CD), was performed before the decision on anti-TNF treatment and before any major therapeutic decision (e.g., switch to another anti-TNF therapy, bowel surgery, and nonresponse).

Patient allocation and statistical analysis

All data were analyzed using R statistical software (version 3.6.0; www.r-project.org). Continuous variables were described as medians and interquartile ranges (IQRs). Categorical variables were described as absolute frequencies and percentages. Missing data were not imputed. The difference between patients treated with ADA and IFX was assessed using the likelihood ratio test on the odds ratio or 2-sample *t* test, as appropriate. Propensity score matching was performed using the R package MatchIt (version 3.0.2). The model for propensity matching consisted of the SES-CD, stricturing behavior, penetrating behavior, perianal disease, *z* score of body mass index, and age at the time of anti-TNF onset. Variables were selected based on the clinical decision, according

Table 1. Characteristics of both study groups before propensity score matching

	ADA (N 5 31)	IFX (N 5 44)	*P* value
Basic characteristics			
Age	14.18 (11.64–16.34), NA 5 0	14.46 (13.24–16.27), NA 5 0	0.36
Sex (male)	21 (0.68), NA 5 0	24 (0.55), NA 5 0	0.25
Smoking	3 (0.1), NA 5 0	1 (0.02), NA 5 0	0.16
Ethnicity (White)	29 (0.94), NA 5 0	43 (0.98), NA 5 0	0.37
Family history of IBD	1 (0.03), NA 5 0	8 (0.08), NA 5 0	0.03
Concomitant immunopathology	3 (0.1), NA 5 0	3 (0.07), NA 5 0	0.66
Body height (cm)	162 (136.95–170.75), NA 5 0	156.55 (148.88–171.52), NA 5 0	0.41
Body height (*z* score)	21.49 (25.56–0.53), NA 5 0	22.12 (23.94–0.26), NA 5 0	0.41
Body weight (kg)	44.3 (28.65–58.5), NA 5 0	47.25 (37.12–56.08), NA 5 0	0.43
BMI (*z* score)	21.77 (22.58–0.53), NA 5 0	21.34 (21.97–0.4), NA 5 0	0.27
Paris classification			
L1	11 (0.35), NA 5 0	10 (0.23), NA 5 0	0.23
L2	1 (0.03), NA 5 0	4 (0.09), NA 5 0	0.3
L3	19 (0.61), NA 5 0	30 (0.68), NA 5 0	0.54
L4a or L4b	22 (0.71), NA 5 0	31 (0.7), NA 5 0	0.96
B1	23 (0.74), NA 5 0	36 (0.82), NA 5 0	0.43
B2	4 (0.13), NA 5 0	5 (0.11), NA 5 0	0.84
B3	4 (0.13), NA 5 0	2 (0.05), NA 5 0	0.19
B21B3	0 (0), NA 5 0	1 (0.02), NA 5 0	0.3
Perianal disease	7 (0.23), NA 5 0	12 (0.27), NA 5 0	0.64
Growth impairment	10 (0.33), NA 5 1	10 (0.23), NA 5 0	0.32
Disease activity and labs			
wPCDAI (points)	22.5 (16.88–40.62), NA 5 7	32.5 (16.88–40), NA 5 4	0.64
CRP (mg/L)	13.6 (8.35–26.25), NA 5 4	17 (4.85–29.85), NA 5 1	0.55
F-CPT (*m*g/g)	1,800 (1,080–2,883), NA 5 20	1,000 (801–1,720), NA 5 11	0.09
Albumin (g/L)	42.8 (40.2–43.8), NA 5 6	41.1 (39.4–43.4), NA 5 3	0.32
ESR (mm/hr)	28.5 (20–41.25), NA 5 3	30 (18–46.5), NA 5 1	0.59
ASCA positivity	23 (0.88), NA 5 5	34 (0.81), NA 5 2	0.4
pANCA positivity	5 (0.19), NA 5 5	6 (0.14), NA 5 1	0.57
SES-CD (points)	20 (13–27), NA 5 2	18 (11.75–21.5), NA 5 4	0.2
Treatment			
Time since dg. to anti-TNF start (yr)	1.04 (0.51–1.61), NA 5 0	0.6 (0.17–1.23), NA 5 0	0.14
EEN during dg.	21 (0.68), NA 5 0	37 (0.84), NA 5 0	0.1
CS during dg.	6 (0.19), NA 5 0	6 (0.14), NA 5 0	0.51
IMM during dg.	29 (0.94), NA 5 0	43 (0.98), NA 5 0	0.37
EEN during anti-TNF start	5 (0.16), NA 5 0	7 (0.16), NA 5 0	0.98
CS during anti-TNF start	1 (0.03), NA 5 0	2 (0.05), NA 5 0	0.77
IMM during anti-TNF start	29 (0.94), NA 5 0	38 (0.86), NA 5 0	0.31

Values are listed as median and interquartile range or median and fraction (%); NA 5 number of missing values.
ADA, adalimumab; ASCA, anti-*Saccharomyces cerevisiae* antibodies; BMI, body mass index; CRP, C-reactive protein; CS, corticosteroids; dg., diagnosis; EEN, exclusive enteral nutrition; ESR, erythrocyte sedimentation rate; F-CPT, fecal calprotectin; IBDinflammatory bowel disease; IFX, infliximab; IMM, immunomodulators; pANCA, antineutrophilic antibodies; SES-CD, simple endoscopic score for Crohn's disease; TNF, tumor necrosis factor; wPCDAI, weighted pediatric Crohn's disease activity index.

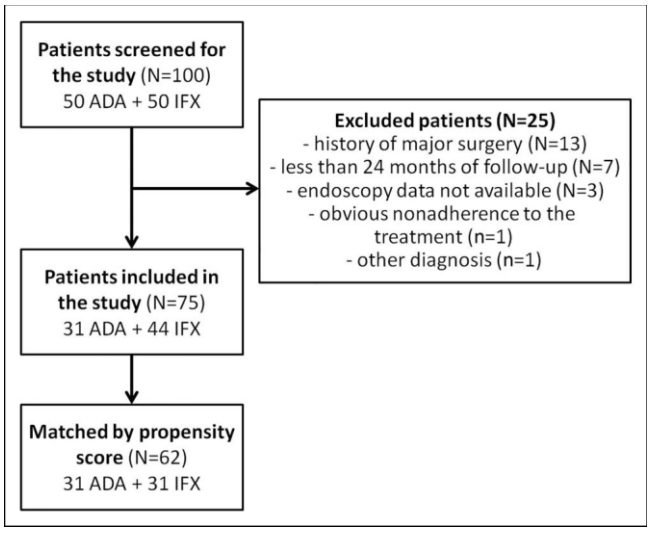

Figure 1. Flowchart of patient recruitment into the study and propensity score matching.

to factors that could influence the outcome or choice of therapy. Matching was performed using nearest neighbor matching with a ratio of 1:1. The covariate balance in the matched sample was checked by visual inspection of plots showing the mean of each covariate against the estimated propensity score, separately by treatment status. The effect of concomitant IMM therapy was evaluated as the percentage of time on concomitant IMM out of the complete follow-up time, as a continuous variable, and as a categorical variable if the patient received IMM for at least 6 months.

The primary outcome of the study was evaluated using a Cox proportional hazards model, subsequently adjusted for the proportion of time on IMM therapy. The preselected predictors were tested using unadjusted Cox regression. To assess the importance of particular variables, we further tested the association of time to relapse with the variables using multivariable Cox proportional hazards models.

We used a generalized linear mixed model to assess the association between SAE and the type of anti-TNF therapy. All mixed models were adjusted for follow-up time and IMM use. When values were missing, the time point was omitted from the current analysis.

P , 0.05 was considered significant. A 95% confidence interval (CI) was used. Figures were constructed using R package ggplot2. According to powerSurfEpi R-package, our study with 31 experimental subjects and 31 control subjects was able to detect hazard ratio (HR) of , 0.34 or . 2.90 with probability (power) 0.80.

RESULTS

Of 100 patients screened for inclusion in the study (50 ADA and 50 IFX), 25 met the exclusion criteria. The basic characteristics of patients in each study group before propensity score matching (31 patients in the ADA group and 44 patients in the IFX group) are presented in Table 1. No significant differences were found, except for family history of inflammatory bowel disease (IBD), which was more frequent in the IFX group (P 5 0.03). Finally, propensity score matching allowed us to directly compare 31 pairs of patients (Figure 1).

Primary outcome—time to treatment escalation

The overall time to treatment escalation in the whole study group (N 5 75) is presented in Figure 2a, showing an approximate rate of 50% during the 3-year follow-up. Neither subanalysis of the whole study group (N 5 75, HR 5 0.68 [95% CI 0.35.1.33], P 5 0.26, Figure 2b) nor of patients matched by propensity score (N 5 62, HR 5 0.63 [95% CI 0.31–1.28], P 5 0.20, Figure 2c) revealed any significant difference in time to treatment escalation between ADA and IFX. The results were not affected by adjusting this model to concomitant IMM (HR 5 0.63 [95% CI 0.31–1.28], P 5 0.20).

When the need for treatment intensification was omitted (situation classified as relapse) (see Figure 3a for pooled data on ADA 1 IFX), there was no significant difference in relapse rate between the ADA and IFX groups, in the whole study group (N 5 75, HR 5 0.83 [95% CI 0.40–1.76], P 5 0.64, Figure 3b), or

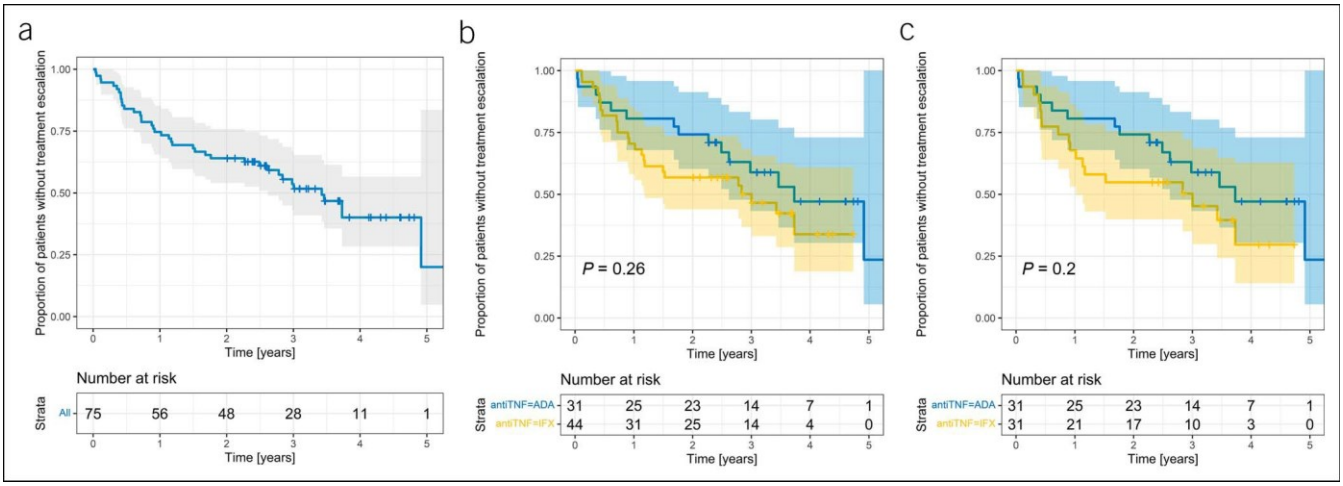

Figure 2. (a) Survival curve of time to treatment escalation in the whole study group (pooled data, N 5 75). (b) Time to treatment escalation according to the type of anti-TNF therapy in the whole study group (N 5 75). (c) Time to treatment escalation according to the type of anti-TNF therapy after propensity score matching (N 5 62). ADA, adalimumab; IFX, infliximab; TNF, tumor necrosis factor.

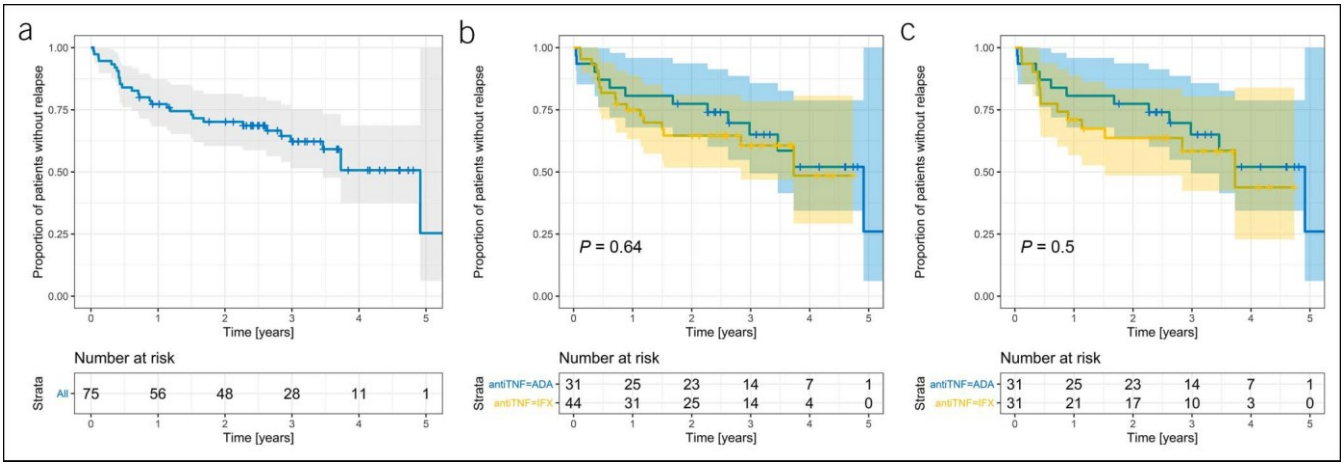

Figure 3. (a) Survival curve of time to relapse (when dose and interval adjustment were omitted as a reason) in the whole study group (pooled data, N 5 75). (b) Time to relapse (when dose and interval adjustment were omitted as a reason) according to the type of anti-TNF therapy in the whole study group (N 5 75). (c) Time to relapse (when dose and interval adjustment were omitted as a reason) according to the type of anti-TNF therapy after propensity score matching (N 5 62). ADA, adalimumab; IFX, infliximab; TNF, tumor necrosis factor.

after propensity score matching (N 5 62, HR 5 0.76 [95% CI 0.35–1.68], *P* 5 0.50, Figure 3c). Adjusting this model to concomitant IMM did not affect the results (HR 5 0.76 [95% CI 0.34–1.67], *P* 5 0.49).

Supplementary Digital Content (see Supplementary Table 2, http://links.lww.com/CTG/A800) presents various reasons for treatment escalation during the follow-up period in both groups after propensity score matching (N 5 62). There was no significant difference between the 2 groups in any of the reasons listed.

Secondary outcomes

Primary nonresponse. There was no statistically significant difference in the primary nonresponse rate before propensity score matching (2/31 [6%] in the ADA group and 3/44 [7%] in the IFX group; *P* 5 0.95) nor after matching (2/31 [6%] in the ADA group and 3/31 [10%] in the IFX group; *P* 5 0.64). There was no significant difference in inflammatory markers (CRP, erythrocyte sedimentation rate, and F-CPT) or wPCDAI between ADA and IFX at the end of the induction period (week 12–16).

Predictors of treatment escalation and relapse. On a univariate analysis of the whole study group (N 5 75, pooled data), antineutrophilic antibody (pANCA) positivity and anti-*Saccharomyces cerevisiae* antibody (ASCA) negativity were identified as potentially strong predictors of treatment escalation. The SES-CD, L3 disease phenotype, and use of concomitant IMM for at least the first 6 months demonstrated a trend toward significance (Table 2). In a subsequent multivariate analysis, the combination of ASCA negativity and pANCA positivity was identified as the only and very strong independent predictor of treatment escalation (HR 5.19, 95% CI 2.41.11.18, *P* , 0.0001, Figure 4). There was no effect of disease phenotype, concomitant IMM, or type of anti-TNF when added to the model (Table 3).

Predictors of relapse (as defined earlier) were similar to those of treatment escalation (L3, SES-CD, pANCA positivity, and B2 being statistically significant (*P* , 0.05) and L1, family history of IBD, and ASCA negativity being of borderline significance). A combination of pANCA and ASCA remained a strong predictor (*P* 5 0.0091). There was no effect of concomitant IMM or type of anti-TNF when added to the model.

SAEs. A comparison of SAE occurrence in the treatment groups before and after propensity score matching is summarized in Tables 4 and 5. No significant difference was identified between the ADA and IFX groups, except for pneumonia after propensity score matching (3 cases in the IFX group and no cases in the ADA group; *P* 5 0.04). A subsequently performed mixed model reflecting the occurrence of SAEs during each patient visit and adjusted to concomitant IMM treatment and length of follow-up did not reveal any differences between the study groups (Table 6). In 1 patient receiving ADA, serious dermatological side effects led to cessation of ADA. In 1 patient receiving IFX, infusion allergic reaction led to the cessation of IFX.

PK. Regarding reactive TDM performed during the study period, TDM data were only available from selected visits (12% of all anti-TNF visits; 4% in the ADA group, and 21% in the IFX group). The median (IQR) trough levels at the time of the primary outcome were 14.05 (10.88–15.40) *m*g/mL in the ADA group and 6.15 (2.08–6.58) *m*g/mL in the IFX group (1 patient in the IFX group had undetectable trough levels). Positive ATI were only detected in the IFX group (5 observations in 3 patients during the follow-up period).

Because we did not intend to compare the PK of both anti-TNF agents, the PK subanalysis on propensity score–matched subgroups was not performed.

Concomitant IMM. After the onset of anti-TNF for at least 6 months, 29/31 (94%) patients in the ADA group and 38/44 (86%) in the IFX group received concomitant IMM therapy (97% AZA and 3% MTX). Adjusting the Cox model of time to treatment escalation (primary outcome) to concomitant IMM treatment did not affect the results (see the section on primary outcome). In the pooled data (N 5 75), concomitant IMM (as a continuous or categorical variable) was not identified as a strong independent predictor of treatment escalation on either univariate or multivariate analysis (Table 2). Because only a minority of patients

Table 2. Risk factors of treatment escalation identified by univariate analysis in the whole study group (N 5 75)

	HR (95% CI)	*P* value
pANCA positivity	3.221 (1.521–6.820)	0.002
ASCA negativity	3.093 (1.469–6.514)	0.003
SES-CD	0.960 (0.923–0.999)	0.045
L3 phenotype	0.571 (0.300–1.087)	0.088
Concomitant IMM (at least 6 mo)	0.472 (0.196–1.134)	0.093
Concomitant immunopathology	0.197 (0.027–1.469)	0.113
B2 disease phenotype	2.011 (0.837–4.833)	0.118
Family history of IBD	1.892 (0.827–4.327)	0.131
L1 disease phenotype	1.580 (0.807–3.094)	0.182
Time to anti-TNF onset	1.213 (0.876–1.680)	0.244
F-CPT	1.000 (1.000–1.001)	0.249
Perianal disease	1.489 (0.749–2.962)	0.257
L2 disease phenotype	1.808 (0.553–5.907)	0.327
B1 disease phenotype	0.704 (0.331–1.497)	0.363
B3 disease phenotype	0.526 (0.122–2.260)	0.388
BMI z score	1.047 (0.901–1.218)	0.547
CRP	0.996 (0.982–1.010)	0.559
ESR	0.996 (0.982–1.010)	0.560
Growth impairment	1.213 (0.610–2.410)	0.582
Concomitant IMM (as continuous)	0.774 (0.286–2.092)	0.614
wPCDAI	0.995 (0.976–1.015)	0.616
Age	0.979 (0.875–1.096)	0.716
Sex (male)	0.907 (0.476–1.727)	0.766
Height z score	0.989 (0.919–1.065)	0.775
Albumin	1.005 (0.959–1.054)	0.826
Year of anti-TNF administration (era)	1.024 (0.721–1.453)	0.896
Smoking	0.945 (0.214–4.178)	0.941

Predictive factors were evaluated during anti-TNF onset. Values are listed as hazard ratio (HR) with 95% confidence interval (CI) and sorted by the raising *P*-value. In the multivariate model, factors in bold were tested, and composite predictive factor (pANCA1 and ASCA-) was used (Table 3).
ASCA, anti-*Saccharomyces cerevisiae* antibodies; BMI, body mass index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; F-CPT, fecal calprotectin; IBD, inflammatory bowel disease; IMM, immunomodulators; pANCA, antineutrophilic antibodies; SES-CD, simple endoscopic score for Crohn's disease; TNF, tumor necrosis factor; wPCDAI, weighted pediatric Crohn's disease activity index.

received anti-TNF monotherapy, and limited PK data were available, a subanalysis investigating the effects of IMM on drug PK was not performed.

DISCUSSION

In accordance with guidelines on the management of pediatric CD, the selection of anti-TNF therapy (ADA vs IFX) in anti-TNF naive patients is based on patient and family preference, drug availability, administration route, and cost (10,11). This approach is based on early adult (mainly retrospective) studies that did not demonstrate any difference in efficacy between ADA and IFX (12–22), and subsequent large adult prospective studies (23,24) and retrospective studies with the longest follow-up to date (up to 5 years) (25,26). Beyond clinical efficacy, no difference was found in mucosal and histological healing (27). Two recent large propensity score–matched comparison studies in adult patients revealed no significant difference in clinical benefit between the 2 therapies. In addition, large nationwide population-based studies revealed no differences in real-world settings (28,29). Even studies showing some differences do not consistently demonstrate an effect in one direction (30–34). Thus, to date, there is no firm evidence that initiating either ADA or IFX in anti-TNF naive adult patients would make a difference, even for long-term prognosis (a median follow-up of 64 months) after switching to second-line anti-TNF (35). Neither a Markov model (3-month cycle) developed to simulate the therapeutic sequences of initiating biological treatment with ADA or IFX revealed any significant differences in persistence after 3 years in patients with active luminal CD (36).

Along with anti-TNF agents, anti-integrins and anti-IL 12/23 biologics have been proven by RCTs to be effective in adults with active CD (37). Because head-to-head trials would require a high number of patients for a noninferiority design and are unlikely to be performed unless funded by academic (nonindustry) institutes, indirect comparisons based on systematic reviews and network meta-analyses may help clinicians to guide first-line biological treatment. In the study by Singh et al. (7), both ADA and IFX were ranked highest among all biologicals as first-line therapy for the induction of remission in adult patients with moderate to severe CD confirming the results of a previous network meta-analysis evaluating various biologics in both the induction and maintenance phases and including IMM as a comparator (3). By contrast, in an older network meta-analysis, despite both ADA and IFX being effective, IFX was found to have the highest probability of being ranked as the most efficacious agent for induction (86%) and ADA for the maintenance of remission (48%) (8).

In pediatric clinical practice, adult data are relied upon because evidence in children is very scarce. In previous RCTs, both ADA and IFX were shown to be effective and safe in pediatric populations (1,2). Early retrospective observational studies revealed no difference between both therapies for up to 3 years of follow-up after the induction phase (6). A recent systematic review and meta-analysis identified 4 prospective cohort studies comparing ADA and IFX in pediatric populations (4). Three of these were abstracts; the only study that was published as a full text evaluated mucosal healing with anti-TNF therapy in 37 patients (12 ADA and 25 IFX) with biologically naive CD (5). No significant difference was found between the 2 therapies in achieving complete mucosal healing over 1 year of follow-up (P 5 0.74). High rates of clinical benefit (remission 1 response 65%–93%) within 2 years of follow-up with no significant difference between ADA and IFX were recently reported among 87 children with CD from a prospective cohort of the Sicilian IBD Network (9).

Thus, to date, there is no evidence that ADA is superior to IFX or vice versa in adult or pediatric patients with CD (38). This is also supported by our data based on propensity score matching. However, our power calculations showed that using 31 experimental and 31 control subjects, we were not able to detect HR of approximately 0.34–2.90. Thus, we can only conclude that there

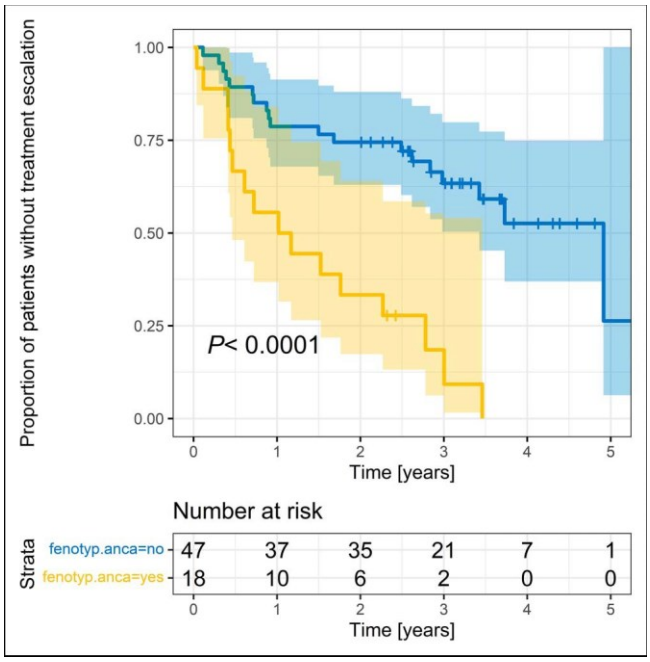

Figure 4. Time to dose and interval adjustment in the whole study group (pooled data, N 5 75) stratified by composite predictor (combination of ASCA negativity and pANCA positivity [fenotyp ANCA 5 yes] vs combination of ASCA positivity and pANCA negativity [fenotyp ANCA 5 no]). ASCA, anti-*Saccharomyces cerevisiae* antibodies; pANCA, antineutrophilic antibodies.

does not seem to be a substantial difference in the efficacy of both drugs.

Nevertheless, in real-life clinical practice, ADA is often considered as a second-line anti-TNF therapy in pediatric patients (39,40). This may be based on historically stronger experience with IFX, which, for many years, has been the only anti-TNF therapy approved for pediatric patients with CD. IFX may also be perceived as being more potent and adjustable than ADA by some clinicians due to the intravenous route of administration and weight-based dosing schedule, which allows a more precise dosage, especially in smaller children (41). In perianal disease, IFX may be preferred in clinical practice; however, available data probably do not allow first-line anti-TNF therapy to be determined based on disease phenotype (24,38,42,43).

The overall time to treatment escalation in our study was approximately 50% within 3 years of follow-up. However, our definition of treatment escalation also included the need for dose and interval adjustment, which increases the rates compared with a recently published systematic review of pediatric cohort studies reporting the probability of continuing IFX therapy 83%–97% after 1 year and 67%–91% and 61%–85% after 2 and 3 years, respectively. No conclusions can be made for ADA in this review due to the limited number of time-to-event studies (44). When we omitted the need for dose and interval adjustment from the definition, our relapse rate was similar (40% during 3 years) to those published. In the study focused on loss of response in primary responders, the reported random effects pooled incidence of dose intensification was 38% (95% CI 28–50) for IFX and 36% (95% CI 30–43) for ADA, with substantial heterogeneity in both cases. In pediatric patients, the mean percentage loss of response was 25.5%, with no possibility to compare anti-TNFs because of the lack of data (45).

Primary nonresponse to anti-TNF therapy is a substantial obstacle in IBD treatment, especially in adults, and is associated with an inferior response to second-line biologics (7). Primary nonresponse rates in our study (6% with the ADA group and 7% with the IFX group) were lower than those reported for both adults and children (1,2,37). However, these rates do not seem to be underestimated because the inflammatory markers and wPCDAI significantly decreased in both groups up to week 12–16. It is unlikely that patients would continue anti-TNF therapy due to the physicians' decision despite any signs of improvement. Moreover, nonresponse rates may be higher in RCTs, which follow a strict protocol, and different definitions of nonresponse are used in various studies, preventing direct comparison of results. Furthermore, a recently proposed tight TDM strategy during the induction phase (11), which could identify early nonresponders by PK, was not performed in our Center during the study. Several predictors of primary nonresponse are described in the literature (38,46); however, we did not perform these analyses because the rates of primary nonresponse were very low in our patient population.

Concerning predictors of long-term anti-TNF response, traditional factors that appear in the literature, and are derived mainly from adult data are younger age (younger than 40 years), being naive to anti-TNF, and concomitant use of IMM (38). In our study, only the latter was considered relevant and is discussed further. In pediatric patients, these data are generally very scarce.

Table 3. Risk factors of treatment escalation tested by multivariate analysis in the whole study group (N 5 75)

	HR (95% CI)	*P* value	Significance
pANCA1 and ASCA2	5.19 (2.41–11.18)	0.00003	***
L3 phenotype	0.49 (0.23–1.07)	0.073	NS
SES-CD	0.98 (0.94–1.03)	0.523	NS
Concomitant IMM (at least 6 mo)	0.78 (0.27–2.28)	0.650	NS
Type of anti-TNF	0.95 (0.45–2.03)	0.901	NS

Predictive factors were evaluated during anti-TNF onset (Table 2). Values are listed as hazard ratio (HR) with 95% confidence interval (CI) and sorted by the raising *P*-value. In the multivariate model, factors in bold were tested, and composite predictive factor (pANCA1 and ASCA2) was used (Table 3).
ASCA, anti-*Saccharomyces cerevisiae* antibodies; CI, confidence interval; HR, hazard ratio; IMM, immunomodulators; pANCA, antineutrophilic antibodies; SES-CD, simple endoscopic score for Crohn's disease; TNF, tumor necrosis factor.
***p , 0.001; NS 5 not significant.

Table 4. SAE according to the treatment group before propensity score matching (N 5 75)

	ADA (N 5 31)	IFX (N 5 44)	*P* value
Pneumonia	0 (0), NA 5 0	3 (0.07), NA 5 0	0.07
Meningitis	0 (0), NA 5 0	2 (0.05), NA 5 0	0.14
Pancreatitis	0 (0), NA 5 0	2 (0.05), NA 5 0	0.14
Leukopenia	1 (0.03), NA 5 0	2 (0.05), NA 5 0	0.77
Anemia	2 (0.06), NA 5 0	4 (0.09), NA 5 0	0.67
HSV	3 (0.1), NA 5 0	6 (0.14), NA 5 0	0.6
VZV	2 (0.06), NA 5 0	1 (0.02), NA 5 0	0.37
Other	12 (0.39), NA 5 0	15 (0.34), NA 5 0	0.68
Hospitalization	14 (0.45), NA 5 0	15 (0.34), NA 5 0	0.33
Any SAE	20 (0.65), NA 5 0	29 (0.66), NA 5 0	0.9

ADA, adalimumab; HSV, herpes simplex virus; IFX, infliximab; SAE, serious adverse event; VZV, varicella zoster virus.

A recently published study within the pediatric inflammatory bowel disease (PIBD) Ahead project identified several risk factors (especially phenotypic, serological, and genetic) for unfavorable disease course; however, predictors of anti-TNF response/relapse were not specifically addressed (47). In the largest pediatric prospective inception cohort study (RISK study) in 913 CD patients, several risk factors of B2 and B3 disease behavior were identified but with no specific conclusions regarding the prediction of anti-TNF efficacy (48). Recent studies identified various serological or genetic predictors of anti-TNF response (49–54); however, these factors were not measured in our patients and thus cannot be discussed. We identified a combination of ASCA negativity and pANCA positivity as the strongest independent predictors of treatment escalation in the multivariate model. To the best of our knowledge, this serological combination has not previously been described in the literature and should be prospectively validated in an independent cohort. Because pANCA positivity is typical for the ulcerative colitis (UC)-like phenotype, and anti-TNF effectivity is generally lower in UC than in CD (9,12), it remains unclear whether the abovementioned serological combination could be a potential proxy marker of distinct disease phenotype of CD with lower sensitivity to anti-TNF treatment.

Based on the results of a network meta-analysis focused on the side effects of anti-TNF, the relative safety profiles of ADA and IFX seem to be comparable (55). In our study, SAE rates were low, did not differ between both groups in the mixed model, and led only occasionally to treatment cessation (only 1 patient in each group), supporting the current opinion that anti-TNF treatment is safe in pediatric CD. Neither a recently published nationwide cohort study among 2018 pediatric IBD patients revealed any association between anti-TNF use and the risk of serious infections (56,57).

Proactive TDM was not performed in our study; thus, limited data did not allow us to fully evaluate the predictive value of PK regarding anti-TNF response. However, data available from selected visits have revealed median levels of both ADA and IFX in the range of recent recommendations in pediatric patients (11). Thus, it is unlikely that our patients were underdosed and that the physicians' approach was affected by this phenomenon. ATI formation was very rare but the transient presence of ATI could have been overlooked due to the reactive TDM approach. Recent studies identified various predictors of IFX levels, such as the presence of ATI, serum albumin concentration, concomitant IMM therapy, body weight, and sex (58). Owing to the scarcity of

Table 5. SAE according to the treatment group after propensity score matching (N 5 62)

	ADA (N 5 31)	IFX (N 5 31)	*P* value
Pneumonia	0 (0), NA 5 0	3 (0.1), NA 5 0	0.04
Meningitis	0 (0), NA 5 0	1 (0.03), NA 5 0	0.24
Pancreatitis	0 (0), NA 5 0	2 (0.06), NA 5 0	0.09
Leukopenia	1 (0.03), NA 5 0	0 (0), NA 5 0	0.24
Anemia	2 (0.06), NA 5 0	4 (0.13), NA 5 0	0.39
HSV	3 (0.1), NA 5 0	4 (0.13), NA 5 0	0.69
VZV	2 (0.06), NA 5 0	1 (0.03), NA 5 0	0.55
Other	12 (0.39), NA 5 0	11 (0.35), NA 5 0	0.79
Hospitalization	14 (0.45), NA 5 0	10 (0.32), NA 5 0	0.3
Any SAE	20 (0.65), NA 5 0	22 (0.71), NA 5 0	0.59

ADA, adalimumab; HSV, herpes simplex virus; IFX, infliximab; SAE, serious adverse event; VZV, varicella zoster virus.

Table 6. Mix model presenting occurrence of any SAE—adjusted to type of anti-TNF, concomitant IMM, and length of follow-up

	OR (95% CI)	*P* value
Type of anti-TNF	1.072 (0.566–2.031)	0.831
Concomitant IMM	1.613 (0.627–4.148)	0.322
Length of follow-up	1.167 (0.934–1.459)	0.175

Numbers in Tables 4 and 5 are listed as No. (and fraction, %) of cases that presented with respective SAE at least once during the follow-up. There were no cases of hepatopathy, thrombosis, malignities, or deaths identified in any of the groups. The subsequently performed mix model (Table 6) did not find any difference in the occurrence of SAE between both groups.
CI, confidence interval; IMM, immunomodulator; SAE, serious adverse event; TNF, tumor necrosis factor.

PK data in our study, we did not intend to identify any predictors of PK in our patients.

The approach to concomitant IMM therapy differs among pediatric IBD centers. In the European Union, AZA is used in most patients; by contrast, in the United States, use of MTX or anti-TNF monotherapy is more popular (39,59). Despite conflicting data, combination therapy is still considered useful (13,60–63), and most pediatric centers use it for at least 6 months from the onset of anti-TNF treatment. In accordance with recent data and current guidelines, IMM has been used less frequently in patients treated with ADA compared with patients treated with IFX (11,64). In our study, IMM treatment was not identified as a strong predictor of relapse on both univariate and multivariate analyses. These results could be affected by the high rate of concomitant IMM treatment in both groups. Owing to limited data, we could not analyze the possible effect of IMM on anti-TNF PK.

In addition to efficacy and safety, cost may be an important factor when selecting an appropriate first-line anti-TNF therapy. In some studies, ADA seems to be less costly than IFX; in others, the opposite seems to be true (65–68). Moreover, biosimilars coming to the market have changed the scenario significantly (69,70). Financial issues may be strongly dependent on the local situation, and other aspects such as quality of life should be considered when selecting an appropriate biological treatment.

Our data show that both ADA and IFX seem to demonstrate comparable efficacy and safety in pediatric CD patients naive to biologics. This study included a relatively small sample size compared with adult trials, preventing us from drawing strong conclusions. Despite its prospective design, some data were missing, and PK data were not available at all time points because TDM was not applied proactively during the study. Results in ADA-treated patients may have been influenced by a lack of adherence to therapy, which we were unable to evaluate. Conversely, this is the first pediatric study using propensity score matching with effective pairing (no dropouts), a prospective design, and a long duration of follow-up. Data comparing both anti-TNFs should be considered with caution in the future because these are derived from the traditional step-up approach. Because the top-down strategy (at least for IFX) may become preferable in children based on recent data (11,71), further research on the efficacy of various biologics as first-line treatment immediately after diagnosis must be performed.

CONFLICTS OF INTEREST

Guarantor of the article: Jiri Bronsky, MD, PhD.

Specific author contributions: J.B.: study design, data analysis, writing of the manuscript, and project supervision. I.C., D.K., T.L., K.M., K.P., M.S., and K.Z.: patient recruitment, data collection, and revision of manuscript draft; O.H.: study design, patient recruitment, data collection, statistics, and revision of manuscript draft.

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Study Highlights

WHAT IS KNOWN

- Both adalimumab (ADA) and infliximab (IFX) are effective and safe in the treatment of pediatric Crohn's disease.

WHAT IS NEW HERE

- This is the first prospective observational study comparing ADA and IFX in pediatric Crohn's disease.
- Propensity score matching did not reveal substantial differences in efficacy or safety between ADA and IFX.
- The ASCA2/pANCA1 combination is a strong predictor of treatment escalation.

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CLINICAL RESEARCH ARTICLE

Sustainability of biologic treatment in paediatric patients with Crohn's disease: population-based registry analysis

Ondrej Hradsky[✉], Ivana Copova[1], Marianna Durilova[1], Denis Kazeka[1], Tereza Lerchova[1], Katarina Mitrova[1], Jan Schwarz[2], Romana Vetrovcova[2], Nabil El-Lababidi[3], Eva Karaskova[4], Maria Veghova-Velganova[4], Astrid Sulakova[5], Lucie Gonsorčíková[6], Marketa Veverkova[7], Ivana Zeniskova[8], Martin Zimen[9], Martin Bortlik[10,11] and Jiri Bronsky[1]

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BACKGROUND: We aimed to evaluate the predictors of sustainability of biologic drugs for paediatric patients with Crohn's disease (CD).
METHODS: The Czech National Prospective Registry of Biologic and Targeted Therapy of Inflammatory Bowel Disease (CREdIT) was used to identify the biologic treatment courses in paediatric patients with CD. Mixed-effects Cox models and propensity score analyses were employed to evaluate predictors of treatment sustainability.
RESULTS: Among the 558 observations of 473 patients, 264 were treated with adalimumab (47%), 240 with infliximab (43%), 41 with ustekinumab (7%), and 13 with vedolizumab (2%). Multivariable analysis revealed higher discontinuation risk with infliximab compared to adalimumab (HR = 0.600, 95%CI 0.389–0.926), both overall and in first-line treatment (HR = 0.302, 95%CI 0.103–0.890). Infliximab versus adalimumab was associated with shorter time to escalation (HR = 0.094, 95%CI 0.043–0.203). Propensity-score analysis demonstrated lower sustainability of infliximab (HR = 0.563, 95%CI 1.159–2.725). The time since diagnosis to treatment initiation (HR = 0.852, 95%CI 0.781–0.926) was the most important predictor. Baseline immunosuppressive therapy prolonged sustainability with infliximab (HR = 2.899, 95%CI 1.311–6.410).
CONCLUSIONS: Given the results suggesting shorter sustainability, the need for earlier intensification and thus higher drug exposure, and the greater need for immunosuppression with infliximab than with adalimumab, the choice of these drugs cannot be considered completely equitable.

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IMPACT:

- Our study identified predictors of sustainability of biologic treatment in paediatric patients with Crohn's disease, including adalimumab (versus infliximab), early initiation of biologic treatment, and normalised baseline haemoglobin levels. Infliximab treatment was associated with earlier intensification, higher drug exposure, and a greater need for immunosuppression.
- Parents and patients should be fully informed of the disadvantages of intravenous infliximab versus adalimumab during the decision-making process.
- This study emphasises the importance of not delaying the initiation of biologic therapy in paediatric patients with Crohn's disease.

INTRODUCTION

A previously published meta-analysis showed that approximately 20% of patients with Crohn's disease (CD) lose response to anti-tumor necrosis factor (anti-TNF) therapy every year.[1] However, these data mostly come from studies lasting less than three years. A more recent study in adults has indicated that sustainability of biologic drugs differs during follow-up, and that the incidence of loss of response was much lower after two years of treatment.[2] We recently showed that the sustainability rate among 75 anti-TNF naïve paediatric patients with CD was 60% during three years of anti-TNF treatment.[3] Long-term data concerning paediatric patients with inflammatory bowel disease (IBD) on biologic treatment are scarce.[4] The predictors of sustainability remain unknown. Among adults and children, one of the most discussed predictors is the delay between diagnosis and the initiation of biologic treatment.[5–7]

[1]Department of Paediatrics, Second Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic. [2]Department of Paediatrics, Faculty of Medicine in Pilsen, Faculty Hospital, Charles University in Prague, Pilsen, Czech Republic. [3]Department of Paediatrics and Inherited Metabolic Disorders, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic. [4]Department of Paediatrics, Faculty of Medicine and Dentistry, University Hospital Olomouc, Olomouc, Czech Republic. [5]Department of Paediatrics, University Hospital Ostrava and Medical Faculty University of Ostrava, Ostrava, Czech Republic. [6]Department of Paediatrics, First Faculty of Medicine, Thomayer University Hospital and Charles University, Prague, Czech Republic. [7]Department of Paediatrics, Faculty of Medicine, Masaryk University and University Hospital Brno, Brno, Czech Republic. [8]Department of Paediatrics, Hospital Ceske Budejovice, Ceske Budejovice, Czech Republic. [9]Department of Paediatrics, Hospital Jihlava, Jihlava, Czech Republic. [10]Department of Gastroenterology, Hospital Ceske Budejovice, Ceske Budejovice, Czech Republic. [11]Department of Internal Medicine and Institute of Pharmacology, First Faculty of Medicine, Charles University, Prague, Czech Republic. ✉email: ondrej.hradsky@gmail.com

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In this study, we aimed to evaluate sustainability among biologic drugs in patients with CD using a prospective national database of patients with IBD treated with targeted therapy (Registry: CREdIT; https://credit.registry.cz). We aimed to identify the predictors of sustainability and develop a model to predict sustainability, if possible. Additionally, we compared the efficacy of anti-TNF biologics with that of non-anti-TNF biologics after previous anti-TNF treatment failure. Finally, we aimed to provide insight into the reasons and dynamics of treatment discontinuation.

MATERIALS AND METHODS

Study design and registry

This study was designed as a pre-planned analysis of retrospectively and prospectively collected data using a nationwide registry of patients with IBD treated with biologics or small molecules, called CREdiT (https://credit.registry.cz). The registry was established in 2016, and after reviewing the protocol, the Ethics Committee issued a favourable opinion. After informed consent was obtained, patients were registered at the time of treatment initiation (prospective arm) or, for a small proportion of patients, at the start of the registry, when treatment was already ongoing for >3 months (retrospective arm). After registration, all visits and drug applications were prospectively recorded. Data were collected by a paediatric gastroenterologist. The collected data consisted of information entered at the time of diagnosis (demographic data, disease classification, and characteristics), at the time of new treatment initiation (therapy, disease activity), and at the time of the visit with subcutaneous or intravenous drugs application (therapy, disease activity). Further details are listed in Table 1.

Nine of 12 paediatric IBD biologic treatment centres in the Czech Republic enroled at least one patient, all of whom participated in the study. Based on our best estimates and personal communication, we assume that this represents approximately 85% of all paediatric patients with IBD treated with biologic therapy in the Czech Republic. The treatment course was considered an individual observation. The inclusion criteria were a diagnosis of CD and starting the course of biologic treatment at the age of <19 years. The exclusion criteria were missing data on the event (discontinuation), time to event, type of treatment, and treatment line.

Outcome and definitions

The primary outcome was the time to treatment discontinuation. Any discontinuation of treatment was considered an event. If an individual child received repeated biologic treatments, all courses that met the inclusion criteria were analysed. Switching to a different drug within the same biologic agent (including a biosimilar or switching from an intravenous to subcutaneous formulation) was not considered a termination and was analysed as one observation. The time to termination was calculated from the last application of the drug to the start date. If a patient did not terminate treatment, the last visit was considered for the follow-up time. The reasons for termination were classified into three categories: treatment failure, side effects, and termination based on patient's preference. The side-effect of the treatment had to be considered the main reason for its termination, to reach the category "side-effect". Escalation was defined as any intensification of treatment over the standard regimen, calculated based on the patient's body weight or body area (including shortening the interval or increasing the dose of the drug). The time to escalation was calculated in a manner similar to that used to calculate the time to termination. Immunosuppressive therapy at the time of treatment initiation was defined as any dose of azathioprine, 6-mercaptopurine, or methotrexate administered at the time of the first biologic drug application. The term "treatment line" is used in the text to refer to the numbered order of biological therapy administered to the patient.

Statistical analysis

All data were analysed using the R statistical software (version 4.2.0; www.r-project.org). Continuous variables were described as median and interquartile range (IQRs). Categorical variables were described as absolute frequencies and percentages. Variables with a high proportion of missing data were excluded from analysis. Other missing data (frequency <45%) were imputed using multiple imputation methods with the R package "mice".

The primary outcome of the study was evaluated using a mixed-effects Cox proportional hazards model with the R package "coxme". The random part of the model consisted of a particular patient, centre, and time-period. The pre-selected predictors were tested using Cox mixed regression. To assess the importance of particular variables, we further tested the association between time to treatment termination and the variables using multivariable Cox proportional hazards mixed models. All these models were adjusted for retrospective data acquisition.

After detecting a difference in sustainability between ADA and IFX, we performed a propensity score analysis to improve the balance between these two treatment groups. Matching was performed on imputed datasets using nearest neighbour matching and a 1:1 ratio within the package "matchThem". As covariates, we selected four variables that best predicted treatment allocation (CRP, centre, height Z score, and retrospective acquisition of data) and added treatment line and time since diagnosis based on clinical decisions. The final Cox proportional hazards mixed model was adjusted for the use of immunomodulators at the beginning of the biologic treatment and the time since diagnosis.

The incidence rate per patient-year was calculated for different time periods. We used parametric survival modelling with Weibull distribution and Wald test for a significant increase/decrease in hazard with longer treatment duration.

The normalisation of haemoglobin was done by equation: (actual haemoglobin—((lower limit + upper limit)/2))/(upper limit—lower limit). The limits were adopted from UpToDate.[8]

Probability (p) values of <0.05 were considered significant. A 95% confidence interval (CI) was used. Figures were constructed using the R package "ggplot2".

The data underlying this article will be shared on reasonable request to the corresponding author.

RESULTS

Among the included (Fig. S1) 558 observations (227 female, 41%) of 473 paediatric CD patients, 264 observations were with adalimumab (47%), 240 with infliximab (43%), 41 with ustekinumab (7%), and 13 with vedolizumab (2%). Most of the observations were from the first line of biologic treatment (first: 418, 75%; second: 102, 18%; third: 34, 6%; fourth and fifth: 3, 1%) and concomitant immunosuppressive therapy at the beginning of the treatment course (448, 80%) (Table 1). The overall sustainability of drugs in paediatric patients with CD, irrespective of the treatment line, is shown in Fig. 1a. After 3 years of treatment, approximately 75% of the patients were still receiving the same biologic treatment.

Sustainability predictors

We identified the time since diagnosis, treatment line, haemoglobin level at the beginning of treatment, and the treatment substance as predictors of sustainability in Cox mixed models adjusted for person, centre, and time-period (Table S1). Among biologic treatment, observations with infliximab (HR 0.57, 95% CI 0.37–0.88, $p = 0.011$) and vedolizumab (HR 0.31, 95% CI 0.11–0.95, $p = 0.040$) had shorter sustainability compared to adalimumab, Fig. 1b.

Multivariable analysis revealed that patients treated with infliximab were at a higher risk of discontinuation than patients treated with adalimumab (Table 2; HR 0.640, 95% CI 0.412–0.993, $p = 0.046$). Furthermore, treatment with vedolizumab also predicted shorter sustainability (HR 0.29, 95% CI 0.087–0.969, $p = 0.044$). In the same model, the time since diagnosis to biologic treatment initiation (HR 0.852, 95% CI 0.781–0.929, $p < 0.001$) and normalised haemoglobin levels (HR 1.783, 1.086–2.924, $p = 0.022$) were associated with the sustainability of biologic treatment in paediatric patients with CD. Because a difference between the treatment groups was detected, we compared wPCDAI, C-reactive protein, and faecal calprotectin levels between these groups (Tables S2 and S3). We did not find any differences even after separation when the endpoints were reached. Given the low power of the predictors, we withdrew from building a prediction model.

Comparing sustainability between ADA and IFX using propensity score matching

Among originally included 504 courses of anti-TNF treatment (Tables S4, Table S5) 240 with infliximab and 240 with adalimumab were matched (Fig. S2). Patients treated with infliximab showed a

Table 1. Baseline characteristic of observations.

	adalimumab ($N = 264$)	infliximab ($N = 240$)	ustekinumab ($N = 41$)	vedolizumab ($N = 13$)	Overall ($N = 558$)
Time since diagnosis [Years]					
Median [Q1,Q3]	0.917 [0.264,2.21]	0.923 [0.159,2.30]	4.67 [3.77,5.45]	2.48 [0.999,4.69]	0.975 [0.226,2.59]
Missing	29 (11.0%)	21 (8.8%)	27 (65.9%)	6 (46.2%)	83 (14.9%)
Sex					
Female	110 (41.7%)	97 (40.4%)	16 (39.0%)	4 (30.8%)	227 (40.7%)
Male	154 (58.3%)	143 (59.6%)	25 (61.0%)	9 (69.2%)	331 (59.3%)
Age at start [Years]					
Median [Q1,Q3]	14.5 [11.9,16.4]	15.0 [12.1,16.9]	14.5 [11.7,16.1]	15.7 [11.7,16.8]	14.8 [12.0,16.7]
Location					
L1	29 (11.0%)	38 (15.8%)	5 (12.2%)	3 (23.1%)	75 (13.4%)
L2	53 (20.1%)	45 (18.8%)	14 (34.1%)	5 (38.5%)	117 (21.0%)
L3	178 (67.4%)	153 (63.8%)	22 (53.7%)	5 (38.5%)	358 (64.2%)
Missing	4 (1.5%)	4 (1.7%)	0 (0%)	0 (0%)	8 (1.4%)
L4					
no	120 (45.5%)	129 (53.8%)	25 (61.0%)	7 (53.8%)	281 (50.4%)
yes	144 (54.5%)	111 (46.3%)	16 (39.0%)	6 (46.2%)	277 (49.6%)
Growth retardation					
G0	190 (72.0%)	174 (72.5%)	23 (56.1%)	10 (76.9%)	397 (71.1%)
G1	74 (28.0%)	66 (27.5%)	18 (43.9%)	3 (23.1%)	161 (28.9%)
Indication for treatment initiation					
extraintestinal	24 (9.1%)	10 (4.2%)	5 (12.2%)	0 (0%)	39 (7.0%)
luminal	181 (68.6%)	164 (68.3%)	32 (78.0%)	10 (76.9%)	387 (69.4%)
perianal	59 (22.3%)	66 (27.5%)	4 (9.8%)	3 (23.1%)	132 (23.7%)
Baseline SES-CD					
Median [Q1,Q3]	17.0 [9.00,25.0]	21.0 [14.0,28.0]	20.0 [17.0,27.5]	16.0 [14.3,19.8]	19.0 [12.0,26.0]
Missing	151 (57.2%)	160 (66.7%)	25 (61.0%)	5 (38.5%)	341 (61.1%)
Baseline fCPT [ug/g]					
Median [Q1,Q3]	1320 [418,1800]	1340 [488,2030]	1400 [402,1820]	814 [274,1550]	1320 [429,1800]
Missing	63 (23.9%)	64 (26.7%)	10 (24.4%)	2 (15.4%)	139 (24.9%)
Baseline CRP [mg/l]					
Median [Q1,Q3]	5.30 [1.60,16.4]	6.10 [1.53,15.1]	3.30 [1.20,10.1]	2.60 [0.500,12.0]	5.40 [1.40,15.7]
Missing	3 (1.1%)	2 (0.8%)	0 (0%)	0 (0%)	5 (0.9%)
Baseline haemoglobin [g/l]					
Median [Q1,Q3]	124 [115,135]	124 [112,136]	126 [120,137]	132 [120,143]	124 [115,136]
Missing	3 (1.1%)	2 (0.8%)	0 (0%)	0 (0%)	5 (0.9%)
Normalised baseline haemoglobin					
Median [Q1,Q3]	−0.288 [−0.561,−0.0455]	−0.325 [−0.608,−0.100]	−0.197 [−0.450,0]	−0.125 [−0.561,0.0750]	−0.288 [−0.575, −0.0500]
Missing	3 (1.1%)	2 (0.8%)	0 (0%)	0 (0%)	5 (0.9%)
Baseline leukocytes					
Median [Q1,Q3]	7.12 [5.90,9.10]	7.50 [5.80,9.72]	8.90 [7.10,11.4]	7.77 [7.40,9.70]	7.50 [5.90,9.60]
Missing	3 (1.1%)	2 (0.8%)	0 (0%)	0 (0%)	5 (0.9%)
Baseline wPCDAI					
Median [Q1,Q3]	20.0 [7.50,35.0]	20.0 [7.50,35.0]	20.0 [7.50,32.5]	22.5 [12.5,25.0]	20.0 [7.50,35.0]
Missing	0 (0%)	1 (0.4%)	0 (0%)	0 (0%)	1 (0.2%)
Baseline BMI Z-score					
Median [Q1,Q3]	−0.658 [−1.36,0.138]	−0.640 [−1.37,0.00926]	0.0187 [−0.728,0.298]	−0.246 [−0.937, −0.00504]	−0.607 [−1.35,0.137]
Missing	2 (0.8%)	0 (0%)	0 (0%)	0 (0%)	2 (0.4%)

Table 1. continued

	adalimumab ($N = 264$)	infliximab ($N = 240$)	ustekinumab ($N = 41$)	vedolizumab ($N = 13$)	Overall ($N = 558$)
Baseline height Z-score					
Median [Q1,Q3]	−0.624 [−1.54,0.239]	−0.762 [−1.60,0.0708]	−0.766 [−1.53, −0.267]	−0.524 [−1.42,0.357]	−0.688 [−1.57,0.141]
Treatment line					
1.	214 (81.1%)	202 (84.2%)	0 (0%)	2 (15.4%)	418 (74.9%)
2.	50 (18.9%)	36 (15.0%)	13 (31.7%)	3 (23.1%)	102 (18.3%)
3.	0 (0%)	1 (0.4%)	28 (68.3%)	5 (38.5%)	34 (6.1%)
4.	0 (0%)	0 (0%)	0 (0%)	3 (23.1%)	3 (0.5%)
5.	0 (0%)	1 (0.4%)	0 (0%)	0 (0%)	1 (0.2%)
Baseline immunomodulator					
no	53 (20.1%)	40 (16.7%)	12 (29.3%)	5 (38.5%)	110 (19.7%)
yes	211 (79.9%)	200 (83.3%)	29 (70.7%)	8 (61.5%)	448 (80.3%)
Start time period [calendar years]					
(2009,2015)	11 (4.2%)	19 (7.9%)	0 (0%)	0 (0%)	30 (5.4%)
(2015,2018)	42 (15.9%)	35 (14.6%)	3 (7.3%)	1 (7.7%)	81 (14.5%)
(2018,2023)	211 (79.9%)	186 (77.5%)	38 (92.7%)	12 (92.3%)	447 (80.1%)
Patients enroled retrospectively					
no	234 (88.6%)	213 (88.8%)	40 (97.6%)	13 (100%)	500 (89.6%)
yes	30 (11.4%)	27 (11.3%)	1 (2.4%)	0 (0%)	58 (10.4%)

Q1 first quartile, *Q3* third quartile, *L1* ileocaecal, *L2* colonic, *L3* ileocolonic disease, *L4* upper gastrointestinal involvement, *SES-CD* simple endoscopic score for Crohn's disease, *CRP* C-reactive protein, *wPCDAI* weighted Paediatric Crohn's Disease Activity Index, *BMI* body mass index

shorter time to drug termination than those treated with adalimumab (adjusted HR 0.563, 95% 0.367–0.863, $p = 0.008$) (Fig. 1c). This Cox mixed model was adjusted for baseline treatment with an immunomodulator, the time since diagnosis, and random effects of patient and time-period (Table S6).

Predictors of sustainability of anti-TNF as a first line treatment
Among the 504 included observations, 214 treatment courses with adalimumab, 202 courses with infliximab, and two courses with vedolizumab were used as first-line biologic treatments. The basic characteristics of anti-TNF treatment are listed in Table S7. Using Cox mixed regression models adjusted for person, centre, and time-period, we identified two predictors of sustainability. Patients treated with infliximab as the first-line treatment showed shorter sustainability of biologic treatment than those treated with adalimumab as first-line treatment (HR 0.575, 95% CI 0.360–0.919, $p = 0.021$). Shorter time to initiation of biological therapy was associated with longer sustainability.. In a multiple Cox mixed model (Table S8) that included infliximab, time since diagnosis to biologic treatment initiation, and baseline normalised haemoglobin we found that they were all associated with sustainability as first-line treatment (infliximab HR 0.302, 95% CI 0.103–0.890, $p = 0.030$; time since diagnosis HR 0.789, 95% CI 0.717–0.867, $p < 0.001$; normalised baseline haemoglobin HR 2.028, 95% CI 1.189–3.46, $p = 0.010$). Moreover, we found an interaction between the substance and baseline immunomodulatory treatment; among patients treated with infliximab, immunomodulators prolonged sustainability (HR 2.899, 95% CI 1.311–6.41, $p = 0.009$). This model was further adjusted for retrospective gathering of data in the fixed part of the model and for individual, centre and time period in the random part of the model.

Predictors of sustainability of anti-TNF after previous treatment with anti-TNF
We identified 138 patients who were treated with biologics after a course of anti-TNF treatment, including 86 who were administered a second anti-TNF treatment (50 with adalimumab and 36 with infliximab) and 52 who were administered other biologics (11 with vedolizumab and 41 with ustekinumab) (Table S9). We found a negative association between baseline CRP levels and sustainability (HR 0.971, 95% CI 0.957–0.985, $p < 0.001$). The sustainability of non-anti-TNF treatment (vedolizumab or ustekinumab) was not significantly longer than that of anti-TNF treatment after the course of anti-TNF treatment (Fig. 1d, Fig. S3, Table S10), even in multiple Cox regression mixed models adjusted for baseline CRP (HR 0.491, 95% CI 0.192–1.256, $p = 0.136$).

Time to escalation
Treatment escalation was recorded in 48% of the treatment courses. Treatment with infliximab (Fig. S4a), time since diagnosis, wPCDAI, and calprotectin levels were identified as predictors of treatment sustainability (Table S11a). Using a multiple Cox regression mixed model, we found that the time to treatment intensification was shorter in patients treated with infliximab than in those treated with adalimumab (HR 0.094, 95% CI 0.043–0.203, $p < 0.001$). Additionally, we identified the time since diagnosis (HR 0.891, 95% CI 0.818–0.970, $p = 0.009$) and baseline wPCDAI (HR 0.980, 95% CI 0.973–0.989, $p < 0.001$) as predictors of sustainability without intensification (Table S12a). We also performed a sub-analysis from which we excluded those observations where intensification occurred within 8 weeks. Although we found an association between ustekinumab treatment and shorter time to intensification in the unadjusted model, this association was lost in the final Cox regression model, and the final result was very similar to the analysis on the whole group (Table S11b, Fig. S4b, Table S12b).

Termination of the treatment during the study follow-up and reason for termination
During the observational period, with a median of 1.4 years, treatment was terminated in 101 cases (18%), five (5%) due to patient preferences, twenty-four (24%) due to side effects, and 72 (71%) due to treatment failure (Fig. 2). These represent 1110 person-years with an event rate of 0.091 (Table S13). The incidence of

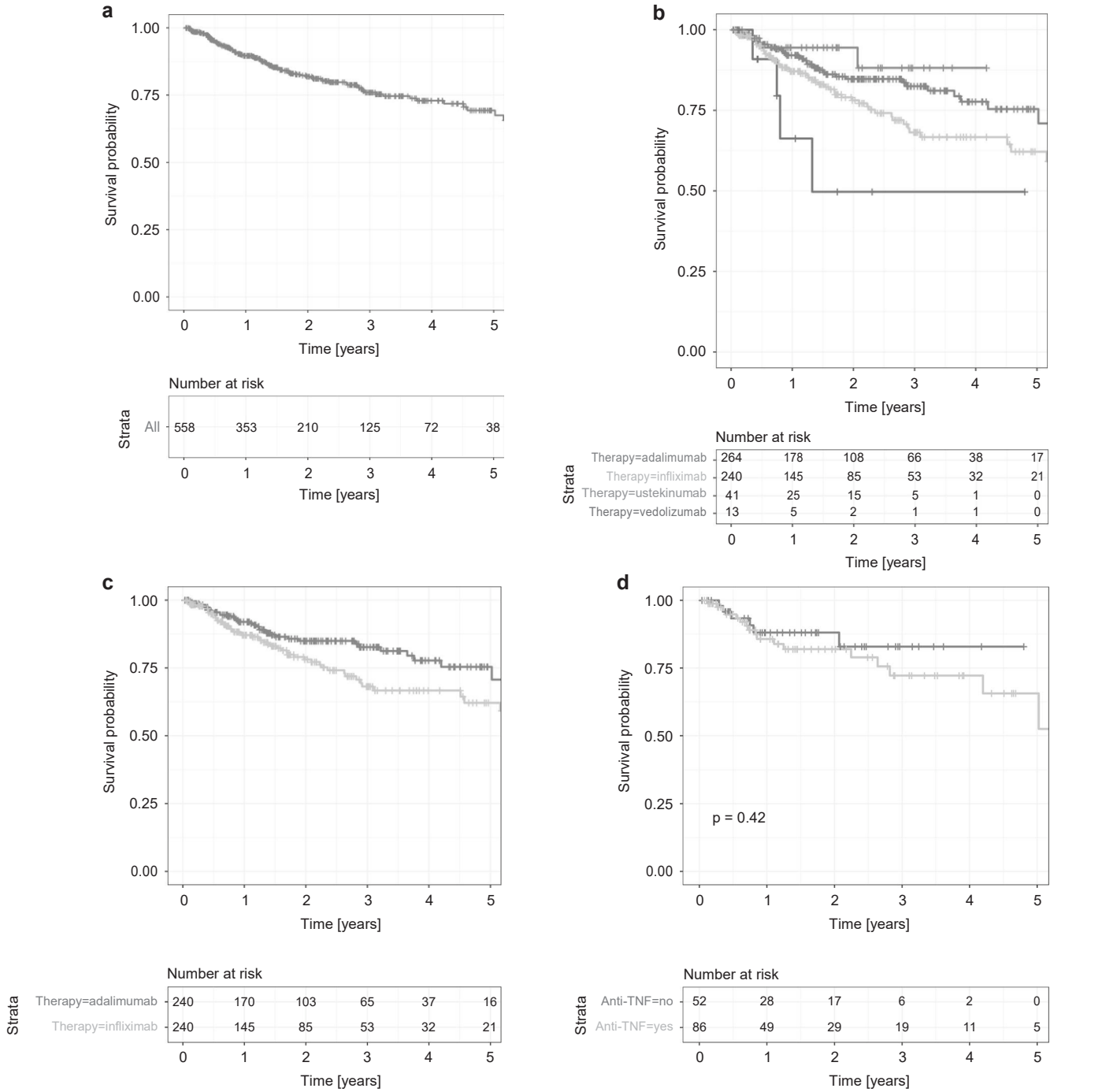

Fig. 1 Sustainability of biologic treatment (Kaplan Meier curves). a All included paediatric CD patients, (b) subgroup analysis per biologic agent, (c) subgroup analysis per biologic agent in propensity score matched subgroup and (d) subgroup analysis in subgroup of patient after failing anti-TNF treatment. Note non-anti-TNF was represented by vedolizumab or ustekinumab treatment.

treatment termination was highest in the first year (event rate of 0.48, 95% CI 0.35–0.63), decreased quickly in the second year (event rate of 0.12, 95% CI 0.08–0.18), reached its lowest in the fourth year (0.01–0.06), and remained quite stable (Fig. 3, Fig. S5). During the entire period of follow-up (1110 person-years) the decrease in termination incidence rate was significant ($p < 2*10^{16}$).

DISCUSSION

Our study, which utilised a nationwide registry of 558 courses of biologic treatment, revealed that the sustainability of infliximab was 40% lower than that of adalimumab in children with CD. In addition, we do not find a difference in disease activity at termination between the treatment groups. This difference persisted even after propensity score matching or when the analysis was restricted to patients treated only with their first biologic therapy. Furthermore, we observed that patients treated with infliximab required 6-fold earlier intensification. According to our study, postponing the initiation of biologic therapy in paediatric patients with CD by one year resulted in 15% reduction in the likelihood of treatment sustainability, underscoring the critical significance of early treatment initiation for achieving

Table 2. Multivariable Cox regression mixed model for sustainability.

predictor	HR	*p*
Infliximab (versus adalimumab)	0.600 (0.389–0.926)	0.021
Ustekinumab (versus adalimumab)	2.778 (0.71–10.87)	0.142
Vedolizumab (versus adalimumab)	0.29 (0.087–0.969)	0.044
Treatment line	1.121 (0.706–1.783)	0.628
Baseline normalised haemoglobin [g/l]	1.783 (1.086–2.924)	0.022
Baseline immunomodulator	1.145 (0.672–1.949)	0.617
Time since diagnosis [years]	0.852 (0.781–0.929)	<0.001

The model were further adjusted for retrospective gathering of data in the fixed part of the model and for individual, centre and time period in random part of the model.

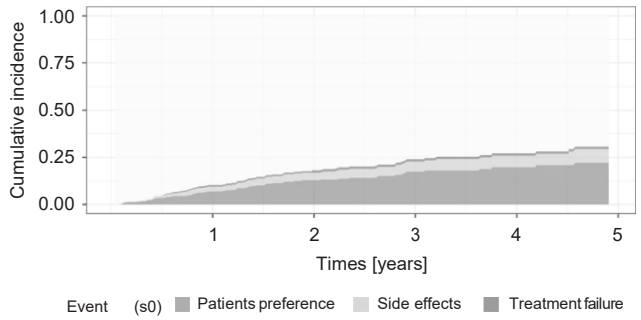

Fig. 2 Cumulative incidence of treatment termination with corresponding reasons.

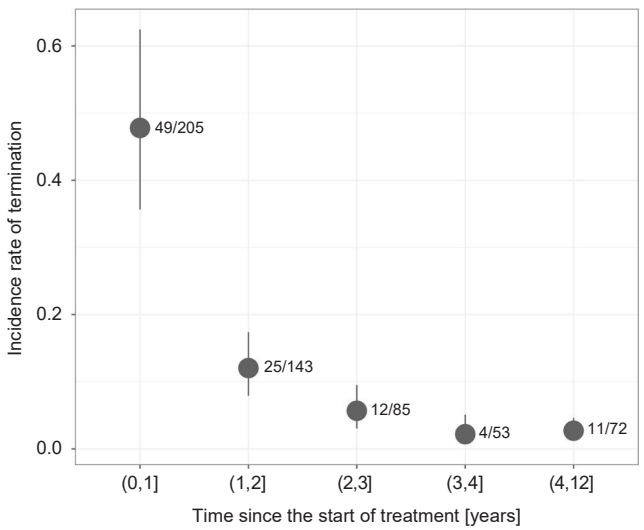

Fig. 3 Incidence of treatment termination according to time since the start of treatment.

optimal treatment outcomes. Among the markers of disease activity at the time of biologic treatment initiation, only lower haemoglobin levels were associated with sustainability in our study. Although we identified these predictors, we were unable to construct a sufficiently robust prediction model because of the strength of their association.

Regarding the sustainability of adalimumab versus infliximab, studies in adults have shown conflicting results.[2,9–11] There are very limited data available on this topic for the paediatric population.[3,12,13] Although the published meta-analysis suggested comparable efficacy, owing to the small sample size and heterogeneity of the included studies, the conclusion was that larger studies were needed for a more definite conclusion.[13] In a previous small-scale study conducted at our referral centre, we used propensity score analysis and found no significant differences between adalimumab and infliximab in terms of treatment intensification or relapse.[3] However, it is important to note that the number of patients included in the study was relatively small, and the follow-up period was not long enough to detect any potential differences. According to a recent abstract by Atia et al.,[14] a propensity score analysis of data from the epi-IIRN database for children and adults that included 760 patients treated with infliximab and 760 treated with adalimumab, found that adalimumab had longer sustainability, yielding the same results as those observed in our largest study to date.

We observed a difference in long-term sustainability between adalimumab and infliximab despite their similar short-term efficacy in randomised controlled trials.[15,16] One possible reason for this difference might be the mode of administration. For example, intravenous administration can result in greater variations in serum concentrations.[17–19] In line with this, newly registered biologics are increasingly administered subcutaneously during the maintenance phase.[20]

In the resulting multiple Cox regression model, we found shorter sustainability in patients who were treated with vedolizumab. Given the low number of included observations with vedolizumab and the isolated outcome, this can only be considered as a starting point for further research that would primarily focus on the difference between e.g., anti-TNF and anti-integrin therapy in paediatric patients. Even though this is an adjusted model, it is likely that the group of patients who received vedolizumab included patients who had already failed previous therapy, i.e., patients who were more refractory. On the other hand, it should be noted that the other non-anti-TNF, ustekinumab, to which similar patients were started, showed the opposite trend, that is protective.

There is no clarity yet on the appropriate anti-TNF agents, and how long they need to be administered for immunosuppressive therapy concomitantly.[21–25] Some data suggest that combination therapy may be more important with infliximab than with adalimumab.[21,22] Additionally, in our paediatric study, we observed that the use of an immunomodulator alongside infliximab resulted in a threefold increase in the rate of sustainability, whereas no such benefit was observed with adalimumab treatment.

Both older and more recent data consistently indicate that delaying the initiation of intensive therapy, typically involving biologic agents, following the diagnosis of CD is associated with a poorer prognosis.[5,6,26] In our study, we demonstrated that for each year biologic therapy was delayed in paediatric CD patients, there was 15% reduction in treatment sustainability. Surprisingly, a recent study from the ImproveCareNow Network registry did not find an association between early initiation of the first biologic treatment and its discontinuation.[4] However, it should be emphasised that this association was only evaluated in a subset of patients for whom a recent clinic visit before biologic initiation was available and whose biologic discontinuation status was known. Therefore, the possibility of selection bias, which may have caused this association to not be found in the analysed subpopulation, cannot be ruled out. Moreover, more than one-third of the cohort consisted of paediatric-onset adult patients.

Although Kaplan et al.[4] identified a weak association between baseline CD disease activity (short PCDAI) and the time to termination, this association was not significant in the multivariable analysis. In contrast, our study demonstrated that the probability of sustainability increased with increasing in normalised haemoglobin values, suggesting that disease activity, as might be reflected by haemoglobin levels, may be a predictor of

treatment sustainability. This association appears to be significant only shortly after treatment initiation, as shown by further analysis using Receiver Operating Characteristic curves at individual time points (data not shown). Since the association of normalised haemoglobin only comes out in the multivariate regression model, is valid shortly after initiation, and is a normalised value, it is difficult to make any recommendation for practice based on such data.

We investigated whether patients who previously failed treatment with an anti-TNF agent experienced prolonged survival when treated with a non-anti-TNF agent. While non-anti-TNF treatment was numerically associated with greater sustainability, our adjusted models did not demonstrate a significant difference. It is important to note that the number of patients in this group was considerably lower and the length of follow-up for these patients, particularly those receiving non-anti-TNF treatment, was relatively short. Moreover, it should be noted that we had no access to the recommended[27] therapeutic drugs in the previous anti-TNF treatment course, and we had no information whether the failure was pharmacokinetic or pharmacodynamic. As there have been no other published data on this topic in paediatric patients, further studies with a larger group, including also patients treated with non-anti-TNF drugs, are necessary to clarify whether other anti-TNF drugs are inappropriate after the failure of anti-TNF treatment. However, it should be noted that in our study, after two years, more than 80% of the patients in both groups were still receiving the same treatment (Fig. 1d).

A recent study of adult patients with CD revealed that the discontinuation rates of anti-TNF therapy decreased over time, with rates of discontinuation being three times lower after four years than those in the first year of treatment.[2] Similarly, we observed a significant decrease in termination rates, which were over 10 times lower after three years compared to those in the first year of a treatment course. As in adults, the most frequent reason for the termination of treatment in children is treatment failure.[2,4] However, in accordance with other literature,[28–30] the percentage of treatment courses terminated due to side effects appears to be much higher in children (24% in our paediatric study and 23% in the ImproveCareNow cohort[4]) than in adult patients (11%[2]). Notably, the overall sustainability of anti-TNF therapy in adult patients appears to be lower than that in paediatric patients. In our paediatric study, we observed a sustainability rate of 75%, which is consistent with other paediatric reports,[4,31] however it was higher than the data in adults showing less than 50% sustainability at 3 years.[2]

Our study has several strengths. First, this was a prospective study conducted by physicians and covered approximately 85% of all patients treated with biologics in the Czech Republic, making it a population-based study. Second, the study included a sufficient number of patients to assess the differences between the anti-TNF agents. However, some limitations should be acknowledged. For instance, it was more challenging to evaluate the potential benefits of concomitant immunomodulators because they were administered to a large proportion of patients. Furthermore, although our study focused on sustainability, we acknowledge that an intervention study could provide more conclusive evidence. Like all fully observational studies, ours involves decisions that are in the hands of the treating physicians and may be influenced by personal preference, price, and whether the drugs are reimbursed by insurance. Specifically, our study includes a relatively large number of patients who were first treated with both types of anti-TNF agents for the latter reasons. However, from a certain point of view, this may be an advantage, at least in relation to the assessment of the applicability of both agents to each other. On the other hand, vedolizumab and ustekinumab, which were initiated only in later lines, are more difficult to compare with anti-TNF agents. The nature of an observational study also does not rule out different practices for different drugs. And it must be acknowledged that this may have been reflected in the willingness of physicians to intensify therapy. Therefore, the finding of a large difference between adalimumab and infliximab intensification should be viewed with caution and may not always mean that intensification was actually needed. On the other hand, even when infliximab intensification was more frequent, lower sustainability were found in our study. Because this difference may be more pronounced, an analysis was also performed excluding observations in which intensification was performed within the first 8 weeks. An additional limitation is the presence of a proportion of retrospective data and the association of the method of data collection with sustainability. The resulting models were therefore adjusted for the type of data collection and this variable was also included in the development of the model for propensity score. However, the results of this sub-analysis were very similar to the analysis of the whole group. In order to include a sufficient number of observations, patients had to be enroled over a longer period of time, during which time the requirements for depth of remission were likely to tighten. Although this approach is unlikely to vary by e.g., preparation, a time period factor was additionally added to all models. Safety-related data were not reported separately in this study but will be addressed in a future publication. Additionally, we compared observations rather than patients, which could be perceived as a disadvantage. However, we consider this an advantage because it allowed us to better treat patients who usually do not respond or have difficulty responding to any treatment. Appropriate statistical methods were used to account for this approach. One drawback of the search for predictors was the incompleteness of the baseline endoscopic data. Because of the large amount of missing baseline endoscopic data (61%), we decided not to impute or use these data in the models. A significant limitation of our study was the lack of therapeutic drug monitoring, which prevented us from drawing definitive conclusions regarding the comparison of anti-TNF and non-anti-TNF treatments in patients who previously failed anti-TNF therapy. Finally, it should be noted that our study did not aim to compare less commonly used biologic treatments, such as ustekinumab and especially vedolizumab. Therefore, caution should be exercised when interpreting the results in the context of these treatments.

In conclusion, we identified several predictors of the sustainability of biologic therapy. Of these, the most important seems to be the time from diagnosis to initiation of therapy and a specific drug. Considering the strengths of the predictors, we were unable to construct a predictive model. However, the key finding was the difference between adalimumab and infliximab. Overall, given that the results are in line with the available evidence suggesting shorter sustainability of infliximab than adalimumab, the need for earlier intensification and thus higher drug exposure and the greater need for immunosuppression, and the likely higher incidence of cutaneous adverse events with infliximab compared with adalimumab observed in other studies,[28,32] the choice of anti-TNF agent cannot be considered completely equitable. Therefore, parents and patients should be fully informed of the disadvantages of intravenous infliximab versus adalimumab during the decision-making process. Because the current prediction models for identifying patients with a low probability of maintaining remission on immunomodulators are not sufficiently robust,[33] it is important to emphasise that the initiation of biologic therapy should not be delayed.

DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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AUTHOR CONTRIBUTIONS

O.H.: Created the conception, study design and data analysis, patient recruitment, first draught of the paper. I.C.: Patient recruitment and revision of the original article. M.D.: Patient recruitment and revision of the original article. D.K.: Patient recruitment and revision of the original article. T.L.: Patient recruitment and revision of the original article. K.M.: Patient recruitment and revision of the original article. J.S.: Patient recruitment and revision of the original article. R.V.: Patient recruitment and revision of the original article. N.L.: Patient recruitment and revision of the original article. E.K.: Patient recruitment and revision of the original article. M.V.V.: Patient recruitment and revision of the original article. A.S.: Patient recruitment and revision of the original article. L.G.: Patient recruitment and revision of the original article. M.V.: Patient recruitment and revision of the original article. I.Z.: Patient recruitment and revision of the original article. M.Z.: Patient recruitment and revision of the original article. M.B.: Leading the registry, patient recruitment and revision of the original article. J.B.: Leading the project team, patient recruitment and revision of the original article.

COMPETING INTERESTS

O.H.: lectures/congress fees/consultancy (outside the scope of the submitted work; MSD, AbbVie, Takeda, Sandoz, Nutricia, Nestlé, and Ferring). I.C.: no conflict. M.D.: congress fees (outside the scope of the submitted work; Nutricia, Nestlé). D.K.: congress fee (outside the scope of the submitted work; Takeda). T.L.: lectures/congress fees/consultancy (outside the scope of the submitted work; Ferring, Nutricia, Biocodex, and AbbVie). K.M.: lectures/congress fees/consultancy (outside the scope of the submitted work; Takeda, Janssen-Cilag). J.S.: lectures/congress fees (AstraZeneca, AbbVie, Nestlé, Nutricia, MSD, and Takeda). R.V.: congress fees (outside submitted work; AbbVie, Nestlé). N.L.: lectures/congress fees/consultancy (outside the scope of the submitted work; AbbVie, Sandoz, Nutricia, Nestlé). E.K.: lectures/congress fees/consultancy (outside the scope of the submitted work; AbbVie, Nutricia, and Nestlé). M.V.V.: lectures, congress fees (outside submitted work) - Nestlé, Nutricia. A.S.: Lectures/congress fees/consultancy —AbbVie, Takeda, Nutricia, Nestlé. L.G.: no conflict. M.V.: no conflict. I.Z.: no conflict. M.Z.: no conflict. M.B.: lectures/congress fees/consultancy (outside the scope of the submitted work; Abbvie, Takeda, Janssen-Cilag, Celltrion, Roche, AstraZeneca, Biogen, Tillotts, Ferring, Alfasigma, PRO.MED.CS, Sandoz, Bristol-Myers Squibb, Pfizer, and Swedish Orphan Biovitrum). J.B.: lectures/congress fees/consultancy (outside the scope of the submitted work; MSD, AbbVie, Sandoz, Danone-Nutricia, and Nestlé).

ETHICAS APPROVAL AND CONSENT FOR PARTICIPATE

This study was approved by the Ethics Committee (EK - 440/30) and all parents or participants provided informed consent.

ADDITIONAL INFORMATION

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Correspondence and requests for materials should be addressed to Ondrej Hradsky.

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ORIGINAL RESEARCH ARTICLE

Is It Useful to Monitor Thiopurine Metabolites in Pediatric Patients with Crohn's Disease on Combination Therapy? A Multicenter Prospective Observational Study

Kristyna Pospisilova[1] · Jitka Siroka[2] · Eva Karaskova[3] · Ondrej Hradsky[1] · Tereza Lerchova[1] · Kristyna Zarubova[1] · Ivana Copova[1] · Lucie Gonsorcikova[1] · Maria Velganova-Veghova[3] · Irena Francova[4] · Lubor Urbanek[2] · Milos Geryk[3] · Vladimir Mihal[3] · Jiri Bronsky[1]

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Abstract

Background The additional value of azathioprine concomitant treatment on infliximab pharmacokinetics in children is not well described yet.

Aims In the present study, we aimed to describe the relationship between thiopurine metabolite levels, infliximab trough levels, anti-IFX antibody formation, and clinical and laboratory markers of disease activity in pediatric patients with Crohn's disease, and to assess non-adherence.

Methods Data were collected prospectively during repeated visits from pediatric patients followed for Crohn's disease in two Czech pediatric inflammatory bowel disease centers between January 2016 and June 2017. Thiopurine metabolites (6-thioguanine and 6-methylmercaptopurine) were measured by high-performance liquid chromatography. Infliximab trough levels and anti-IFX antibody serum levels were measured routinely by ELISA. The risk of loss of response to infliximab therapy was also assessed.

Results A significant association between infliximab serum levels and 6-thioguanine erythrocyte levels was observed when tested as categorical variables (63 patients, 321 observations). To predict infliximab levels > 5 µg/mL, we propose a 6-thioguanine cutoff of 278 pmol/8 × 10^8 erythrocytes (sensitivity, 0.799; specificity, 0.347). A higher loss-of-response-to-infliximab rate (tested in a subgroup of 51 patients) was observed in patients with undetectable 6-thioguanine levels than in those with detectable levels ($p = 0.026$). Non-adherence to azathioprine therapy was suspected in 20% of patients.

Conclusion Thiopurine metabolite monitoring in pediatric patients with Crohn's disease is useful when optimizing combination therapy. Pediatric patients with undetectable 6-thioguanine levels are more likely to lose response to infliximab therapy. When targeting optimal infliximab levels, the 6-thioguanine cutoff levels in children appear to be higher than in adults.

1 Introduction

In adults, concomitant azathioprine administration during infliximab therapy is associated with lower levels of antibodies against infliximab (anti-IFX) and increased infliximab trough levels, resulting in better clinical outcomes than with infliximab monotherapy [1]. However, after 6 months of combination therapy, the additive effect of long-term

Key Points

We evaluated the association between infliximab and 6-thioguanine (6-TGN) levels in children with Crohn's disease on combination therapy (infliximab and azathioprine).

Patients with undetectable 6-TGN levels are more likely to lose response to infliximab therapy (compared with those with detectable 6-TGN levels).

Thiopurine metabolite monitoring is useful even in combination therapy.

✉ Kristyna Pospisilova
potuznikovakris@gmail.com

Extended author information available on the last page of the article

azathioprine co-administration has been reported to be marginal [2–4].

As 6-thioguanine nucleotides (6-TGN) are active metabolites of azathioprine, they also function as therapy effectors [5]. The simplified azathioprine metabolism is presented in Fig. 1 [6, 7]. Azathioprine in monotherapy has been demonstrated to be more effective when a patient's 6-TGN levels are > 230 pmol/8 × 10^8 red blood cells (RBCs). In combination therapy, 6-TGN levels > 125 pmol/8 × 10^8 RBCs seem to be sufficient to achieve effective trough infliximab levels in adults [8–13]. In children, the dosing of azathioprine in combination therapy may not need to be as high as in monotherapy [13].

Some patients with a *normal* thiopurine *S*-methyltransferase (TPMT) genotype are known to shunt mercaptopurine metabolism in favor of 6-methylmercaptopurine (6-mMP) production (shunters), resulting in higher TPMT activity, low 6-TGN, and high 6-mMP levels [5, 12, 14, 15].

The primary aim of this study was to prospectively observe a group of pediatric patients with Crohn's disease (CD) on combination therapy in order to find suitable 6-TGN cutoff levels in RBCs associated with the optimal serum infliximab trough levels and the absence of anti-IFX. Secondary aims were to investigate azathioprine metabolites as potential predictors of relapse, to reveal non-adherence to azathioprine therapy, to reveal 'shunters', and to evaluate the possible relationship between infliximab, anti-IFX, and 6-TGN levels to clinical and laboratory markers of disease activity.

Fig. 1 Simplified scheme of AZA metabolism. AZA, after its absorption from the gut, is quickly converted into 6-MP. 6-MP is then metabolized producing 6-TGN, 6-mMP and TUA. The proportion of end products varies interindividually. 6-TGN is considered to be the therapy effector, whereas 6-mMP and TUA "only" side products. TPMT plays an important role in the final 6-TGN vs 6-mMP ratio, other factors including other enzymes' activity interfere with the final 6-TGN levels [6, 7]. *6-mMP* 6-methyl mercaptopurine, *6-MP* 6-mercaptopurine, *6-TGN* 6-thioguanine nucleotide, *AZA* azathioprine, *HGPRT* hypoxanthine-guanine phosphoribosyltransferase, *TPMT* thiopurine methyltransferase, *TUA* thiouric acid, *XO* xanthine oxidase

2 Material and Methods

2.1 Ethical Clearance

The protocol for this prospective, longitudinal, multicenter, observational study was approved by the Ethics Committees of the University Hospital Motol and the 2nd Medical Faculty of Charles University in Prague, the Czech Republic. Written informed consent was obtained from all patients and guardians before study enrollment.

2.2 Patients and Medication

All eligible patients (N = 63) who were treated at the University Hospital Motol between January 2016 and June 2017 and Olomouc University Hospital between August 2016 and July 2017 for CD and who fulfilled the inclusion criteria were included. Inclusion criteria to participate in the present study were as follows: previous diagnosis of CD based on Porto criteria or revised Porto criteria [16]; 2–18.9 years old at time of enrollment; treated with combination therapy of infliximab and azathioprine for a minimum of 3 months; and informed consent of a guardian (or of patient if 18 years of age or older). Exclusion criteria were as follows: unavailable laboratory data on infliximab and azathioprine from at least one timepoint of combination therapy; pregnancy; and unwillingness of patient or guardian to continue in the study. Patients were included as soon as they fulfilled the inclusion criteria. Data and blood samples were collected prospectively at repeated visits during the follow-up period. Only observations reporting both thiopurine metabolite levels and infliximab serum levels were used for data analysis (if either of the data were missing, the observation was not considered). Details on recruitment and patient flow are presented in Fig. 2.

Disease activity was defined both clinically (Weighted Pediatric Crohn's Disease Activity Index [wPCDAI]) [17, 18] and through using laboratory markers (C-reactive protein [CRP], leukocyte count, platelet count, erythrocyte sedimentation rate [ESR], and fecal calprotectin [F-CPT]). Optimization of azathioprine dose as well as infliximab dose and/or application interval (shortened when considered necessary) was allowed during the study period (based on the decision of the treating physician). The infliximab dose was escalated with either patient's weight gain or in case of suspected insufficient effect of therapy (based on clinical and/or laboratory signs). Endoscopy was performed in case of the suspicion of loss of response to therapy (LOR, as defined in section 2.2.1). Patients' infliximab and anti-IFX levels were uncovered to the treating physician only when demanded due to unsatisfactory patient status, thus were not the primary treatment target (no proactive therapeutic drug

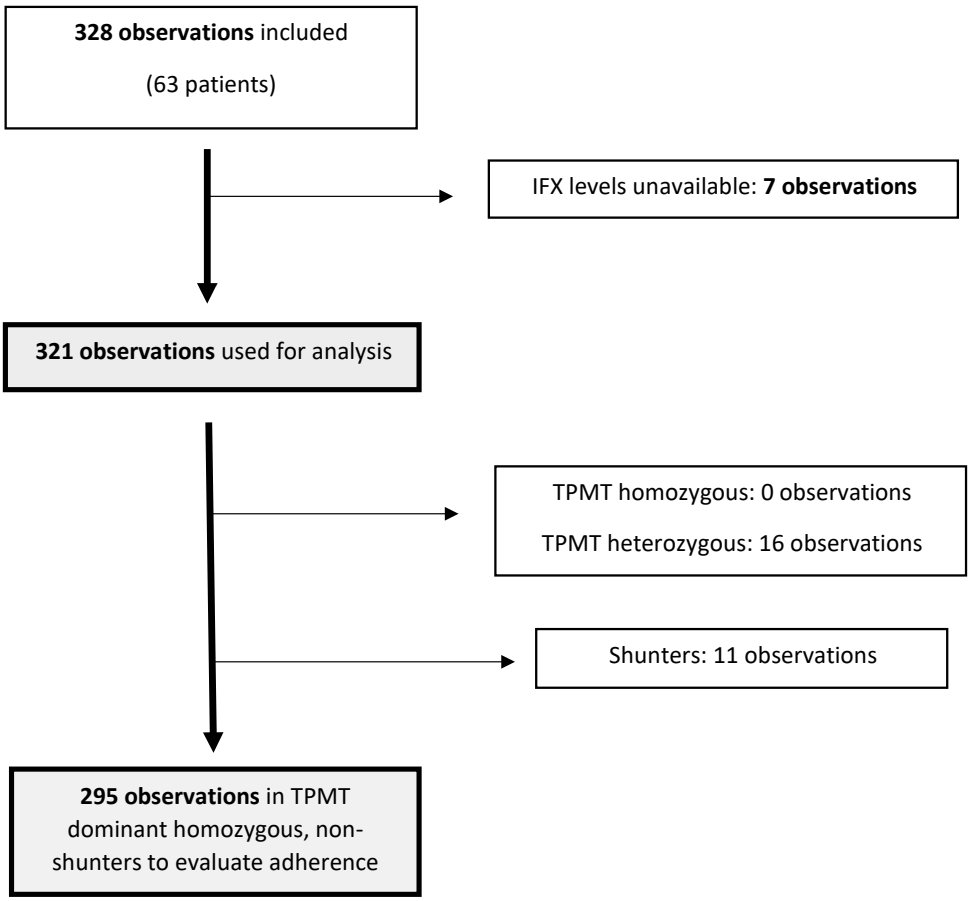

Fig. 2 Recruitment and patient flow. We used 321 observations for data analysis. Evaluation of adherence to AZA therapy was performed on a cluster of 295 observations (patient visits). *AZA* azathioprine, *IFX* infliximab, *TPMT* thiopurine methyltransferase

monitoring was performed). If cessation of infliximab or azathioprine therapy was required, patients were followed up only until the last dose was administered. At 19 years of age, patients were transferred to adult care and were no longer followed up in the present study.

The *TPMT* gene was screened for known polymorphisms. Patients were divided into subgroups according to their *TMPT* genotype. Shunters cannot be spotted by routine screening of the *TPMT* gene, thus patients in our study were considered shunters if the 6-mMP RBC levels were 11 times higher than 6-TGN RBC levels [5]. Patients with TPMT heterozygous genotype and suspected shunters were excluded from the analysis of adherence to thiopurine therapy (Fig. 2).

2.2.1 Survival Study Subpopulation—The Incidence of Loss of Response to Infliximab Therapy Regarding 6-TGN Levels

In patients followed up at University Hospital Motol ($N = 51$), clinical outcome regarding their possible LOR to infliximab therapy was recorded. LOR was defined as the requirement for a major change in therapy (switch or swap to other biologic agents or surgical procedures, such as ileocecal resection) after the ineffectiveness of infliximab was suspected. The decision to change therapy was made by the treating physicians based on clinical, laboratory, and/or endoscopic disease activity. Therefore, LOR was evaluated prospectively. Minor changes in infliximab treatment—dose or interval adjustments—were not classified as LOR. Data obtained from the Motol subpopulation were used to evaluate the LOR by survival analysis (survival study subpopulation).

2.3 Samples and Laboratory Methods

Blood and stool samples and clinical data were prospectively collected at the repeat visits (during which infliximab was administered). The interval between infliximab infusions (and thus visits) ranged from 4 to 8 weeks, based on the decision of the attending physician. Blood samples were taken prior to infliximab administration.

Standard blood tests (blood count parameters—RBC count, leukocyte count and differential, thrombocyte count, hemoglobin; ESR; serum parameters—CRP, lipase, γ-glutamyl transferase, alanine-aminotransferase, aspartate aminotransferase, albumin) were performed routinely. Serum

infliximab trough levels and anti-IFX levels were assessed using the infliximab ELISA test (TANI Medical, Ankara, Turkey). Anti-IFX levels above 5 AU/mL were considered positive. If a stool sample was available, F-CPT was determined using the EliA Calprotectin 2 test (Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden).

2.4 Measurement of Thiopurine Metabolites

Whole blood was collected from each patient into ethylenediaminetetraacetic acid (EDTA) tubes, and hematocrit and RBC counts were subsequently determined. Immediately after sampling, the blood was centrifuged, plasma and leukocytes were removed, and RBCs were washed twice. RBCs were then diluted (1:1) with an equal volume of water and frozen at −20 °C until determination of thiopurine metabolites. The sample preparation protocol was adopted from Dervieux and Boulieu [19–21]. Samples were analyzed by Agilent 1100 high-performance liquid chromatography equipped with a multi-wavelength detector.

2.5 Data Analyses

Collected data were analyzed using R statistical software (version 3.5.2, https://cran.r-project.org/bin/windows/base/old/3.5.2/). For repeated measurements, linear mixed model (LMM) and generalized linear mix model (GLMM) analyses were performed using packages *lme4* and *lmerTest*. If not mentioned otherwise, all included results with all included patients were used for analysis.

The TPMT *normal* activity subgroup (patients with two wild-type alleles in the *TPMT* gene according to routine *TPMT* gene screening), was tested separately when assessing TPMT activity and association with metabolite levels. To assess changes in blood count parameters (leukocyte, neutrophil, lymphocyte, and platelet counts), LMMs were performed using 6-TGN RBC levels, infliximab trough levels, CRP, sex, and age as predictors. 6-TGN RBC levels or azathioprine daily dose adjusted to body weight (BW), serum albumin, CRP, wPCDAI, and BW-adjusted infliximab dose and calculated to 8-week dose together with sex, age, and time on infliximab therapy were chosen as predictors for models assessing the association with infliximab and anti-IFX levels. We chose those parameters to primarily evaluate the assumed association between 6-TGN RBC levels and infliximab or anti-IFX levels with potential predictors.

6-TGN cutoff values of 125 pmol/8 × 10^8 RBCs and 230 pmol/8 × 10^8 RBCs were chosen and tested separately in each model using 6-TGN as a categorical variable. After consideration of the selected parameters and sensitivity of the chosen laboratory method, levels of 6-TGN under 60 pmol/8 × 10^8 RBCs were considered *undetectable*. For the adherence assessment, *undetectable* assessments were assigned only to those samples in which both 6-TGN and 6-mMP levels were lower than the detection limit of the method (the 6-mMP detection limit was 500 pmol/8 × 10^8 RBCs). 6-TGN levels above 430–450 pmol/8 × 10^8 RBCs were considered toxic with respect to risk of leukopenia and 6-mMP levels above 5700 pmol/8 × 10^8 RBCs were considered relevant with respect to hepatopathy [12, 22].

When considering disease activity markers as categorical variables, remission was defined as CRP < 5 mg/L, ESR < 20 mm/h, F-CPT <100 µg/g, or wPCDAI < 12.5 points (tested separately if not mentioned otherwise). For the purposes of using infliximab as categorical variables, 3 µg/mL and 5 µg/mL (preferred) cutoffs were used [23–26].

To determine the optimum cutoff values for infliximab trough levels regarding CRP-based remission (CRP <5 mg/L), respective receiver operating characteristic (ROC) curves were plotted (package *pROC*). For repeated measurements (one patient—potentially multiple observations), we calculated the area under the curve using cross-validated area under the ROC curve estimates for pooled repeated measures data (R package "cvAUC").

Survival analysis (presented as a Kaplan-Meier plot) was performed to assess the risk of LOR according to 6-TGN levels (using package *survplot*). Hazard ratio (HR) was also calculated for each of the survival analysis subgroups separately.

All plots were constructed using the R package *ggplot2*.

3 Results

3.1 Study Population

In total, 63 patients were included in the present study (19% females, aged 6.7–18.8 years, 3 patients TPMT heterozygous; Table 1), comprising laboratory measurements from 328 visits. Seven visits were excluded from analysis due to the unavailability of infliximab data; thus, 321 observations were used in the final analyses (Fig. 2). While azathioprine is usually introduced prior to infliximab therapy, in three patients (totaling 16 observations), azathioprine therapy was added after infliximab therapy was initiated. Laboratory results (medians and interquartile ranges) are shown in Table 1. F-CPT was available only in 231 observations.

3.2 Primary Outcome: Optimal 6-TGN Cutoff

An association between serum infliximab levels and 6-TGN RBCs levels was observed when 6-TGN levels were analyzed as categorical variables using a previously suggested cutoff of 230 pmol/8 × 10^8 (LMM, adjusted model; $N = 321$; 95% CI 0.036–3.149; $p = 0.047$) but not when the 6-TGN

Table 1 Characteristics of the study population

	At enrolment/first visit ($N = 63$)	Overall visits ($N = 321$)	Last visit ($N = 63$)
Median number of visits per one patient (range)		5 (1–12)	
Motol/Olomouc hospital	51/12	243/78	51/12
Female gender (%)	19 (33.8)	87 (27.1)	19 (33.8)
Median age (range), y	15.6 (6.7–18.8)	15.6 (6.7–19.0)	16.6 (7.1–19.0)
Median time with IBD (range), mo	28 (5–97)	34 (5–114)	39 (5–114)
Median time on AZA (range), mo	25 (5–97)	32 (5–114)	38 (5–114)
Median time on IFX (range), mo	14 (3–73)	21 (3–84)	26 (3–84)
Receiving mesalamine (%)	6 (9.7)	41 (7.8)	5 (7.9)
History of ileocecal resection (%)	12 (19.3)	64 (19.9)	13 (20.6)
Frequency of TPMT heterozygosity (%)	3 (4.8)	16 (4.9)	3 (4.8)
TPMT heterozygous			
Median AZA dose (range), mg/kg/d	0.8 (0.5–1.2)	0.7 (0.5–1.2)	0.7 (0.5–1.1)
TPMT dominant homozygous			
Median AZA dose (range), mg/kg/d	1.7 (0.4–2.6)	1.6 (0.4–2.6)	1.6 (0.4–2.5)
Median IFX dose (IQR), mg/kg	5.4 (4.9–6.3)	5.4 (4.9–6.3)	5.6 (5.0–5.9)
Median IFX administration interval (IQR), wk	8 (7–8)	8 (6–8)	8 (6–8)
Median IFX dose adjusted to administration interval (IQR), mg/kg/8w	5.6 (4.9–6.7)	5.9 (5.1–7.9)	6.1 (5.3–8.0)

Sixty-three patients were included, from which 321 observations were used for analysis. TPMT heterozygous were rare among included patients

AZA azathioprine, *IBD* inflammatory bowel disease, *IFX* infliximab, *IQR* interquartile range, *TPMT* thiopurine methyltransferase

cutoff of 125 pmol/8 × 10^8 was used (LMM, adjusted model; $N = 321$; 95% CI − 0.161 to 3.402; $p = 0.085$). Using ROC curves, the optimum 6-TGN cutoff for predicting infliximab >5 µg/mL was 278 pmol/8 × 10^8 RBCs (cross-validated ROC; $N = 321$; AUC 0.533; 95% CI 0.469–0.596; sensitivity 0.799, specificity 0.347) (Fig. 3a). For infliximab >3 µg/mL, ROC curves revealed a promising 6-TGN cutoff of 116 pmol/8 × 10^8 RBCs (cross-validated ROC; $N = 321$; AUC 0.535; 95% CI 0.465–0.605; sensitivity 0.819, specificity 0.342) (Fig. 3b). No significant association was observed between anti-IFX antibody levels and 6-TGN RBCs levels, even when previously proposed 6-TGN cutoffs (230 and 125 pmol/8 × 10^8 RBCs) were tested.

3.3 Secondary Outcomes

3.3.1 Loss of Response to Infliximab Therapy

In the survival analysis subgroup ($N = 53$), seven patients experienced relapse during their follow-up in the present study and needed a major change in the therapy (discontinuation of infliximab resulting in switch or swap or surgical procedure) and thus were marked as LOR. Patients were divided into two subgroups regarding 6-TGN levels—patients with 6-TGN RBC levels under the detection limit (*undetectable* levels) and those with *detectable* levels. A significantly higher relapse rate was observed in the subgroup of patients with *undetectable* ($N = 12$, HR 4.71; 95% CI 1.05–21.11) 6-TGN levels, than in patients with *detectable* ($N = 39$, HR 0.212; 95% CI 0.047–0.951) 6-TGN RBC levels (survival analysis; $N = 51$; *undetectable* vs *detectable*; $p = 0.026$). However, for 6-TGN cutoffs of 230 or 125 pmol/8 × 10^8 RBCs, the difference was not significant. The Kaplan–Meier curve and the associated risk table are shown in Fig. 4. Characteristics of this study subgroup are shown in Table 2.

3.3.2 Infliximab Levels and Antibody Formation

No association was found between serum infliximab levels and 6-TGN levels in RBCs (both tested as continuous variables), not even when adjusted for variables selected according to their clinical relevance (infliximab levels adjusted to 8-week intervals, anti-IFX levels, albumin serum levels, age at sample collection, and duration of therapy). Infliximab trough levels were positively associated with patient age at the time of sample collection (LMM; $N = 321$; 95% CI 0.233–0.971; $p = 0.003$), but not with the duration of combination therapy. No significant association was observed between 6-TGN and infliximab trough levels when a subgroup of patients with an 8-week interval of infliximab administration was tested alone (LMM, adjusted model; $N = 192$; 95% CI − 0.0007 to 0.0113; $p = 0.096$). Detailed results are presented in Table 3.

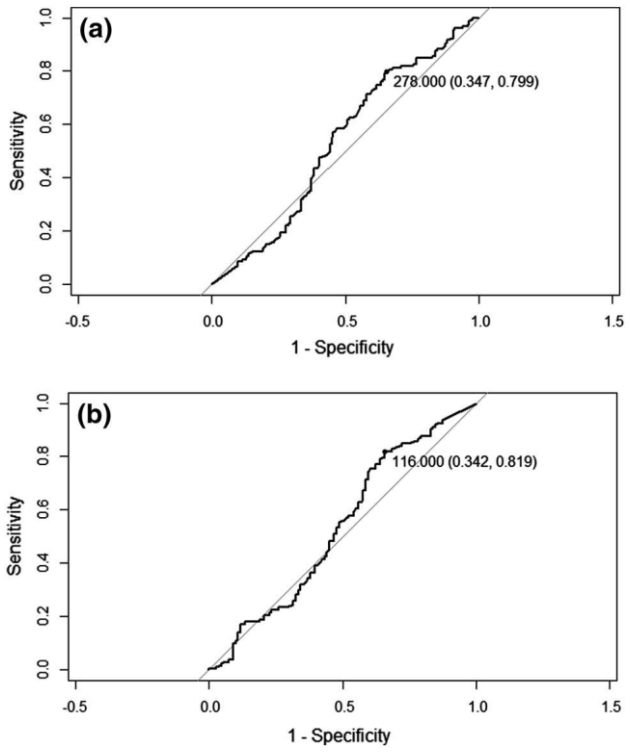

Fig. 3 ROC curves suggesting the best 6-TGN cutoff predicting IFX >5 µg/mL (**a**) and 3 µg/mL (**b**). IFX trough levels 5 µg/mL or higher were previously reported to be optimal for the treatment of inflammatory bowel diseases [23–26]. According to our data analysis, when predicting IFX >5 µg/mL, the best 6-TGN cutoff seemed to be (cross-validated ROC analysis) 278 pmol/8 × 10^8 RBCs. *6-TGN* 6-thioguanine nucleotide, *IFX* infliximab, *RBCs* red blood cells, *ROC* receiver operating characteristic

Positive anti-IFX levels (>5 AU/mL) were detected in seven observations.

3.3.3 Adherence, Thiopurine Metabolite Levels, and Azathioprine-Related Adverse Events

The median levels of thiopurine metabolites, infliximab, and anti-IFX are reported in Table 4 (no TPMT recessive homozygous cases were included). We suspected shunters in 11 observations, which were excluded from the adherence analysis. Thus, adherence to thiopurine therapy was studied in a subgroup of patients from which patients with TPMT heterozygous genotype and suspected shunters were excluded (Fig. 2, N = 295 observations). 6-TGN levels <125 pmol/8 × 10^8 RBCs were detected in 76 out of 295 (26%) visits. In 17 out of 76 low 6-TGN observations (6% of total number), azathioprine daily doses < 1 mg/kg were prescribed, resulting in suspected underdosing. In the remaining 59 observations (20% of total number), non-adherence to therapy was considered probable.

A significant association between prescribed azathioprine daily dose adjusted for BW and 6-TGN was recorded in TPMT-dominant homozygous patients (LMM; N = 305; 95% CI 67.09–159.19; p < 0.001). 6-mMP levels were found to be strongly positively correlated with 6-TGN levels in TPMT dominant homozygous patients (LMM; N = 305; 95% CI 2.970–4.439; p < 0.001), and to BW-adjusted azathioprine daily dose (LMM; N = 305; 95% CI 224.4–853.7; p = 0.001). Six patients (comprising 44 visits) received oral mesalamine, but no significant association between mesalamine administration and 6-TGN or 6-mMP levels was observed. No azathioprine-induced adverse events were recorded.

6-mMP levels > 5700 pmol/8 × 10^8 RBCs were detected during only two visits (comprising one patient); normal liver transaminase (AST, ALT, or GGT) levels were observed in both instances. No cases of severe cytopenia were reported. However, a negative association between 6-TGN RBC levels and lymphocyte count was observed (LMM; N = 321; 95% CI − 0.0013 to − 0.0006; p < 0.001). In addition, the absolute neutrophil count was negatively associated with infliximab serum levels (LMM; N = 321; 95% CI − 0.061 to − 0.016; p = 0.001). However, absolute neutrophil count was not associated with the RBC levels of 6-TGN or azathioprine daily dose adjusted to BW.

3.3.4 Disease Activity Markers and Association with Thiopurine Metabolites and Infliximab Trough Levels

No association between CRP levels and 6-TGN levels was observed. However, there was a trend observed in association between CRP levels and infliximab levels, but not reaching statistical significance (LMM; N = 320; 95% CI − 0.216 to 0.015; p = 0.09). When CRP was tested as a categorical variable (less or higher than 5 mg/mL), the significance of the association with infliximab levels slightly increased (LMM; N = 320; 95% CI − 0.00007 to 0.015; p = 0.05). The significance increased even more when both CRP and infliximab levels (cutoff 5 µg/mL) were tested as categorical variables (LMM; N = 320; 95% CI 0.032–0.168; p = 0.004). No association was observed between other clinical (wPCDAI) or laboratory (ESR or F-CPT) markers and either infliximab or 6-TGN levels.

4 Discussion

4.1 Optimal 6-TGN Cutoff and Loss of Response to Infliximab Therapy

The optimal 6-TGN cutoff in pediatric patients concomitantly treated with biological agents (e.g., infliximab) for CD remains unknown. To simplify this evaluation for clinicians, we have attempted to determine a 6-TGN cutoff level

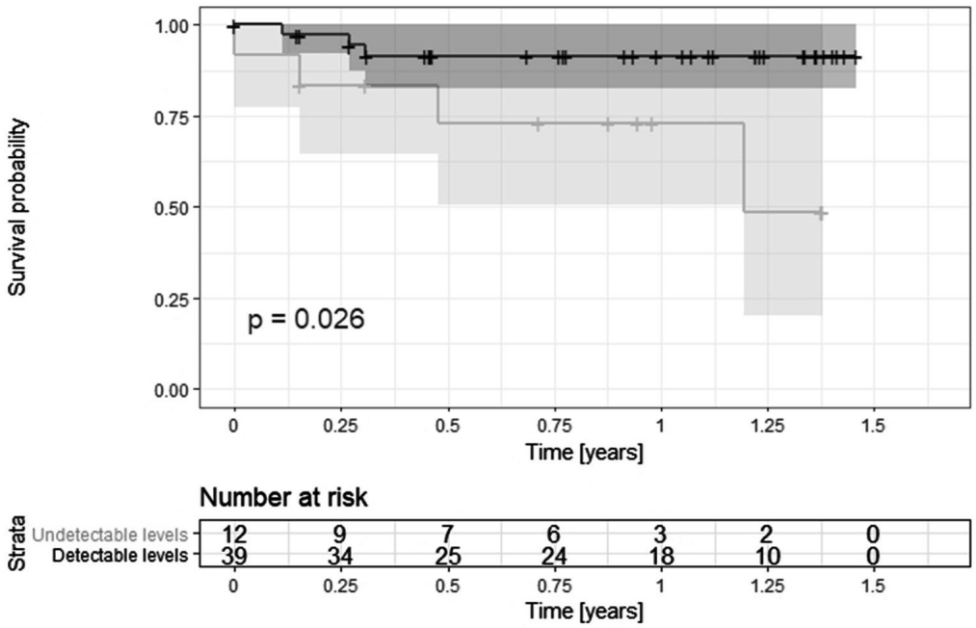

Table 2 Characteristics of the survival study subpopulation ($N = 51$)

Female gender (%)	19 (37.3)
Median age on first visit (range), y	15.8 (6.7–18.8)
Median age on last visit (range), y	16.6 (7.1–19.0)
Median length of follow-up (range), m	11 (0–18)
Overall LOR count during follow-up (%)	7
Median 6-TGN RBC levels on last visit (IQR), pmol/8 × 10^8 RBCs	160 (100–224)
Non-LOR ($N = 44$)	166 (115–231)
LOR ($N = 7$)	0 (0–149)[a]
Median IFX trough levels on last visit (IQR), µg/mL	6.2 (3.8–8.6)
Non-LOR ($N = 44$)	6.4 (4.5–9.5)
LOR ($N = 7$)	4.1 (3.0–8.2)

Data obtained from patients followed up at the University Hospital Motol were used to evaluate LOR. For this purpose, LOR was defined as the requirement for a major change in therapy based on the decision of the treating physician. In the subgroup of patients with 6-TGN levels under the detection limit (*undetectable* levels), a significantly higher relapse rate was observed compared with those with *detectable* 6-TGN RBC levels (survival analysis; $N = 51$; $p = 0.026$). The Kaplan–Meier curve and the associated risk table are presented in Fig. 3

6-TGN 6-thioguaninenucleotide, *IFX* infliximab, *LOR* loss of response to therapy, *RBC* red blood cell

[a]Levels under the detection limit of the method are considered as undetectable or 'zero' ('0')

predicting higher infliximab levels (and thus, as expected, better outcomes).

In previous studies, trough levels of infliximab > 5 µg/mL were reported to be optimal for the treatment of inflammatory bowel diseases [23–26]. In adult patients, effective azathioprine doses appear to be lower in combination therapy than in monotherapy [27, 28]. Furthermore, effective levels of 6-TGN may also be lower in combination therapy [8, 9]. To demonstrate this hypothesis in the pediatric population, we first set and tested a 6-TGN cutoff of 125 pmol/8 × 10^8 RBCs. In our study, the potential association between infliximab levels and 6-TGN observed for this cutoff did not reach statistical significance. However, the significance increased slightly with a 6-TGN cutoff of 230 pmol/8 × 10^8 RBCs. Based on a ROC analysis to predict infliximab levels >5 µg/mL, the best 6-TGN cutoff was 278 pmol/8 × 10^8 RBCs. It is unlikely that this phenomenon is mediated through the formation of anti-IFX as in our cohort the positivity of anti-IFX was rare. Moreover, this is the first study proposing a 6-TGN cutoff for children on combination therapy for CD, and even in adults the data are scarce—the 125-pmol/8 × 10^8 RBCs 6-TGN cutoff has been demonstrated only in one cross-sectional study [9]. The reason why effective 6-TGN levels on combination therapy seem to be higher in the pediatric population than in adults is unclear and needs further

Table 3 Association of IFX trough levels and multiple study variables according to LMM ($N = 321$)

	Estimate (beta)	95% CI		*p* value
↑ 6-TGN levels (as cont. variable)	0.003	− 0.002	0.008	0.29
↓ Anti-IFX levels	− 0.204	− 0.381	− 0.029	**0.029***
↑ IFX dose adjusted to 8-w interval	0.962	0.659	1.254	**< 0.001***
↑ Albumin serum level	0.269	− 0.051	0.488	**0.016***
↑ Age at visit (y)	0.603	0.233	0.971	**0.003***
↑ Time on IFX (m)	0.031	− 0.026	0.087	0.304

No association between 6-TGN and IFX levels was observed when both were tested as continuous variables. IFX trough levels were found to be positively associated with patient age at the time of sample collection, but not with the duration of combination therapy

Bold font indicates statistical significance

6-TGN 6-thioguanine, *IFX* infliximab, *LMM* linear mixed model, *m* months, *w* weeks, *y* years

Table 4 Laboratory results and wPCDAI collected at enrollment, during repetitive visits and on the last visit with respect to administered medication, its effect and possible adverse events and disease activity

	At enrolment/first visit ($N = 63$)	Overall visits ($N = 321$)	Last visit ($N = 63$)
Median 6-TGN levels (IQR), pmol/8 × 10^8 RBCs	229 (138–296)	204 (124–284)	165 (85–224)
6-TGN levels above 125 pmol/8 × 10^8 RBCs (%)	48 (76)	240 (75)	41 (65)
6-TGN levels above 250 pmol/8 × 10^8 RBCs (%)	21 (33)	134 (42)	12 (19)
Median 6-mMP levels (IQR), pmol/8 × 10^8 RBCs	385 (159–814)	357 (139–761)	215 (0–465)
Median IFX serum levels (IQR), µg/mL	4.0 (2.1–6.8)	4.7 (2.4–8.0)	5.6 (2.8–8.5)
Median anti-IFX serum levels (IQR), AU/mL	1.0 (0.9–1.1)	1.0 (0.9–1.1)	1 (0.8–1.1)
Median wPCDAI (IQR)	2.5 (0–5)	0 (0–5)	0 (0–5)
Laboratory results			
Median serum albumin level (IQR), g/L	44.8 (42.7–47.4)	44.8 (43.2–46.6)	45.0 (43.4–46.7)
Median serum CRP level (IQR), mg/L	0.7 (0.5–2.0)	0.7 (0.5–2-4)	0.7 (0.5–2.2)
Median hemoglobin level (IQR), g/L	135 (125–149)	137 (128–146)	137 (127–146)
Median WBC count (IQR), × 10^9/L	6.2 (5.3–7.2)	5.9 (5.1–7.0)	5.9 (5.1–6.9)
Median platelet count (IQR), × 10^{12}/L	339 (279–370)	321 (272–363)	301 (263–349)
Median neutrophil count (IQR), × 10^9/L	3.3 (2.6–4.4)	3.3 (2.7–4.1)	3.3 (2.7–4.0)
Median lymphocyte count (IQR), × 10^9/L	1.6 (1.4–2.1)	1.6 (1.4–2.0)	1.7 (1.4–2.0)
Median ESR (IQR), mm/h	20 (9–34)	18 (9–34)	10 (4–15)
Median F-CPT level (IQR), g/kg	203 (42–716) $N = 44$	189 (50–705) $N = 226$	182 (48–691) $N = 40$

6-TGN levels <125 pmol/8 × 10^8 RBCs were detected in 25% observations. The observed median IFX serum level was 4.7 µg/mL (overall visits). No leukopenia, thrombopenia or anemia (as potential adverse events) were spotted

6-mMP 6-methylmercaptopurine, *6-TGN* 6-thioguaninenucleotide, *anti-IFX* anti-infliximab antibodies, *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate, *F-CPT* fecal calprotectin, *IQR* interquartile range, *RBCs* red blood cells, *WBC* white blood cell, *wPCDAI* weighted Pediatric Crohn's disease activity index

research, going deeper into the pathophysiology of azathioprine metabolism and pharmacokinetics.

Our results did not confirm the previous findings reporting lower optimal levels for combination therapy, when sufficient or higher levels of infliximab were considered the therapy target. However, it is questionable whether we should aim for cutoffs for pharmacokinetics only or base our decisions on clinical outcomes. In clinical practice, predicting LOR might be of even greater importance than predicting 'optimal' infliximab levels alone. According to our survival analysis, 6-TGN levels <60 pmol/8 × 10^8 RBCs (*undetectable*) were found to be positively associated with higher LOR rates in the study cohort. Thus, according to our data, when considering the clinical outcome, target 6-TGN levels appear to be much lower than those previously reported in adults. When we look closer at the results, we can easily conclude that those patients with *undetectable* 6-TGN levels mimic those treated with infliximab in

monotherapy who do not receive azathioprine (or any other immunomodulator) at all (anymore). In our study, LOR is described as a need for major change in therapy (switch or swap of infliximab; thus, infliximab discontinuation or the need for surgery). It has been previously published that children with CD are more likely to remain on infliximab when receiving an immunomodulator (such as azathioprine or others) concomitantly [29, 30]. Thus, it is not surprising that patients with *undetectable* 6-TGN levels are more prone to LOR leading to infliximab discontinuation.

A model for predicting low 6-TGN levels has recently been published. Thus, even clinicians who do not have access to routine thiopurine metabolite measurements may benefit from considering 6-TGN levels as categorical variables [31].

An association was observed between infliximab and CRP only when they were both tested as categorical variables, but no association was revealed between CRP and 6-TGN. We did not prove an association between other disease activity markers (wPCDAI, F-CPT, and ESR) and either infliximab levels or 6-TGN levels. Thus, this may suggest a potential direct effect of azathioprine that is not mediated via infliximab pharmacokinetics. However, the design of our study was not appropriate to evaluate these associations as the majority of patients were in stable remission during the study—there were basically no patients with wPCDAI >12.5 points included in our study, and median CRP was quite low (0.7 mg/L) as well. Some F-CPT results were missing, which made the chance to detect a significant association with infliximab and/or 6-TGN levels even smaller. A larger and possibly more heterogenic study cohort would be needed to confirm (or refute) our observations.

4.2 Infliximab Levels and Antibody Formation

We revealed an association between infliximab levels and patient age (at the time of sample collection), but not with the duration of therapy. These results are in accordance with previously published data suggesting that intensified infliximab dosing is required in younger children to achieve adequate trough levels [32, 33]. At the time of our study, we did not have a proactive strategy in infliximab therapeutic drug monitoring; thus, some of our younger patients may have only achieved lower infliximab levels after standard dosing based on clinical decisions.

The association of infliximab trough levels with infliximab dose adjusted to an 8-week interval is not surprising as it was previously shown that both dosage and interval of infliximab are highly associated with infliximab pharmacokinetics [33–35]. The observed association of infliximab trough levels with albumin serum levels has also been previously well described [35–37].

An advantage of combination therapy, in comparison with infliximab monotherapy, is the added protection against anti-IFX production [1, 38], although this is thought to depend on 6-TGN levels [9]. In contrast to what has been published, we did not find an association between 6-TGN levels and anti-IFX levels. Our results might be (at least partially) biased by the fact that only a few patients developed high anti-IFX levels during follow-up. To confirm (or refute) this hypothesis, a larger pediatric study cohort is required.

4.3 Adherence, Thiopurine Metabolite Levels, and Azathioprine-Related Adverse Events

We suspected nonadherence in 20% of observations, which is lower than what has been previously reported [39]. Despite the supposedly nonadherent patients included in our study, a significant correlation between prescribed azathioprine daily dose adjusted to BW and 6-TGN levels was observed. When azathioprine treatment is initiated, it takes about 55 days to reach stable thiopurine metabolite concentrations in RBCs [40]. Thus, it is highly probable that the amount of time required for the concentration of thiopurine metabolites in RBCs to reach zero (or undetectable levels) in non-adherent patients is not insignificant. We assume that infrequent skipping of azathioprine doses is difficult to observe from an assessment of 6-TGN and 6-mMP levels. It is likely to be even more difficult when the 6-TGN nonadherence cutoff is set to a low value due to the relatively low recommended azathioprine daily dose (1–1.5 mg/kg) used in combination therapy [27]. More- over, some of our patients are considered ‘underdosed’ with azathioprine daily doses < 1 mg/kg because in our center, we do not usually increase the dose of azathio- prine following weight gain in young patients receiving combination therapy if the patient is in clinical and laboratory remission. This effectively complicates the meaning of nonadherence in a subgroup of patients with low doses. Although it is likely that not all of the nonadherent patients were identified, thiopurine metabolite monitoring still seems to be useful in evaluating adherence to thiopurine therapy.

Due to the small number of patients with TPMT heterozygous genotype included in our study, we were not able to study this subgroup separately. The percentage of TPMT heterozygous patients included in our study did not correspond to what would have been expected from a consideration of the whole population [6]. We may have lost to our study some of the potentially suitable TPMT heterozygous patients due to azathioprine intolerance (before the study started). Thus, azathioprine side effects may have led to azathioprine discontinuation months or even years before the study started. In addition, we revealed five supposed shunters (11 observations; Fig. 2). If the present

therapy had proved ineffective in these patients, adding allopurinol into the therapy may have made their treatment more efficient [13, 14, 41–43].

We did not observe any azathioprine-related adverse events. This was probably (at least partially) because all our patients had been on combination therapy for > 3 months before inclusion in the study. Thus, because idiosyncratic adverse events are more likely to occur at the beginning of therapy (and may potentially be respon- sible for azathioprine discontinuation), patients experienc- ing azathioprine-related adverse events were likely not included in the study simply because they stopped taking azathioprine before fulfilling the 3-months-or-more crite- ria. Moreover, dose-dependent adverse events were less likely to be observed because of the relatively low dose of azathioprine administered (as previously discussed).

4.4 Limitations and Strengths of the Study

Some observations had to be excluded because of missing data. Patients were included after different periods of time on combination therapy and this may have affected the out- comes. Finally, some subgroups were under-represented (e.g., shunters, TPMT intermediate metabolizers, 5-ASA co-administrated, history of CD-related surgery).

The main advantages of our study include the compre- hensive multicentric cluster of patients, the prospective design, and the use of well-known reliable methodolo- gies for thiopurine metabolite measurements. Because of the great deal of data collected, we were able to study many aspects of combination therapy in children, includ- ing predictors.

5 Conclusions

As undetectable levels of 6-TGN seem to be associated with loss of response on combination therapy, thiopurine metabo- lite monitoring could be useful when optimizing combina- tion therapy and assessing treatment adherence in clinical practice.

Declarations

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Disclosure of potential conflicts of interest Pospisilova K: lectures/ congress fees/consultancy (outside submitted work)—MSD, Nutri- cia, Nestlé and Mead Johnsons; Karaskova E: lectures/congress fees/ consultancy (outside submitted work)—MSD, AbbVie, Nutricia, and Nestlé; Hradsky O: lectures/congress fees/consultancy (outside sub- mitted work)—MSD, AbbVie, Nutricia, Nestlé, Ferring, and Falk; Lerchova T: lectures/congress fees/consultancy (outside submitted work)—Nutricia, Ferring and Biocodex; Zarubova K: lectures/con- gress fees/consultancy (outside submitted work)—Nutricia and Nestlé; Velganova-Veghova M: congress fees/consultancy (outside submitted work)—MSD, AbbVie, Nutricia, and Nestlé; Bronsky J: lectures/ congress fees/consultancy (outside submitted work)—MSD, AbbVie, Nutricia, Nestlé, Ferring, Biocodex, and Walmark; Gonsorcikova L, Francova I, Geryk M, Copova I, Mihal V, Siroka J, and Urbanek L report no conflicts of interest.

Ethics approval The protocol for the observational study was approved by the Ethics Committees of the University Hospital Motol and the 2nd Medical Faculty of Charles University in Prague.

Informed consent Written informed consent was obtained from legal guardians before study enrollment.

Consent for publication Non applicable.

Study registration The study has been registered retrospectively in the ENCePP registry (encepp.eu), it can be found under the registration number EUPAS38918. Registered on 12 January 2021.

Data availability The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Code availability The code is available from the corresponding author on reasonable request.

Author contributions PK: study design, literature search, data col- lection, patient recruitment, thiopurine metabolite measurement, data analysis, manuscript writing; SJ: data collection, thiopurine metabolite measurement and laboratory supervision, manuscript critical revision; KE: data collection, patient recruitment, manuscript critical revision; HO: study design, data analysis and interpretation, manuscript criti- cal revision; LT: study design, data collection, patient recruitment, manuscript critical revision, ZK: data collection, patient recruitment, manuscript critical revision; CI: data collection, patient recruitment, manuscript critical revision; GL: data collection, patient recruitment, manuscript critical revision; V-VM: data collection, patient recruit- ment, manuscript critical revision; FI: data collection, infliximab levels and antibodies measurement supervision, manuscript critical revision; UL: thiopurine metabolite measurement—method optimization, data collection, manuscript critical revision; GM: data collection, patient recruitment, manuscript critical revision; Mihal V: data collection, supervision, manuscript critical revision; BJ: study design and super- vision, literature search, manuscript writing. All authors read and approved the final manuscript.

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Authors and Affiliations

Kristyna Pospisilova[1] · Jitka Siroka[2] · Eva Karaskova[3] · Ondrej Hradsky[1] · Tereza Lerchova[1] · Kristyna Zarubova[1] · Ivana Copova[1] · Lucie Gonsorcikova[1] · Maria Velganova-Veghova[3] · Irena Francova[4] · Lubor Urbanek[2] · Milos Geryk[3] · Vladimir Mihal[3] · Jiri Bronsky[1]

Jitka Siroka
sirokajitka@gmail.com

Eva Karaskova
e.karasko@seznam.cz

Ondrej Hradsky
ondrej.hradsky@lfmotol.cuni.cz

Tereza Lerchova
terezadrskova@gmail.com

Kristyna Zarubova
zarubova.kristyna@gmail.com

Ivana Copova
icopova.ic@gmail.com

Lucie Gonsorcikova
Lucie.Gonsorcikova@fnmotol.cz

Maria Velganova-Veghova
mariavelganova@post.cz

Irena Francova
Irena.Francova@vfn.cz

Lubor Urbanek
urbanek.l@centrum.cz

Milos Geryk
milos.geryk@gmail.com

Vladimir Mihal
vladimir.mihal@fnol.cz

Jiri Bronsky
jiri.bronsky@lfmotol.cuni.cz

[1] Department of Pediatrics, 2nd Faculty of Medicine, Charles University in Prague and Motol University Hospital, V Uvalu 84, 150 06 Prague 5, Czech Republic

[2] Laboratory of Growth Regulators, Palacky University Olomouc and Institute of Experimental Botany AS CR, Slechtitelu 27, 783 71 Olomouc, Czech Republic

[3] Department of Pediatrics, Faculty of Medicine and Dentistry, Palacky University and University Hospital, I.P. Pavlova 185/6, 779 00 Olomouc, Czech Republic

[4] Institute of Medical Biochemistry and Laboratory Diagnosis, General University Hospital and 1st Faculty of Medicine, Charles University in Prague, Na Bojisti 3, Prague 2, 121 08 Prague, Czech Republic

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ORIGINAL ARTICLE

Time to Relapse in Children with Crohn's Disease Treated with Azathioprine and Nutritional Therapy or Corticosteroids

Ondrej Hradsky[1] · Ivana Copova[1] · Kristyna Zarubova[1] · Jiri Nevoral[1] · Jiri Bronsky[1]

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Abstract
Background The duration of remission has been shown to be longer in patients initially treated with exclusive enteral nutrition (EEN) compared to corticosteroids (CS). However, no published studies required concurrent immunomodulator [6-mercaptopurine or azathioprine (AZA)] use at the time of diagnosis.
Aims The aims of this retrospective study were to compare the duration of remission between patients initially treated with AZA in combination with CS or EEN and identify predictors of early relapse in these patients.
Methods Data from 65 newly diagnosed children with CD in clinical remission on either EEN or CS and commencing AZA at diagnosis were included. We compared duration of remission using physician global assessment and carried out Cox regression analysis to identify predictors of early relapse. Patients were followed up to the time of first relapse or for at least 12 months.
Results There were no differences in the duration of remission between patients initially treated with EEN or CS (p = 0.978). We identified younger age at diagnosis [hazard ratio (HR) 0.87, 95 CI 0.78–0.98, p = 0.016], lower height Z score at diagnosis (HR 0.61, 95 CI 0.44–0.85, p = 0.003), involvement of the upper gastrointestinal tract (HR 2.69, 95 CI 1.27–5.66, p = 0.009), and elevated platelet count at remission (HR 1.004, 95 CI 1.001–1.008, p = 0.021) as independent predictors of early relapse.
Conclusions Neither induction regime demonstrated longer duration of remission of CD in patients treated with immunomodulators since the time of diagnosis.

Keywords Exclusive enteral nutrition · Corticosteroids · Crohn's disease · Follow-up · Immunosuppressive therapy, azathioprine

Introduction

Exclusive enteral nutrition (EEN) has been shown to be as effective as systemic corticosteroid (CS) therapy at inducing remission in children with Crohn's disease (CD) [1–5]. Previous studies have demonstrated short-term EEN efficacy with better side effect profile [6] improved growth velocity, weight gain, lean body mass, and bone health [6–13]. On the basis of such evidence demonstrating efficacy and other beneficial effects, EEN is considered a first-line induction therapy in pediatric CD [14].

Long-term effects of EEN are not as well studied. Recently, the work of Cameron et al. [15] suggested that EEN may improve weight and BMI Z scores, but not height Z scores in agreement with previously published data [16]. To date, only three published studies have compared the long-term outcome of induction strategies of EEN and CS in newly diagnosed children with CD [17–19]. In two of these, longer duration of remission was observed in patients initially treated with EEN [17, 19]. However,

& Ondrej Hradsky
ondrej.hradsky@lfmotol.cuni.cz

Ivana Copova
icopova.ic@gmail.com

Kristyna Zarubova
kristyna.zarubova@seznam.cz

Jiri Bronsky
jiri.bronsky@gmail.com

[1] Department of Pediatrics, University Hospital Motol and Second Faculty of Medicine, Charles University in Prague, V Uvalu 84, 150 06 Prague 5, Czech Republic

concomitant immunomodulator (IMM, 6-mercaptopurine or AZA) was not mandated at the time of diagnosis in any of these reports.

Predictive markers of long-term outcomes in children are missing. Based on adult data, young age at diagnosis, extensive disease, involvement of upper gastrointestinal tract, perianal disease, smoking, and need for systemic CS at diagnosis have been considered as predictors of unfavorable outcome [20–22].

The primary aim of this study was to compare the duration of remission between patients initially treated with CS and EEN in combination with AZA. The secondary aim was to find potential predictors of early relapse in these patients.

Methods

Subjects

From hospital electronic medical records, registered between 2008 and 2012, we identified 127 newly diagnosed pediatric patients with CD. The inclusion criteria were age under 18 years at diagnosis, remission achieved solely with EEN or CS until 12 weeks, diagnosis of CD established based on Porto criteria [23, 24], treatment with AZA started within the first week of the diagnosis (usually at the day of diagnosis), and obtained informed consent. The exclusion criteria were active perianal disease, AZA intolerance, or missing data. According to internal standards, patients with active perianal disease should be treated with anti-TNF treatment. Even if these patients were treated with EEN or CS and not with anti-TNF therapy, we decided to exclude five patients to keep homogeneity of the study group. Eligibility of patients is described as a flowchart in Fig. 1. All patients were followed at the University Hospital Motol up to the time of first relapse or for at least 12 months (the longest follow-up period without relapse was 40 months). Patients were followed up every 3 months or more often and at the time of change of their clinical status until the end of the study or until transition to the adult care (usually at the age of 19 years).

Treatment

During the study period, we gradually changed our protocols for preference of induction therapy. Initially, we used CS as a first-line therapy, followed by a short time period when patients were allowed to choose between CS and EEN. Finally, we advocated for EEN as the first-choice therapeutic option. Other standards remained constant: For all patients included in the analysis, we initiated immunosuppressive therapy with AZA at the time of diagnosis. We usually started AZA at 1.5 mg/kg/day and after 1–2 weeks increased the dose to 2 mg/kg/day. Patients treated with CS received prednisolone 1–2 mg/kg/day (up to 40 mg/day, exceptionally 60 mg/day) for approximately 2 months with slow tapering. Patients treated by EEN received single or combinations of any polymeric enteral formula available at our department (‘‘Fresubin,’’ Fresenius; ‘‘Nutridrink,’’ Nutricia; “Ensure,” Abbott; “Modulen,” Nestlé) according to the patient’s own preferences for a period of 6–10 weeks. Individuals who could not complete feeds orally were fed via nasogastric tube. The goal intake for EEN corresponded to approximately 120 % of the daily caloric needs of the patient. The resting energy expenditure was calculated according to Schofield equation [25]. At the end of the period of exclusive formula, solid food was gradually reintroduced over 1–2 weeks as formula intake decreased, according to the patient’s tolerance [26].

Remission and Relapse

Remission was evaluated by physician global assessment on visit between 6 and 12 weeks after diagnosis. PCDAI has been calculated retrospectively and had not been used at the time of remission for remission evaluation. Patients who did not enter the remission until 12 weeks needed other induction strategy and thus were excluded. Relapse was defined as an increase in disease activity with the need for additional re-induction therapy (CS, EEN, anti-TNF therapy, or surgery).

Data Collection

We retrospectively collected clinical, anthropometric, and laboratory data from the electronic database and clinical records at the time of diagnosis, at the time of remission (week 6–12), and at the time of relapse, or at the end of observation when the patient was still in remission. National references have been used for Z score calculations [27]. Due to 80 % of missing values in CS group, we decided to analyze fecal calprotectin as predictor only in patients initially treated with EEN.

Statistical Analysis

All data analysis was performed using R statistical software (version 3.1.1). Continuous variables were described as median and interquartile range (IQR). Categorical variables were described as absolute frequencies and percentages. Welch’s two-sample t test was used for comparing continuous variables between the two groups. When testing our hypothesis using categorical data, the likelihood ratio test was used to evaluate odds ratios with 95 % confidence intervals (95 % CI). Due to right censored data, we used survival curves and Cox proportional hazards regression

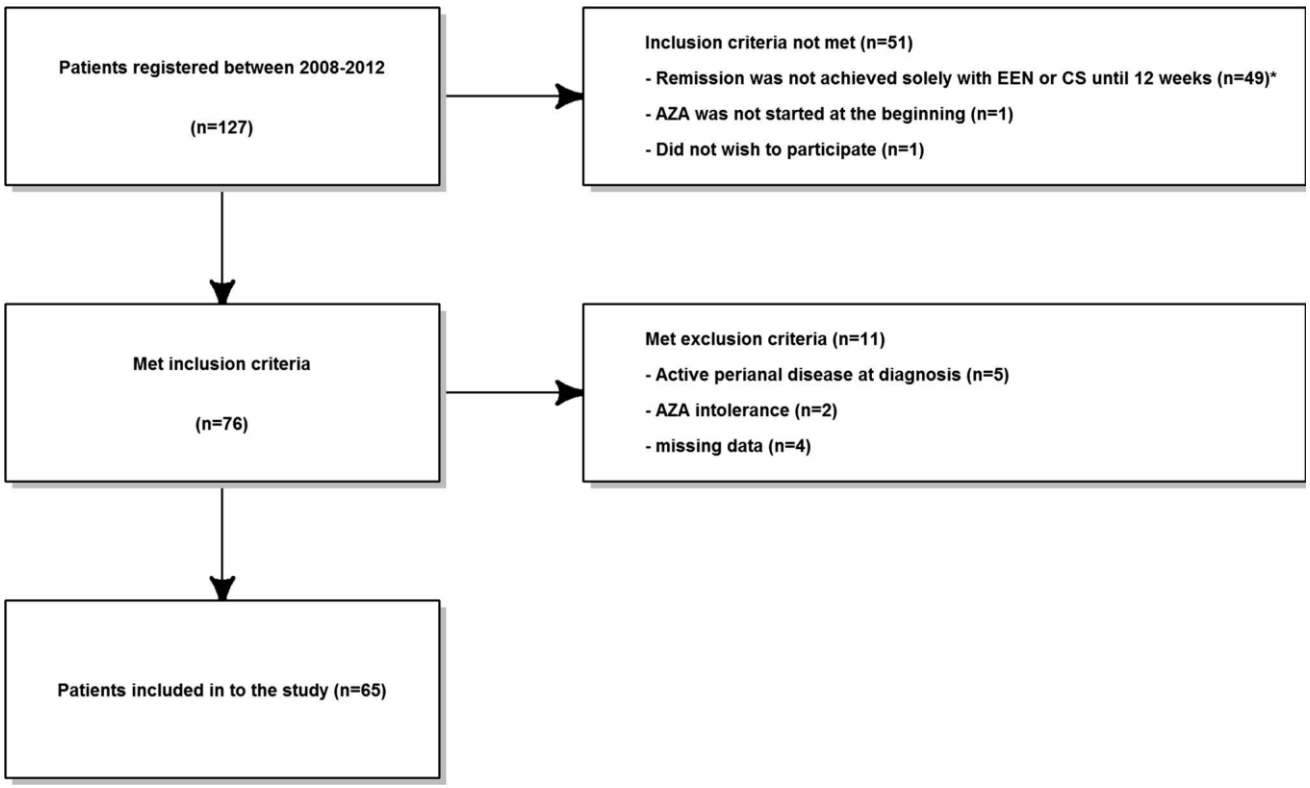


Fig. 1 Flowchart of included and excluded patients. *EEN* exclusive enteral nutrition, *CS* systemic corticosteroids, *AZA* azathioprine. * These patients received monotherapy with anti-tumor necrosis factor-alpha (anti-TNF) (28 patients) or 5-aminosalicylates (5-ASA) (4 patients), or were primarily treated surgically (7 patients). Combined induction therapy with EEN and CS or switching between the two regimens was given to 6 patients

models (R package ''A Package for Survival Analysis in S,'' R package version 2.37-7) [28]. For plot construction, we used an R package ggplot2 [29]. p value less than 0.05 was considered statistically significant. The selection of the variables included into the regression models was based on the Akaike's information criterion (AIC).

Ethical Consideration

The study was approved by the Ethics Committee of the authors' institution, and informed consent was obtained from patient's parents.

Results

Differences Between Patients Treated Initially with Exclusive Enteral Nutrition and Corticosteroids Both at Diagnosis and at Remission

Sixty-five patients fulfilled inclusion and exclusion criteria. Baseline characteristics according to the Montreal classification [30] did not differ between groups (Table 1). The clinical, laboratory, and anthropometric parameters of all 65 included patients at diagnosis and remission are described in Table 2. We found a higher erythrocyte sedimentation rate (ESR) at remission in patients initially treated with EEN versus CS (20 vs. 10 mm/h, $p \setminus 0.001$). We also found differences in treatment regimens between the study groups: AZA was started at a higher dose in the EEN study group (1.49 vs. 1.31 mg/kg/day, $p = 0.008$). However, there were no differences in the doses of AZA between groups at the time of remission, at the end of the observation period, or at relapse. Patients initially treated with EEN were less often treated with 5-ASA during the observation period (7 vs. 38 %, $p \setminus 0.001$).

Duration of Remission

Using the Cox proportion hazard regression model, we found no differences in the duration of remission between patients initially treated with EEN or CS ($p = 0.978$, Fig. 2). When we adjusted the association for all predictors that may differ between the study groups (with $p \setminus 0.1$, e.g., height Z score and dose of AZA at the time of

Table 1 Characteristics of patients according to Montreal classification at the time of diagnosis

	All	EEN	CS	*p* value
Number	65	29	36	
Median age at diagnosis, years*	14.1 (11.49–15.53)	13.91 (12.3– 15.02)	14.85 (11.25– 15.57)	0.809
Gender male	34 (0.52)	14 (0.48)	20 (0.56)	0.559
Disease location				
L1	18 (0.28)	11 (0.38)	7 (0.19)	0.098
L2	11 (0.17)	5 (0.17)	6 (0.17)	0.951
L3	36 (0.55)	13 (0.45)	23 (0.64)	0.124
Upper GI (L4)**	36 (0.55)	13 (0.45)	23 (0.64)	0.124
Disease behavior				
B1	45 (0.69)	19 (0.66)	26 (0.72)	0.561
B2	20 (0.31)	10 (0.34)	10 (0.28)	0.561
Perianal disease***	2 (0.03)	1 (0.03)	1 (0.03)	0.877
EIM	5 (0.08)	1 (0.03)	4 (0.11)	0.23

All quantitative data are reported as medians (IQR), categorical data as numbers (percentage)

EEN exclusive enteral nutrition, *CS* systemic corticosteroids, *GI* gastrointestinal, *EIM* extraintestinal manifestation

* Patients did not differ in frequency of age at diagnosis older than 16 years of age

** The UGI disease was based on macroscopic appearance of mucosal ulceration or bowel wall thickening on radiography

*** Perianal disease according to Montreal classification inactive at the time of diagnosis

diagnosis, ESR and platelets at the time of remission, and 5-ASA anytime during the study period), there was still no difference in the duration of remission (data not shown). Similarly, when we constructed the best fit regression model from all available predictors, the resulting model did not contain initial therapy. We did not find any association between the initial treatment strategy and duration of remission using the different models.

Prediction of Duration of Remission

As a secondary aim of the study, we attempted to identify predictive factors for duration of remission in the study group as a whole. According to the AIC, we constructed a Cox proportion hazard regression model for the prediction of duration of remission from all tested parameters at the time of diagnosis and relapse (Table 4 in ''Appendix''). Age at diagnosis and height *Z* scores both at diagnosis and at time of remission were associated with time to remission in the unadjusted Cox model. Younger age at diagnosis, lower height *Z* score at diagnosis, involvement of the upper gastrointestinal tract, and elevated levels of platelets in blood count ([440 9 10^9/l) at the time of remission were found to be an independent predictor of early relapse (Fig. 3a–c; Table 3). We did not find any difference between EEN and CS when adjusted for negative predictors of duration of remission [hazard ratio (HR) 1.12, 95 CI 0.53–2.34, $p = 0.77$]. We did not find fecal calprotectin higher than 200 ug/g to be a predictor of early relapse in subgroup analysis of patients treated initially with EEN ($p = 0.713$).

Discussion

Primary Aim: Comparison of the Long-Term Efficacy of Exclusive Enteral Nutrition or Corticosteroid Therapy

Recently published European Crohn's and Colitis Organization/European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (ECCO/ESPGHAN) guidelines recommended EEN as first-line therapy to induce remission in children with active luminal CD [14]. Most of the literature informing this recommendation has examined short-term outcomes comparing between therapy with EEN and CS; none has shown differences in the rate of achieving remission [2, 31]. The long-term outcomes based on early choice of EEN versus CS therapy are poorly documented [15, 16], while the duration of remission remains one of the most important parameters of long-term outcome. Several recently published studies comparing long-term outcomes of EEN and CS have assessed and compared rates of remission between groups (Fig. 4 in ''Appendix'') [15–19, 32–35].

Most studies comparing long-term outcomes between EEN vs. CS did not mandate the use of AZA at diagnosis [17, 19]. Meanwhile, our results were comparable to Riello

Table 2 Laboratory and anthropometric markers and doses of azathioprine at the time of diagnosis and remission

		All	EEN	CS	*p* value
PCDAI	Diagnosis	30 (25 to 37.5)	35 (27.5 to 37.5)	30 (21.88 to 35.62)	0.267
	Remission	10 (7.5 to 12.5)	10 (10 to 12.5)	10 (6.88 to 10.62)	0.580
CRP (mg/l)	Diagnosis	19 (9.3 to 40.4)	19 (10.5 to 57)	17.85 (5.4 to 36.35)	0.298
	Remission	2.3 (0.9 to 6.1)	2.3 (0.9 to 5.6)	2.65 (0.38 to 6.12)	0.338
ESR (mm/h)	Diagnosis	32 (24 to 49)	33 (25 to 52)	30.5 (22.75 to 43.5)	0.603
	Remission	15 (8 to 21)	20 (15 to 30)	10 (5.75 to 15.5)	\0.001
Platelets (910^9/l)*	Diagnosis	486 (408 to 597)	487 (442 to 628)	480.5 (406 to 587.25)	0.410
	Remission	405 (313 to 476)	375 (291 to 450)	434 (351.25 to 490.25)	0.086
Hemoglobin (g/dl)	Diagnosis	11.5 (10.7 to 12.3)	11.5 (10.7 to 12.2)	11.55 (10.7 to 12.33)	0.503
	Remission	12.3 (11.6 to 13.1)	12.2 (11.6 to 12.8)	12.5 (11.52 to 13.43)	0.273
Albumin (g/l)	Diagnosis	40.5 (38.2 to 43)	41.6 (39.1 to 43.2)	40.25 (38.1 to 42.92)	0.933
	Remission	44.9 (42.9 to 46.4)	45 (43.8 to 46.4)	44.2 (42.6 to 46.3)	0.622
Height *Z* score SD**	Diagnosis	−0.79 (−1.64 to 0.04)	−0.98 (−1.57 to 0.48)	−0.47 (−1.81 to 0.31)	0.094
	Remission	−0.85 (−1.72 to 0.03)	−0.97 (−1.72 to 0.4)	−0.54 (−1.62 to 0.24)	0.142
Weight *Z* score SD	Diagnosis	−1.12 (−1.74 to 0.42)	−1.12 (−1.8 to 0.2)	−1.12 (−1.74 to 0.55)	0.725
	Remission	−0.5 (−1.22 to 0.32)	−0.69 (−1.22 to 0.09)	−0.35 (−1.13 to 0.33)	0.360
AZA mg/kg/day	Diagnosis	1.42 (1.2 to 1.63)	1.49 (1.31 to 1.7)	1.31 (1.04 to 1.6)	0.008***
	Remission	1.7 (1.43 to 1.98)	1.92 (1.51 to 2.04)	1.62 (1.34 to 1.77)	0.117

All quantitative data are reported as medians (IQR)

EEN exclusive enteral nutrition, *CS* systemic corticosteroids, *PCDAI* pediatric Crohn's disease activity index, *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate, *AZA* azathioprine

* Patients did not differ in frequency of platelets [440 at remission

** Patients did not differ in frequency of height *Z* score \−1.64 SD at diagnosis

*** Between years 2008 and 2009, AZA was started at lower doses; however, during first 2 months, the dose was rapidly increased

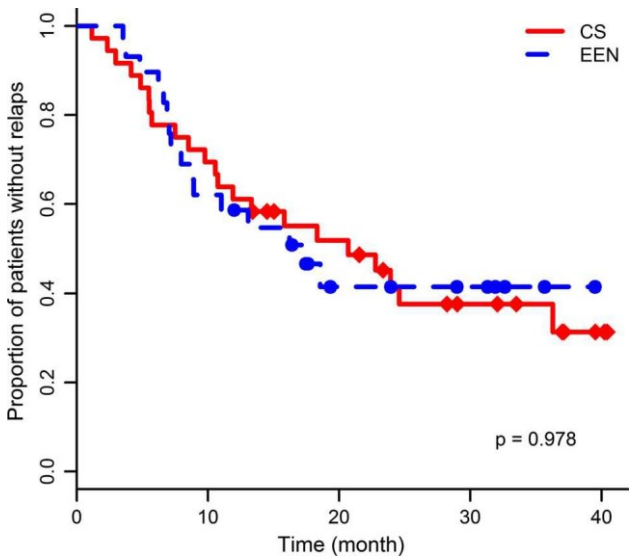
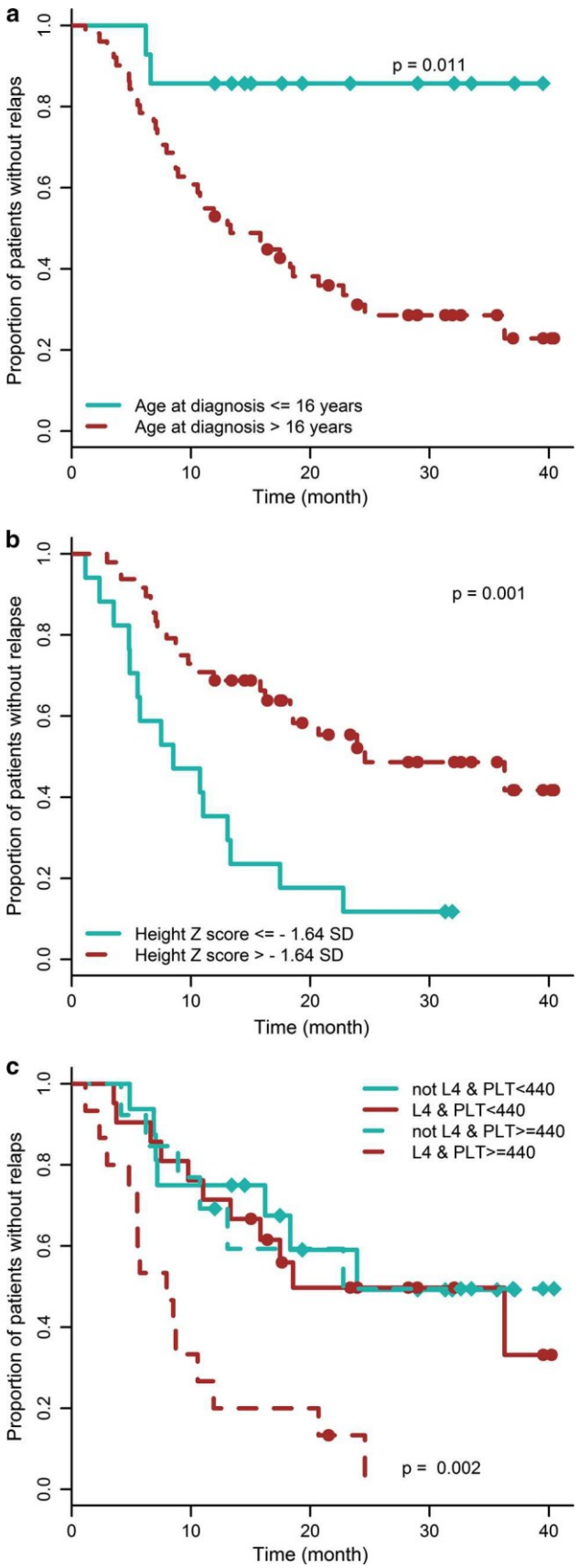


Fig. 2 Relapse-free survival in patients treated with AZA and simultaneously EEN versus CS. An estimate of the hazard ratio (HR) was 1.01 with 95 % CI 0.527–1.93, $p = 0.978$. *CS* corticosteroids, *EEN* exclusive enteral nutrition. *Number* of patients at risk of relapse in EEN, CS group: 10th month 25, 18; 20th month 16, 7; 30th month 8, 5; 40th month 2, 0, respectively

et al. [34] in the setting of early AZA use in both groups. A recent pediatric study comparing long-term outcomes of CS vs. EEN induction therapy with ''early'' use of thiopurines (\6 months from diagnosis) documented reduction in linear growth failure, CS dependency, and better primary sustained response to IFX, but similar combined CS-/IFX-free remission and surgical resection rates [35].

Our study directly compared the effects of EEN and CS on long-term remission in patients concomitantly treated with AZA from the diagnosis. According to published studies, long-term remission rates are comparable between induction strategies in children receiving maintenance therapy with IMM (Fig. 4 in the appendix, column 1), except the RCT by Markowitz et al. [36], where 89 % of patients treated with IMM and induced by CS were still in remission at week 18. In studies without early IMM use, the remission rates seemed to be higher when EEN was used as induction treatment (Fig. 4 in ''Appendix,'' column 2, 3) [15, 32, 33]. Contrary to CS, positive impact of EEN on mucosal healing may potentially increase durability of remission [6, 9]. This difference may only be apparent without IMM treatment; even so, the effects of IMM seem



b Fig. 3 Kaplan–Meier curves of relapse-free survival for independent predictors. a Kaplan–Meier curves of relapse-free survival in pediatric patients younger than or equal to 16 years of age versus patients older than 16 years at the time of diagnosis (HR 6.29, 95 % CI 1.51–26.1, $p = 0.011$). b Kaplan–Meier curves of relapse-free survival in patients with height Z score B-1.64 SD at diagnosis (HR 3.12, 95 % CI 1.61–6.05, $p = 0.001$). c Kaplan–Meier curves of relapse-free survival in the presence or absence of either upper gastrointestinal involvement (L4) and elevated platelet count (PLT) at the time of remission (estimate of HR for simultaneous presence of L4 and elevated PLT was 6.29, 95 % CI 1.51–26.1, $p = 0.011$). The upper gastrointestinal involvement was based on macroscopic appearance of mucosal ulceration or bowel wall thickening on radiography

to be more pronounced than the effects of choice of induction therapy, especially over longer time periods (beyond month 18). While the new ECCO/ESPGHAN guidelines [14] do not clearly indicate when treatment with IMM should be initiated, the overall understanding is that it is in the majority of pediatric patients with CD. The goal for further studies will be the identification of low-risk patients, who will not need IMM and who will benefit from long-term effect of EEN induction therapy.

Secondary Aim: Predictive Factors of Early Relapse

Generally, the age at diagnosis (\40 years), corticosteroids at diagnosis, upper gastrointestinal tract and ileocolonic involvement, and perianal disease are considered to be long-term predictive factors of unfavorable course in adult patients [20–22, 37, 38]. We identified a lower height Z score, involvement of the upper gastrointestinal tract, a lower age at diagnosis, and an elevated platelet count at remission as predictive factors for early relapse in patients treated with AZA at diagnosis. These risk factors can be applied only for prediction of time to relapse in pediatric patients with CD who were treated with IMM at diagnosis. However, predictors of long-term AZA efficacy in sustaining remission in the pediatric literature are scarce. Riello et al. [34] did not find any predictors from baseline data in patients treated by AZA since the time of diagnosis. In the previously mentioned study by Levine et al. [18], the authors found that normal C-reactive protein (CRP) and steroid-free remission at week 12 were predictive factors for remission at week 52. However, not all of the patients were treated with AZA. We were unable to confirm the observation that the CRP (whether as a quantitative or categorical variable with a 0.5, 2, nor 5 mg/l cutoff) was associated with the duration of remission.

Fecal calprotectin lower than 200 ug/g at week 4 has been shown to predict longer duration of remission in pediatric CD with EEN as induction therapy [39]. Due to

Table 3 Cox regression model showing estimates of hazard ratio of included predictors

	Estimates of hazard ratio (95 CI)	Pr(|z|)
Age at diagnosis	0.871 (0.778–0.975)	0.016
Upper GI (L4)*	2.685 (1.273–5.663)	0.009
CRP at diagnosis	1.012 (0.999–1.026)	0.072
Platelets at diagnosis	0.997 (0.994–1.001)	0.103
Height *Z* score at diagnosis	0.609 (0.437–0.848)	0.003
Platelets at remission	1.004 (1.001–1.008)	0.021

L4 upper gastrointestinal (GI) involvement, CRP C-reactive protein

The model was constructed based on Akaike's information criterion from all available predictors at the time of diagnosis and remission

* The UGI disease was based on macroscopic appearance of mucosal ulceration or bowel wall thickening on radiography

the large amount of missing values of fecal calprotectin in CS group of patients, we tested only the patients initially treated with EEN. We did not find fecal calprotectin at the time of remission to be a predictor of time to relapse in that subgroup. Similar to our study, Wine et al. [40] found an association between growth retardation and severity of the disease, and height Z scores were identified as predictor of relapse in a recent study in pediatric patients treated with infliximab [41]. Observations highlight the issue of whether early anti-TNF therapy or surgery could lead to better results in high-risk patients (e.g., younger than 16 years of age, height *Z* score lower than −1.5 SD, and presence of upper gastrointestinal involvement). On the other hand, recently published study comparing the induction therapy with EEN to anti-TNF monotherapy in pediatric CD found similar efficacy in decreasing mucosal inflammation [42]. To clarify this issue, a prospective randomized trial with anti-TNF treatment (or surgery) in one arm and EEN with early AZA in the other is needed.

Advantages and Limitations of the Study

There were several advantages and limitations in the present study. One of the most important advantages was the relative homogeneity of the study group. All patients were treated in one center with little variations in the aspect of patient care, and all patients were treated with AZA for the duration of the study. However, we could not exclude a potential slight effect of changing in care standards over the study period. We do not believe that the differences in proportion of patients treated with 5-ASA between groups played a significant role; moreover, 5-ASA was not found to be a predictive factor when tested in a logistic regression model applied to all patients. Based on published data, we did not anticipate that the type of EEN should influence the induction of remission [1, 2, 43]. Surprisingly, the dosage of AZA was lower than recommended in our internal guidelines. However, due to the similar dose of AZA at the time of remission in both groups (CS and EEN), we do not believe that the time to relapse was influenced by dosage of AZA although the thiopurine levels were not measured. On the other hand, sub-optimal dosing of AZA was a limitation, which did not allow to make a more firm conclusion. However, the main disadvantage is the retrospective nature of the study design and all the inherent difficulties associated with this approach. One of these limitations was the inability to evaluate the efficacy of the induction regimes (the therapeutic approach being changed according to the course of the disease), unavailability of endoscopic scores at diagnosis, subjective judging of remission by physician global assessment, and calculating PCDAI retrospectively. The PCDAI nor physician global assessment did not correlate with ESR nor CRP at the time of remission in our patients, so not all of them were in the laboratory or even endoscopic nor histologic remission. However, we demonstrated that the PCDAI and CRP did not differ between the EEN and CS groups, and moreover, PCDAI or CRP at the time of remission has not been shown as a significant predictor of early relapse in our study. Due to the retrospective design of our study, a few patients were excluded because of missing values. Because of the unavailability of the median survival times of patients on EEN or CS treatment (or the HR of the control treatment relative to the experimental treatment) from previously published studies, we were unable to calculate the study power.

Conclusion

In conclusion, no benefit of EEN over CS was observed in the durability of remission in the patients with newly diagnosed CD concurrently treated with AZA. In this population, younger age at diagnosis, lower height *Z* score

at diagnosis, involvement of the upper gastrointestinal tract, and elevated platelet count at remission were all identified as independent predictors of early relapse.

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Compliance with ethical standards

Conflict of interest None.

Appendix

See Table 4 and Fig. 4.

Table 4 Predictors of the time to first relapse among all included subjects

	Estimates of hazard ratio (95 CI)	*p* value
Age at diagnosis	0.876 (0.799–0.961)	0.005
Gender male	0.813 (0.430–1.539)	0.525
PCDAI at diagnosis	1.009 (0.976–1.043)	0.592
CRP at diagnosis	1.003 (0.993–1.013)	0.557
ESR at diagnosis	0.988 (0.973–1.004)	0.136
Hemoglobin at diagnosis	0.888 (0.721–1.095)	0.266
Platelet at diagnosis	1.002 (0.999–1.004)	0.146
Albumin at diagnosis	1.000 (0.930–1.076)	0.995
Height *Z* score at diagnosis	0.725 (0.540–0.972)	0.031
BMI at diagnosis	0.832 (0.624–1.110)	0.211
AZA at diagnosis	1.234 (0.513–2.971)	0.639
PCDAI at remission	1.038 (0.956–1.126)	0.377
CRP at remission	1.030 (0.996–1.065)	0.087
ESR at remission	1.013 (0.984–1.044)	0.384
Hemoglobin at remission	0.789 (0.621–1.002)	0.052
Platelet at remission	1.003 (1.000–1.006)	0.049
Albumin at remission	0.937 (0.849–1.035)	0.202
Height *Z* score at remission	0.734 (0.550–0.980)	0.036
BMI at remission	0.761 (0.556–1.042)	0.089
AZA at remission	2.477 (1.061–5.786)	0.036
5-ASA any time	0.956 (0.494–1.852)	0.895
Localization of disease		
L1	0.504 (0.222–1.145)	0.102
L2	0.812 (0.339–1.945)	0.640
L3	1.902 (0.972–3.722)	0.061
Upper GI (L4)**	1.951 (0.997–3.819)	0.051
Behavior of disease		
B1	0.795 (0.400–1.580)	0.513
B2	1.258 (0.633–2.500)	0.513
Perianal disease	0.573 (0.078–4.195)	0.583

The displayed hazard ratios were calculated for univariate models

PCDAI Pediatric Crohn's disease activity index, *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate, *BMI* body mass index, *AZA* azathioprine, *5-ASA* 5-aminosalicylates, localization of disease: L1 ileal, L2 colonic, L3 ileocolonic, L4 upper gastrointestinal (GI) involvement, behavior of disease: B1 luminal, B2 stricturing

* Between years 2008 and 2009, AZA was started in lower dose; however, during first 2 months, the dose was rapidly increased

** The UGI disease was based on macroscopic appearance of mucosal ulceration or bowel wall thickening on radiography

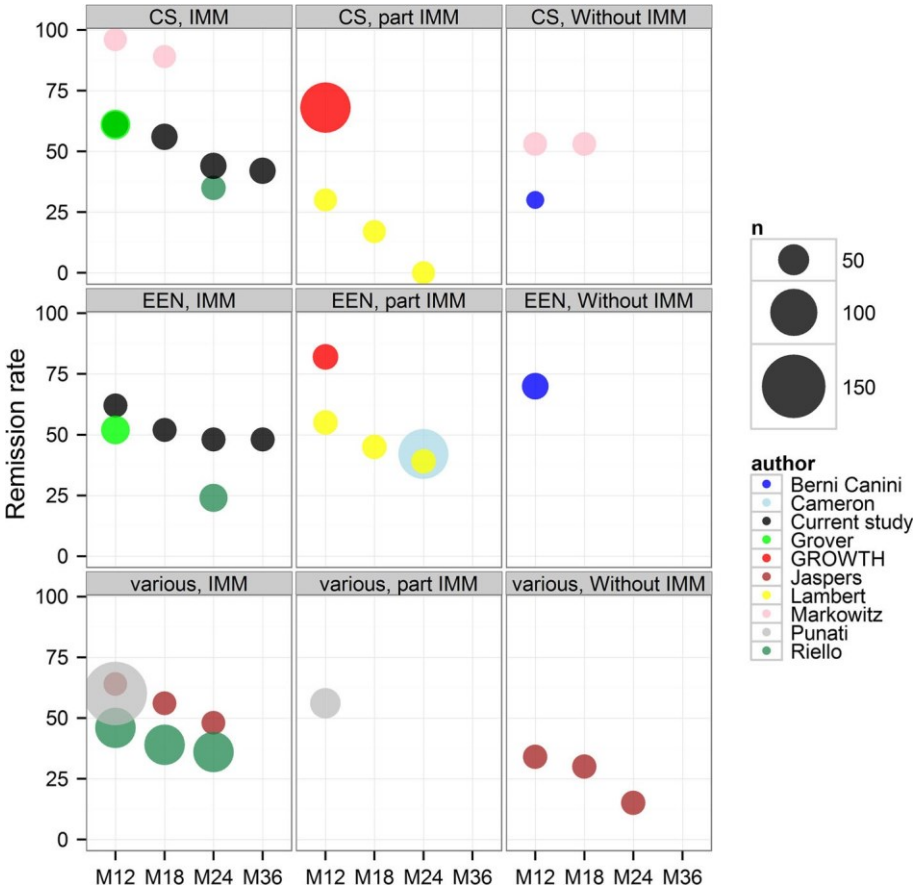

Fig. 4 Remission rate at month 12, month 18, month 24, and month 36 from published studies. The *first row of plots* shows the remission rate in patients initially treated with systemic corticosteroids (CS), the second those treated with exclusive enteral nutrition (EEN), and the third patients who underwent various induction therapeutic modalities (various). The *first column* shows patients treated with immunomodulators (IMM) such as 6-mercaptopurine or azathioprine, the second patients partly treated with IMM, and the third column without IMM. The *widths of boxes* represent the number of included patients. *Each color* represents a different author. Six retrospective studies (by Berni Canini et al. [17], Cameron et al. [15], the current study, Jaspers et al. [31], Lambert et al. [19], and Riello et al. [33]), two prospective studies (the GROWTH study [18], and the study by Puanti et al. [32]), and a randomized controlled trial by Markowitz et al. [34] were included in the analysis. The PCDAI score at diagnosis varied in the majority of studies from 30 to 40; a higher PCDAI was reported in the studies by Riello et al. [33] and Markowitz et al. [34]

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Evaluation of Infliximab Therapy in Children with Crohn's Disease Using Trough Levels Predictors

Jan Ohem[a] Ondrej Hradsky[a] Kristyna Zarubova[a] Ivana Copova[a]
Petra Bukovska[b] Richard Prusa[b] Karin Malickova[c] Jiri Bronsky[a]

[a]Gastroenterology and Nutrition Unit, Department of Paediatrics, and [b]Department of Medical Chemistry and Clinical Biochemistry, 2nd Faculty of Medicine, Charles, University and Motol University Hospital, and [c]Institute of Medical Biochemistry and Laboratory Diagnostics, 1st Faculty of Medicine, Charles, University and General University Hospital, Prague, Czech Republic

Keywords
Biological therapy · Anti-tumour necrosis factor · Antibodies to infliximab · Infliximab levels · Paediatric

Abstract
Background: In adults, infliximab (IFX) levels correlate with disease activity, and antibodies to IFX (ATIs) predict treatment failure. We aimed to determine the association of IFX levels and ATIs with disease activity in a paediatric population. We prospectively collected blood, stool, and clinical data from 65 patients (age 10.5–15.1 years) with Crohn's disease (CD) before IFX administration, and measured IFX trough levels, ATIs, and faecal calprotectin levels (CPT). Samples were collected during maintenance therapy. We used multivariate analysis to identify the predictors of IFX levels. ***Summary:*** Lower levels of IFX were associated with ATIs positivity (OR 0.027, 95% CI 0.009–0.077). Higher C-reactive protein (CRP) level, erythrocyte sedimentation rate, and CPT levels were found in patients with lower IFX levels. The optimal combination of sensitivity (0.5) and specificity (0.74) for disease activity was calculated for IFX levels ≥1.1 µg/mL using CRP level <5 mg/L as a marker of laboratory remission. In a model that used CPT ≤100 µg/g as the definition of remission, the optimal IFX trough level was 3.5 µg/mL. No independent association between remission and ATIs was found in our study population. However, we found an independentz association between IFX levels and serum albumin levels (OR 1.364, 95% CI 1.169–1.593), $p < 0.001$. ***Key Messages:*** The paediatric population was similar to adult populations in terms of the association between IFX and ATIs as well as between IFX and disease activity.

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Introduction

The incidence of Crohn's disease (CD) in children is increasing [1], and the course of the disease is more complicated in children than in adults [2]. Thus, in paediatric populations, it is important to treat CD with maximum efficacy. Treatment failure may lead to growth retardation and compromised quality of life for a prolonged period. Clinically, deep remission, characterised by mucosal healing, is the ultimate treatment goal.

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E-Mail karger@karger.com
www.karger.com/ddi

Jan Ohem, MD
Department of Paediatrics, University Hospital Motol, V Uvalu 84
CZ–150 06 Prague (Czech Republic)
E-Mail jan.ohem@ yahoo.com

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Biologic treatment with anti-tumour necrosis factor (TNF) is widely used in the most complicated cases. Globally, biologic treatment has most commonly involved the use of infliximab (IFX), the first TNF agent approved for use in a paediatric population. The efficacy and safety of IFX have been demonstrated in induction and maintenance therapy. However, anti-TNF treatment is expensive; in addition, treatment failure and loss of response have been observed in a significant proportion of the patients over time [3]. Therefore, studies on avoiding failure and rapidly optimizing the treatment are currently underway; a potential method to achieve these may be the measurement of the IFX levels and antibodies to IFX (ATIs). In paediatric populations, the role of these measurements in optimizing IFX dosage with respect to clinical outcomes remains unclear because the predictors influencing IFX levels have not been identified, and insufficient data exists to establish precise guidelines for therapeutic drug monitoring in paediatric CD. In adult populations, ATIs, which has an incidence between 9% and 63.5%, seem to cause a threefold increase in the risk of loss of response. Several IFX cut-off levels for predicting remission have been reported. Higher levels are associated with remission defined by low C-reactive protein (CRP) levels [4] and fistula healing [5]. According to a recent study, lower IFX levels and higher faecal calprotectin (CPT) can be used to predict relapse in adult populations [6]. Another question is whether the application interval should be shortened or the dose elevated to achieve higher IFX levels as reported in the study by Hofmekler et al. [7]; however, this or the effect of the concomitant therapy was not the main objective of our study [8].

Primary and Secondary Aims of the Study

The primary aim of this study was to determine whether the levels of IFX and ATIs are associated with laboratory remission (defined as CRP level ≤5 mg/L). The secondary outcomes were defined as erythrocyte sedimentation rate (ESR) ≤20 mm/h and CPT ≤100 μg/g during IFX maintenance treatment. We also sought to identify the predictive factors of FX levels using a multivariate mixed model because the identification of the most influential factors can aid the treatment procedure [9, 10]. The study was performed according to the STROBE methodology [11].

Materials and Methods

Recruitment of Patients

This was a prospective observational study conducted during maintenance IFX therapy. From 2012 to 2014, we collected blood samples, stool samples, and clinical data from the study population comprising CD patients (n = 65) treated with IFX. Samples were collected repeatedly from every patient in the morning before each IFX infusion (infusion interval was 4 [q4] to 8 [q8] weeks) at the centre for biological treatment of our paediatric department. Inclusion criteria were age <18 years at the time of enrolment; diagnosis of CD based on the Porto criteria and the revised Porto criteria [12, 13]; current IFX maintenance treatment, including intensified therapy, at the time of enrolment (shortened interval, increased dose); and willingness of both the patients and the parents for participation in the study. Patients on IFX induction therapy or adalimumab therapy were excluded.

Study Objectives

As described previously, laboratory remission, defined as CRP level ≤5 mg/L, was chosen as the primary outcome [14]. We also assessed the predictive role of the IFX and ATIs levels when remission was defined as ESR ≤20 mm/h and CPT ≤100 μg/g. As potential covariates, we also considered other factors, including body weight (BW), serum haemoglobin level, albumin level, platelet count, paediatric CD activity index (PCDAI), extraintestinal manifestations, and adverse events.

Laboratory Analysis

We collected a total of 539 blood samples for determining the IFX trough levels and ATIs. Samples were collected in the morning before IFX infusion, centrifuged immediately after collection, and stored at –20 °C in aliquots until analysis.

Trough levels of IFX and ATIs were measured using an enzyme-linked immunosorbent assay (ELISA) sandwich assay (Q-ATI, Q-Infixi, Matriks Biotek, Gazi Üniversitesi Gölbaşı Yerleşkesi Tekonoplaza Binası B Blok Zemin Kat BZ17 06830, Ankara, Turkey). The lower limit of detection for both IFX and ATIs levels was 30 ng/mL according to the ELISA kit. ATIs were detected using the bound IFX, and a specific enzyme-linked antibody was then added to produce a colour reaction. In order to measure the trough levels of the drug (the lowest level of drug during the application period), we collected samples before each IFX infusion as recommended in the instructions of the ATIs/IFX kit. IFX levels were detected using IFX reactant coated on the wells of the assay plate, and captured IFX was then linked to the specific antibody with bound enzyme. Further colour reaction was measured using spectrophotometry at 450 nm.

Stool samples were collected from each patient (if supplied by the patient) before each visit for the determination of CPT and stored at –20 ° C until analysis. Samples were subsequently measured using the Bühlmann bedside test according to the lateral flow method (Bühlmann, Postfach 110325, 28083 Bremen, Germany).

Statistical Analyses and Ethical Approval

All data analyses were performed using R statistical software (250 Northern Ave, Boston, MA 02210, version 3.2.3 version 3.2.3) [15]. Continuous variables are presented as medians and interquartile range. Categorical variables are presented as absolute frequencies and percentages. For testing our hypothesis using categorical data, the likelihood ratio test was used to evaluate the ORs with 95% CI. To test the differences in the distribution among quartiles of IFX trough levels, we used the Kruskal-Wallis rank sum test. We used receiver operating characteristic (ROC) curves to define the optimal cut-off values for the inflammatory

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Table 1. Basic patients' characteristics

Number of patients	65
Gender, male, *n* (%)	37 (57)
Age at diagnosis, years, median (IQR)	12.6 (10.5–15.1)
Localization, *n* (%)	
L1	10 (15)
L2	16 (25)
L3	38 (58)
Upper GI involvement	28 (43)
Behavior, *n* (%)	
B1	35 (54)
B2	22 (0.34)
B3	7 (11)
Perianal disease, *n* (%)	13 (20)
IFX before study, months, median (IQR)	16 (6–30)
Months from daignosis to first IFX, median (IQR)	9.4 (2.7–22.7)
Age at first IFX, years, median (IQR)	14 (11.4–16.4)
Switch needed, *n* (%)	3 (5)
IC resection needed, *n* (%)	8 (12)
Number of applications per patient, median (IQR)	9 (5–13)

markers (R package "pROC" [16]). Given the repeated measurements, we calculated the area under the curve using cross-validated area under the ROC curve estimates for pooled repeated measures data (R package "cvAUC" [17]). Furthermore, a linear mixed model (R packages "lme4" [18] and "lmerTest" [19]) was used to determine the relationships between remission (defined by inflammatory markers) and predictors (categorical variable of IFX trough level based on ROC, ATIs as a numerical variable, serum albumin level, gender, age, dose of IFX, and dose of azathioprine, AZA). The predictors were selected based on clinical relevancy. p values <0.05 were considered statistically significant. For all the tests, 2-sided p values have been reported. The CPT data of several patients was missing; therefore, we used multiple imputation with sensitivity analysis (R packages "mice" [20] and "SensMice" [21]) and reporting results as complete cases, multiple imputation and sensitivity analysis with assumed deviation among missing values of 200 μg/g. All other variables were treated as complete cases. This study was approved by the Ethical Committee of our university hospital. All patients and/or their parents signed informed consent forms.

Results

Basic characteristics of the study participants are presented in Table 1, and the data related to IFX application are presented in Table 2.

Relationship between ATIs and IFX Trough Levels

Figure 1 shows the relationship between IFX trough levels and ATIs in all the samples. The relationship between IFX trough levels and ATIs, using detection limits of the respective assay, is described in the table embedded in Figure 1. A statistically significant association was observed between positive (>30 ng/mL) ATIs levels and undetectable (<30 ng/mL) IFX trough levels (OR 0.027, 95% CI 0.009–0.077).

Relationship between IFX Trough Levels and Laboratory Markers: Quartile Analysis

Quartile analysis of IFX trough levels showed that CRP, ESR, and CPT were elevated in lower quartiles of IFX levels (Fig. 2).

Defining the Optimal Cut-Off Values for Inflammatory Markers Using ROC/AUC

We used ROC curves of CRP, ESR, and CPT to determine the optimal cut-off values for IFX levels (Fig. 3). By using CRP level <5 mg/L as a marker of laboratory remission, the best combination of sensitivity (0.5) and specificity (0.74) was calculated for IFX level of 1.1 μg/mL. For other markers (ESR and CPT), both the sensitivity and specificity were lower. We were unable to collect stool samples from all patients for the determination of CPT. However, CPT is an important marker; therefore, we used the multiple imputation method with sensitivity analysis. This method was used although the lack of CPT values can be considered an accidental phenomenon. ROC curves were constructed for CRP at the optimal cut-off values for IFX trough levels under conditions of positive (>30 ng/mL) versus negative (<30 ng/mL) ATIs levels. At ATIs positivity, ROC for CRP showed a sensitivity of 0.25 and a specificity of 1 at an IFX level of 0.2 μg/mL. When we considered ATIs positivity as any detectable value above 0, ROC for CRP showed a sensitivity of 0.5 and a specificity of 0.86 at an IFX level of 1.97 μg/mL.

We used the same method to determine the optimal cut-off values for ATIs. However, AUC for CRP (0.48, 95% CI 0.29–0.66) and ESR (0.46, 95% CI 0.29–0.64) was <0.5; therefore, it was not possible to determine the optimal cut-off values for ATIs. AUC for CPT was 0.53 (95% CI 0.33–0.73), and the optimal cut-off level for ATIs was 2.09 (specificity 0.86, sensitivity 0.19) when CPT was used as a marker of remission.

Associations and Predictors

Remission Defined by CRP

Table 3a shows that when cut-off values of IFX trough levels from the ROC curve for CRP in a multiple regression mixed model were used, a significant independent association was observed between the IFX trough levels (above the cut-off of 1.1 μg/mL) and remission, defined as CRP level ≤5 mg/L. Although CRP is a non-specific

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Table 2. Basic characteristics of all IFX administrations/at first IFX administration

Characteristics	All IFX administrations	First IFX administration
Number of applications	539	65
PCDAI, median (IQR)	10 (5-15)	10 (5–15.6)
Erythrocyte sedimentation rate, mm/h, median (IQR)	15 (7–25)	15 (8–22)
C-reactive protein, mg/L, median (IQR)	1.1 (0.5–3.7)	1 (0.5–5)
Serum haemoglobin, g/dL, median (IQR)	13.4 (12.7–14.1)	13.4 (12.6–14)
F-calprotectin, μg/g, median (IQR)	754 (157–1,800)	580 (235.8–1,800)
Platelets, 10^9/L, median (IQR)	291 (253–343)	293.5 (248.8–348.5)
Serum albumin, g/L, median (IQR)	43.9 (42.2–45.6)	43.9 (42–45.9)
IFX levels, μg/g, median (IQR)	2.1 (0.8–3.9)	1.4 (0.5–3.8)
ATI levels, ng/g, median (IQR)	0 (0–0)	0 (0–0)
IFX dose, mg per kg of body weight per 8 weeks, mg, median (IQR)	6.5 (5.2–8.3)	5.7 (5–6.8)
AZA, *n* (%)	408 (76)	51 (0.78)
ASA, *n* (%)	26 (5)	5 (0.1)
Weeks between doses, median (IQR)	8 (6–8)	–
Intensification by dose, *n* (%)	28 (5)	–
Intensification by interval, *n* (%)	19 (4)	–
Age at determination, years, median (IQR)	15.3 (13.4–17.2)	15.2 (12.7–17.1)

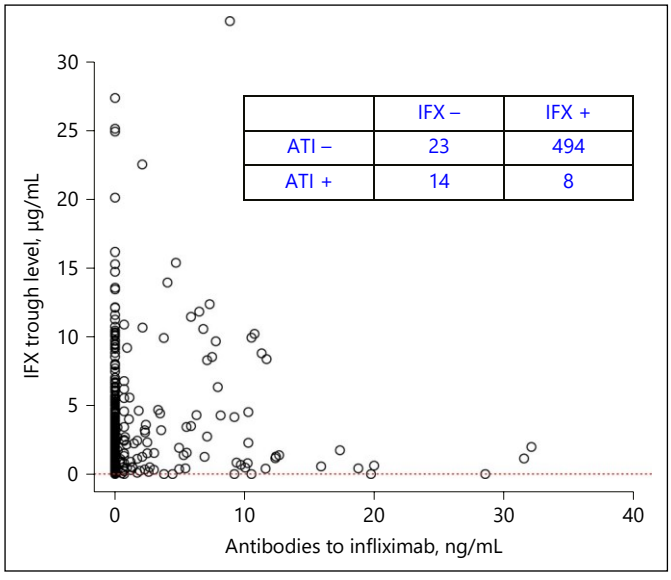

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Fig. 1. The relationship between IFX levels and ATIs. IFX, infliximab. The embedded table shows the number of measurements with positive (kit detection limit: 30 ng/mL) and undetectable IFX levels and antibodies (above or below the detection limit of 30 ng/mL).

marker, it can be used to evaluate remission in combination with IFX level <1.1 μg/mL. Using the same model, no association was found between remission and ATIs (as either a continuous or a dichotomous/categorical variable). However, we found an independent association of remission with serum albumin levels (OR 1.364, 95% CI 1.169–1.593; p <0.001), and a borderline association with the AZA dose (OR 0.434, 95% CI 0.190–0.992; p = 0.048).

Remission Defined by ESR

In a similar model, IFX trough level of 2.1 μg/mL was independently associated with remission defined by ESR ≤20 mm/h. Other associated predictors were serum albumin level, male gender and age at the time of sample collection; however, neither ATIs nor the AZA dose was found to be a predictor (Table 3b).

Remission Defined by CPT

In a model using CPT ≤100 μg/g as the definition of remission, IFX trough level of 3.5 μg/mL was independently associated with remission using only the available CPT values (complete case analysis n = 315; Table 3c). Using a multiple imputation method, we also found CPT to be an independent predictor of IFX levels (p = 0.046). However, we were unable to confirm this association in a sensitivity analysis with expected CPT levels +/– 200 μg/g (p = 0.047 and p = 0.077, respectively).

As the correlation between IFX clearance and BW is not linear and weight-based dosing of IFX does not entirely correct for higher clearance in patients with a higher BW, we also performed analysis by adding BW as a covariate to the existing model. Adding BW caused no significant change in the results (data not shown).

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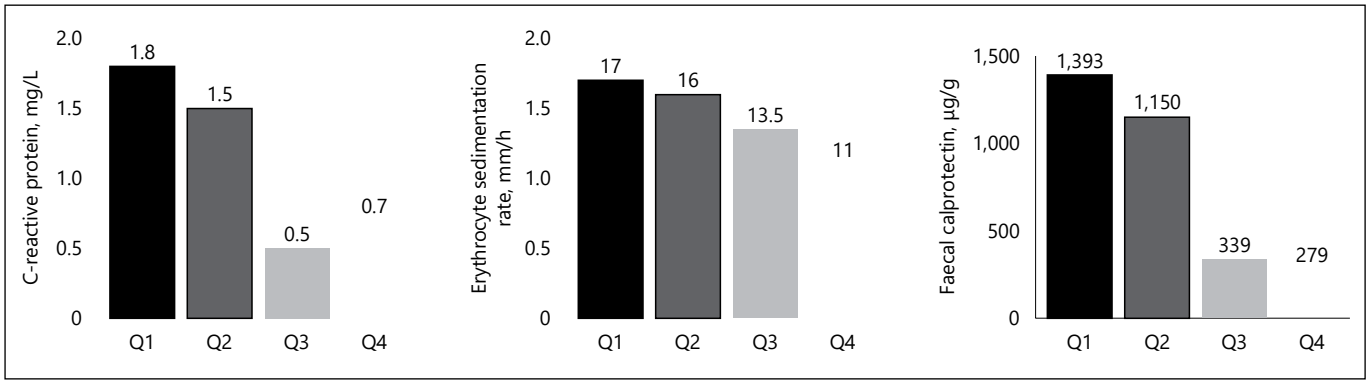

Fig. 2. Quartile analysis of the median levels of inflammatory markers divided according to 4 quartiles of IFX levels (Q1–Q4). *p* values derived from the Kruskal-Wallis rank sum test analysis for C-reactive protein, erythrocyte sedimentation rate and faecal calprotectin were $p < 0.001$, 0.0016 and 0.0013, respectively. IFX concentration (µg/mL) quartiles were as follows: Q1 <0.8; 0.8 ≥Q2 <2; 2 ≥Q3 <3.8; and Q4 ≥3.8.

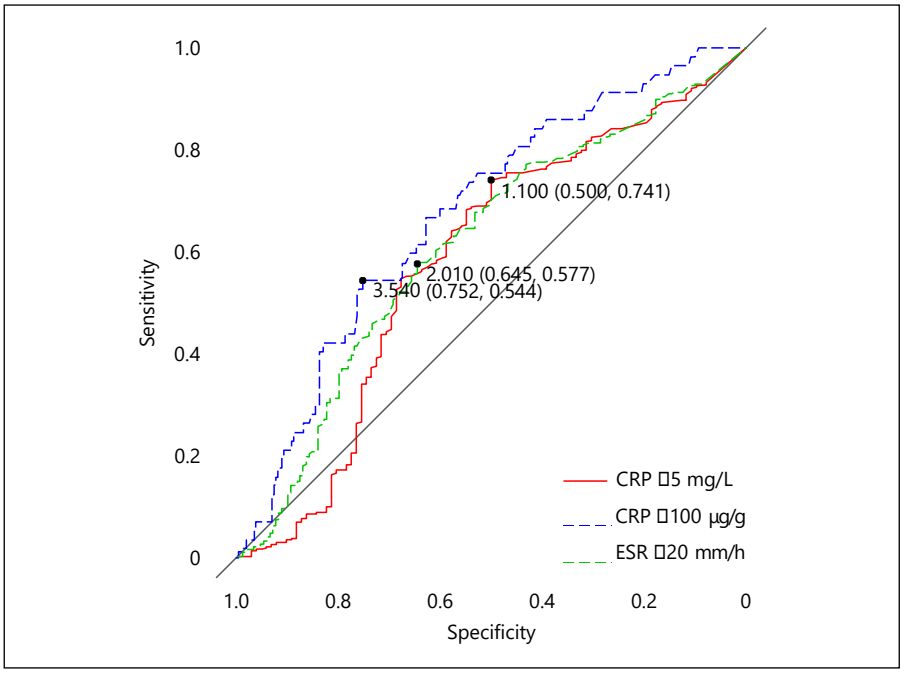

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Fig. 3. ROC curves for inflammatory markers for optimal cut-off values of IFX trough levels CRP, C-reactive protein, ESR, erythrocyte sedimentation rate, CPT, and faecal calprotectin. Area under curve (AUC) for CRP was 0.58 (95% CI 0.46–0.71); optimal cut-off level for IFX with specificity and sensitivity are shown. AUC for ESR was 0.61 (95% CI 0.51–0.71); optimal cut-off level for IFX was 2 µg/mL (specificity 0.65 and sensitivity 0.58). AUC for CPT was 0.67 (95% CI 0.55–0.80); optimal cut-off level for IFX level was 3.5 µg/mL (specificity 0.75, and sensitivity 0.54).

Sensitivity analysis performed after excluding the patients on q4 dosing did not significantly change the association between IFX levels and the outcomes (CRP, ESR and CPT-complete case).

Discussion

The recent European Crohn's and Colitis Organisation/The European Society for Paediatric Gastroenterology Hepatology and Nutrition guidelines on medical management of CD in children state that the evaluation of the induction treatment is recommended after the third IFX dose; however, the evaluation should not be conducted using the IFX levels or ATIs. During the maintenance treatment, it is possible to reduce the IFX dose if remission is achieved and the IFX level is >8–10 µg/mL. Measurement of the IFX levels and ATIs is recommended in cases of insufficient response; however, no specific cut-off values are provided [2].

In adult patients, some studies have reported an association between disease remission and higher IFX lev-

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Table 3

a Remission defined by C-reactive protein <5 mg/L

	OR	95% CI	*p* value
IFX level >1.1 μg/mL	3.096	1.394–6.874	0.005
ATI levels, μg/mL	0.998	0.992–1.004	0.402
Serum albumin, g/L	1.364	1.169–1.593	<0.001
Gender, male	1.237	0.365–4.194	0.733
Duration of IFX therapy, weeks	1.003	0.996–1.011	0.444
Age at IFX application, years	0.843	0.674–1.054	0.136
IFX dose, mg per kg of body weight per 8 weeks	0.909	0.804–1.029	0.129
AZA dose, mg per kg of body weight	0.434	0.19–0.992	0.048

b Remission defined by erythrocyte sedimentation rate <20 mm/h

	OR	95% CI	*p* value
IFX level >2 μg/mL	2.872	1.273–6.477	0.011
ATI levels, μg/mL	1.000	0.994–1.005	0.873
Serum albumin, g/L	1.560	1.3–1.872	<0.001
Gender, male	13.615	2.279–81.342	0.004
Duration of IFX therapy, weeks	0.996	0.987–1.006	0.495
Age at IFX application, years	1.403	1.016–1.939	0.040
IFX dose, mg per kg of body weight per 8 weeks	0.887	0.761–1.033	0.123
AZA dose, mg per kg of body weight	1.113	0.423–2.924	0.829

c Remission defined by f-calprotectin (complete case) <100 μg/g

	OR	95% CI	*p* value
IFX level >3.5 μg/mL	3.332	1.324–8.387	0.011
ATI levels, μg/mL	0.956	0.872–1.048	0.339
Serum albumin, g/L	1.138	0.933–1.387	0.200
Gender, male	1.330	0.313–5.648	0.699
Duration of IFX therapy, weeks	1.002	0.992–1.012	0.733
Age at IFX application, years	1.139	0.864–1.501	0.356
IFX dose, mg per kg of body weight per 8 weeks	0.878	0.716–1.077	0.211
AZA dose, mg per kg of body weight	0.693	0.278–1.726	0.431

els. On the basis of their systematic review, Moore et al. [22] have stated that adult patients in remission during IFX maintenance therapy have higher mean trough IFX levels than those who are not in remission. A threshold of 2 μg/mL has been reported although the predictive value of IFX levels with respect to treatment failure has not been mentioned [23]. Another study in adults has revealed more frequent adverse reactions in patients with positive ATIs. In this study, no association was found between IFX levels and treatment outcomes [24]. In sum, it seems that higher IFX levels in adults are associated with better treatment outcomes. Based on a recent study in adults, the use of an algorithm including IFX levels and ATIs has higher economic feasibility than simply intensifying the dose after treatment failure. The lower limit of the therapeutic range for IFX levels in this study was 0.5 μg/mL [3]. Marits et al. [26] showed significantly higher IFX levels in patients in remission than in those experiencing disease flare and reported that patients with an IFX level >4.1 μg/mL would be in remission with 87% sensitivity and 44% specificity. Moreover, regular measurement of IFX levels followed by faster dose escalation can increase the probability of continued IFX treatment [25]. In a review, Vande Casteele et al. reported a cut-off value of 3 μg/mL for remission in adult patients [26, 27]. The same author performed an-

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other study in 483 adult patients in which the cut-off was calculated as 2.7 μg/mL using CRP level ≤5 mg/L as a marker of remission. In a prospective randomised controlled trial, target doses of 3–7 μg/mL were recommended; however, after dose optimization, continued concentration-based dosing was not superior to clinically based dosing [28].

Fewer studies have been performed among paediatric populations. High IFX levels in a study by Hamalainen et al. [29] were negatively associated with disease activity, and all patients with undetectable IFX levels presented ATIs [30]. Singh et al. [31] found that IFX levels at week 14 after treatment initiation, together with CRP levels, can predict remission at week 54. Cornillie et al. [32] have reported similar results. It also appears that, in both, children and adults, positive ATIs contribute to lower IFX levels. Higher ATIs are possibly associated with a higher risk of surgery in paediatric patients. It is clear from these studies on adult and paediatric populations that optimal IFX trough levels may vary widely, probably depending on the study population and the method used for the determination of IFX levels and ATIs. In our study, IFX trough level of ≥1.1 μg/mL, as determined using ELISA, was associated with remission (defined as CRP level ≤5 mg/L) with acceptable sensitivity and specificity. Our findings are in agreement with those of a study on an adult population where the IFX levels were associated with inflammatory markers [27]. Recently, it has been discussed whether measurement of mid-interval IFX levels rather than trough levels would enable optimal results [33]. The determination of the optimal cut-off value for IFX levels is complicated and should always be related to a clinically relevant outcome. Different cut-offs are recommended by various kit manufacturers, depending on the disease type [34, 35, 36]. The kit used in the present study stated a therapeutic range starting at 1 μg/mL, which correlates with our results. However, values and detection ability may vary among commercially available kits as reported by Vande Casteele et al. [27]. Therefore, our data may not be generalized and applicable to situations where other methods are selected for determining IFX levels [26].

CPT and ESR can also be used for defining laboratory remission in CD patients. With respect to patients treated with IFX, however, it seems that low CRP level is the most suitable marker for evaluating IFX trough levels. We also found an association between serum albumin and trough IFX levels; this was also reported by Frymoyer et al. [37]. This observation may have implications for clinical practice, potentially implying that a normal serum albumin level should be attained before considering further therapeutic steps based on low trough IFX levels.

According to our assessments, we found that many samples with high ATIs had undetectable IFX levels; however, in many other samples, it was possible to detect IFX levels in the presence of ATIs. In our study, there was pronounced heterogeneity in the patients in terms of treatment length, concomitant AZA therapy, and intensification of therapy. All these factors were considered and tested during statistical analyses. Both, the IFX and ATIs kits used in the study are based on the sandwich immunoassay principle and are reportedly precise with a given recovery rate of 98% for IFX and 97% for ATIs in serum of known concentrations [35, 36, 38]. Data from Vande Casteele's study showed that higher ATIs are associated with higher disease activity despite adequate IFX trough levels [19]. We also evaluated the relationship between ATIs and disease activity. ATIs measurement did not appear to provide any additional benefit in predicting disease activity; however, it may contribute important information when assuming low trough IFX levels.

Based on recently published data, it appears that PCDAI is a weak predictor of clinical outcome and endoscopic and histological lesions [39, 40], and none of the PCDAI versions can perform a valid assessment of mucosal healing [41]; therefore, we used PCDAI only as a covariate, not as a primary outcome.

The strength of our study is that, to our knowledge, our sample size is the highest among all studies conducted on this subject. We systematically observed patients longitudinally and collected data over an extended period of time. We confirmed, in a paediatric population, the previously published conclusions for adult patients, such as the association between high ATIs and lower trough IFX levels as well as that between higher trough IFX levels and lower levels of the markers of disease activity. We also demonstrated the suitability of the combined use of CRP levels and trough IFX levels >1.1 μg/mL as a marker of remission. However, our study also had certain limitations such as the lack of sufficient CPT samples due to low patient compliance. We also did not assess the relationship between IFX trough level and AZA dose, which has been reported to affect the development of ATIs. Another limitation of our study is that the ELISA method that we used is not the most precise method for ATIs measurement, although it is the most commonly used method. ATIs measurement can also be performed using fluid-phase radioimmunoassay and homogeneous mobility shift assay

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(HMSA), which are reportedly more precise. However, a gold standard measurement method and corresponding optimal cut-off levels for each method have not yet been established [4].

Conclusion

Based on our results, we conclude that the paediatric population is similar to the adult population in terms of the association between IFX levels and ATIs, as well as between IFX levels and disease activity. The optimal combination of sensitivity and specificity for disease activity using CRP <5 mg/L was calculated for IFX levels ≥1.1 μg/mL. Moreover, serum albumin levels, and possibly concomitant AZA treatment, should be considered when interpreting IFX levels. No association was found between remission and ATIs.

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Endoscopic Recurrence 6 Months After Ileocecal Resection in Children With Crohn Disease Treated With Azathioprine

*Kristyna Zarubova, *Ondrej Hradsky, *Ivana Copova,
yBlanka Rouskova, yLucie Pos, yRichard Skaba, and *Jiri Bronsky

ABSTRACT

Objectives: Intestinal surgery is an important part of Crohn disease (CD) treatment in children. The aim of the present study was to compare the rate of endoscopic recurrence at the sixth month after ileocecal resection (ICR) in children with CD treated with azathioprine between patients who received prior antitumor necrosis factor alpha (anti-TNF-α) therapy and those who were not administered this therapy. Moreover, we tried to identify the potential risk factors for disease recurrence and describe the schedule of long-term follow-up after surgery.

Methods: We prospectively collected data from pediatric patients with CD, who underwent ICR between October 2011 and June 2015 at our hospital and were treated with azathioprine monotherapy after ICR. We evaluated the endoscopic recurrence (Rutgeerts score) at the sixth month after ICR in all included patients.

Results: Among 21 included patients, 13 achieved endoscopic remission (Rutgeerts score < i2) at the sixth month after ICR. No difference was found between patients who received prior anti-TNF-α therapy and those who did not. We did not find any clinically relevant factors associated with endoscopic recurrence rate at the sixth month.

Conclusions: Prior anti-TNF-α therapy does not seem to be a strong risk factor for endoscopic recurrence within 6 months after ICR. Further studies on large sample of patients are needed to identify potential predictors of disease recurrence.

Key Words: azathioprine, Crohn disease, endoscopy, ileocecal resection, pediatric, recurrence

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What Is Known

- Up to 70% of the adult patients need intestinal surgery during the first 10 years after diagnosis.
- One-third of adult patients have clinical recurrence during the first year after intestinal resection, although more than 80% have endoscopic recurrence.

What Is New

- Endoscopic evaluation at 6 months after ileocecal resection is a reliable tool for the assessment of disease recurrence.
- Prior antitumor necrosis factor alpha therapy does not seem to be a strong risk factor for endoscopic recurrence.

Crohn disease (CD) is mostly diagnosed during adulthood; nevertheless, 7% to 20% of the patients are diagnosed at the age of 0 to 19 years (1). Childhood-onset CD is described as more aggressive, with higher disease activity and greater need of immunosuppressive therapy than adulthood-onset CD (2,3). Moreover, pediatric CD is associated with growth retardation and pubertal delay (4–6). It could be one of the reasons for elective surgery (7). Up to 70% of the adult patients need intestinal surgery during the first 10 years after diagnosis (8,9). In children, the cumulative incidence of surgery increases in a disease duration-dependent manner and within 5 years after diagnosis, 17% to 34% of the pediatric patients undergo surgery (10,11). One-third of adult patients have clinical recurrence during the first year after intestinal resection, although >80% have endoscopic recurrence (12,13). Smoking, penetrating disease, perianal disease, and previous intestinal surgery were identified as risk factors for postoperative recurrence in adult patients in several retrospective studies and the prospective study, Postoperative Crohn Endoscopic Recurrence study (14). These conditions have, however, not been identified as risk factors in pediatric patients thus far. For preventing the postoperative recurrence in adults, mostly metronidazole (15), thiopurines (alone or in combination with metronidazole) (14,16,17), and/or antitumor necrosis factor alpha (anti-TNF-α) therapy (14,18) are used. Studies reporting the use of 5-aminosalicylic acid (proving its effect only on clinical, but not endoscopic remission) (19) and enteral nutrition in adult patients are available as well (20).

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From the *Gastroenterology, Hepatology and Nutrition Unit, Department of Pediatrics, and the yDepartment of Pediatric Surgery, 2nd Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic.

Address correspondence and reprint requests to Kristyna Zarubova, MD, University Hospital Motol and Second Faculty of Medicine, V Uvalu 84, Prague 5, 150 06, Czech Republic (e-mail: zarubova.kristyna@gmail.com).

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The primary aim of the present study was to compare the rate of postoperative endoscopic recurrence after ileocecal resection (ICR) in children treated with azathioprine (AZA) monotherapy between patients who received prior anti-TNF-α therapy before surgery and those who did not. Moreover, as a secondary aims, we tried to identify the potential risk factors for disease recurrence and describe the long-term follow-up after surgery.

METHODS

Subjects

We prospectively collected data from pediatric patients with CD, who underwent intestinal surgery between October 2011 and June 2015 at our university hospital (tertiary referral center for inflammatory bowel diseases and for pediatric surgery), only information about diagnosis and preoperative treatment was collected retrospectively. According to our internal standards, patients without residual disease after surgery were treated with AZA monotherapy, whereas patients with residual disease were treated with anti-TNF-α agents. Decision on postoperative treatment was based solely on the presence of residual disease and preoperative anti-TNF treatment had no influence on the decision. Residual disease was defined as any region of active inflammation outside of the resected ileocecal area; an ulcer or aphthous lesions or stricture with inflammation present in any part of the gastrointestinal tract, which can be visualized endoscopically (from the oral cavity to the duodenum and from the terminal ileum to the anus), a perianal disease, and/or significant active inflammation of the small intestine or the upper gastrointestinal tract (identified by or by magnetic resonance enterography [MRE]). In minority of patients where MRE could not be done before surgery (mostly because of emergent need of surgery), the residual disease was evaluated only by endoscopy and manually by surgeon during operation (lumen of the bowel was not checked).

Inclusion criteria were as follows: an age of 0 to 19 years, a diagnosis of CD (according to Porto criteria and revised Porto criteria) (21), an indication of ICR (including combination with evacuation of abscess [n ¼ 5], other ileal [n ¼ 3] or partial (segmental) colonic resection [n ¼ 3], strictureplasty [n ¼ 1] or fistulectomy [n ¼ 3]—none of the fistulas was in the perianal area), no residual disease, undergoing endoscopy/MRE before ICR, AZA monotherapy after ICR, patients and their legal representatives agreed to participate in the study, and signed informed consents.

Exclusion criteria were as follows: an active colonic inflammation not suitable for surgery (confirmed by endoscopy before ICR), an active or previous perianal disease, change of therapy (an interruption of AZA therapy or a newly started anti-TNF-α therapy) before the first endoscopy after ICR, and a follow-up in another hospital after surgery.

All ICRs were performed by pediatric surgeons. During the perioperative period, all patients had short-term prophylactic antibiotics as indicated by the surgeon. No patient had long-term prophylactic therapy (metronidazole, aminosalicylates, or enteral nutrition) other than AZA after ICR. We included all patients who underwent elective surgery and patients who underwent an acute procedure for management of the complications but had neither residual disease nor complicated course after surgery. Median (interquartile range [IQR]) of AZA dosage was 1.95 mg/kg (1.48–2.14), patients with intermediate activity of thiopurine methyltransferase received lower doses of AZA, as recommended. Only 1 patient had no AZA therapy before surgery. We did not routinely monitor AZA metabolites or levels and antibodies of infliximab and adalimumab. Twenty patients were treated with AZA from diagnosis to surgery, 1 patient underwent ICR soon after diagnosis. Median (IQR) duration of anti-TNF-α therapy was 15.5 month (5.3–34) until surgery.

During the study period, 54 intestinal surgeries were performed in 51 patients of which 47 were eligible to be considered for the study (underwent ICR). To describe the extent of the disease, we performed endoscopy in 44 patients (93.6%) with a maximal interval of 100 days before the intestinal surgery. In 21 patients, who fulfilled inclusion criteria, median (IQR) time from endoscopy to surgery was 26.5 (14.5– 72.5) days. Among all patients, 27 patients (57%) fulfilled the inclusion criteria. During follow-up, 6 patients (21%) were excluded: 1 patient had epidural abscess postoperatively (AZA therapy had to be terminated), 1 patient had wound complication, and 4 patients were lost during the follow-up. The inclusion of patients in the study is shown in detail in Figure 1.

Among the remaining 21 patients who fulfilled the inclusion criteria and completed the follow-up, 10 were exposed to anti-TNF-α therapy (1 to adalimumab and 9 to infliximab) before surgery, whereas 11 patients did not receive this therapy (had no indication for starting anti-TNF therapy—no perianal disease, no growth failure or relapse on immunomodulators, or intolerance of immunomodulators).

The present study was approved by the ethical committee of our university hospital. All patients and/or their parents signed informed consents.

Data Collection

The following data were collected retrospectively from the hospital electronic medical records: clinical data (Table 1 and Supplemental Digital Content 1, Table, *http://links.lww.com/MPG/A853* for details); previous endoscopic and imaging methods findings; and surgical data (length of resection and dilatation of the bowel).

The following data were collected prospectively after surgery during at least 6 months of follow-up: clinical data (Pediatric Crohn Disease Activity Index [PCDAI], development of new fistulizing or perianal disease after ICR), laboratory results (blood count, erythrocyte sedimentation rate, C-reactive protein, serum albumin), body mass index *z* score, and endoscopic findings. Fecal calprotectin (CPT) was examined only in 14 patients (67%) before surgery with a maximal interval of 4 months before ICR and in 13 patients (62%) during the follow-up. Clinical characteristics of the patients are listed in Table 1 and in Supplemental Digital Content 1, Table, *http://links.lww.com/MPG/A853*.

Endoscopic Follow-up

Gastroscopy and ileocolonoscopy were performed between the sixth and eighth month after ICR by a pediatric gastroenterologist. Endoscopic recurrence was described using Rutgeerts score (13) as follows:

1. i0 (no aphthous lesions in the distal ileum) and i1 (S5 aphthous lesions) were defined as remission.
2. i2 (2:5 aphthous lesions with normal mucosa between the lesions, skip areas of larger lesions, or lesions confined to the ileocolonic anastomosis that is <1 cm in length), i3 (diffuse aphthous ileitis with diffusely inflamed mucosa), and i4 (diffuse inflammation with larger ulcers, nodules, and/or narrowing) were defined as recurrence.

In case of recurrence of the disease (anastomotic or ileal recurrence alone or in combination with recurrence in other places, perianal, colonic, or small bowel), anti-TNF-α treatment was started and the condition was considered as a ''relapse.''

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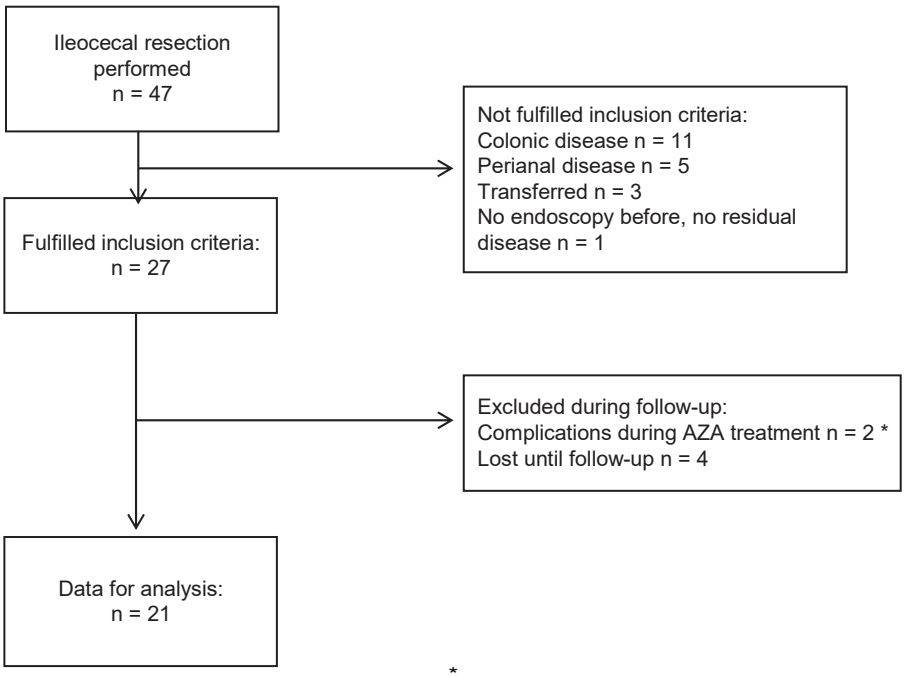

FIGURE 1. Flowchart of the inclusion criteria of patients into the study. * One patient had incisional fistula 1.5 months after ileocecal resection, 1 patient ceased azathioprine (AZA) treatment because of sinusitis and epidural abscess.

After the first postoperative endoscopy, we performed endoscopies in various intervals (6–12 months) or at the time of clinical relapse.

Primary and Secondary Outcomes

The primary outcome was defined as the proportion of patients with a Rutgeerts score less than i2 six months after ICR. Secondary outcomes were to find potential predictors of disease recurrence and to describe long-term endoscopic follow-up of patients after ICR (Supplemental Digital Content 2, Fig., *http://links.lww.com/MPG/A854*).

Statistical Analysis

All data analyses were performed using R statistical software (version 3.2.3) (22). The median and the IQR were used as continuous variables. The absolute frequencies and percentages

TABLE 1. Patients' characteristics

	Rutgeerts <i2	Rutgeerts ≥i2	P^*	P^y	Corrected P^z
Number of patients	13	8			
Sex male (%)	8 (0.62)	6 (0.75)	0.874	0.656	1
Age at diagnosis, y, median (IQR)	14.8 (13.9–16.3)	14.1 (11.3–17.1)	0.75	0.75	1
Age at surgery, y, median (IQR)	16.7 (15.3–17.6)	16.9 (16.7–17.2)	0.772	0.764	1
ESR at the time of surgery, mm/hod, (IQR)	23 (13–29)	39.5 (23.8–62)	0.158	0.153	0.541
Albumin at the time of surgery, g/L, (IQR)	44.7 (42.7–47.5)	42.2 (38.7–44.1)	0.046	0.042	0.528
CPT at the time of surgery, *m*g/g, (IQR)	1800 (1278.5–1800)	1000 (876–1357)	0.237	0.24	0.565
PCDAI the time of surgery (IQR)	15 (7.5–17.5)	23.8 (15.6–46.2)	0.1	0.097	0.534
Anti-TNF therapy before surgery (%)	6 (0.46)	4 (0.5)	1	1	1
Dilatation on MRE (%)	9 (0.69)	2 (0.25)	0.128	0.08	0.528
Acute surgery (%)	2 (0.15)	4 (0.5)	0.227	0.146	0.541
Perforation found (%)	6 (0.46)	5 (0.62)	0.781	0.659	1
Azathioprine, $mg \cdot kg^{-1} \cdot day^{-1}$, (IQR)	2 (1.6–2.1)	2 (1.5–2.3)	0.885	0.874	1
5-ASA (%)	1 (0.08)	1 (0.12)	1	1	1
Rutgeerts, i0 to i4, (IQR)	0 (0–1)	2 (2–3)	0	0	0

5-ASA ¼ 5-aminosalicylic acid, CPT ¼ fecal calprotectin, ESR ¼ erythrocytes sedimentation rate, IQR ¼ interquartile range; MRE ¼ magnetic resonance enterography, PCDAI ¼ pediatric Crohn disease activity index; TNF ¼ tumor necrosis factor.

P values from Wilcoxon rank-sum test or chi-squared test.

[y]*P* values from Wilcoxon permutation test or permutation-based chi-squared test of independence.

[z]False discovery rate correction of the permutation-based tests for multiple testing.

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were employed as categorical variables. Wilcoxon rank-sum test and Wilcoxon permutation test (23) (using R package ''coin'' version 1–1.2) were used for comparing the continuous variables between the 2 groups due to the small sample size. To test our hypothesis using the categorical data, the chi-squared test and the permutation-based chi-squared test of independence (using R package ''coin'' version 1–1.2) were used due to the small sample size (24). Kaplan-Mayer curve was used to display the time to relapse and the Cox-regression analysis was used to test the significance of the difference between patients with and without prior anti-TNF-α therapy. A P value <0.05 was considered to be statistically significant. We used the false discovery rate correction method for multiple testing.

RESULTS

Primary Outcome

Among the 21 included patients, 13 had endoscopic remission (Rutgeerts score < i2) at the sixth month after ICR. There was no difference in remission rate between patients who received anti-TNF-α therapy before surgery (6/10, 60%) and those who did not (7/11, 64%), see Table 1.

Factors Associated With Endoscopic Recurrence

A lower concentration of serum albumin (P ¼ 0.042) was found to be associated with higher endoscopic recurrence rate at the sixth month. After correction for multiple testing, we could not find any difference (Table 1). Other factors, see Table 1 and Supplemental Digital Content 1, Table, *http://links.lww.com/MPG/A853*, were not found to be associated with endoscopic recurrence even without correction for multiple testing. Six patients underwent acute surgery; the other underwent ICR electively, so there was no statistical difference found between groups.

CPT was examined only in 62% of the patients during 6 months of follow-up. The cut-off concentration for remission was set at 100 mg/g; the CPT in 10 patients was in accordance with the endoscopic findings (CPT < 100 mg/g þ Rutgeerts score < i2 and CPT > 100 mg/g þ Rutgeerts score 2: i2). One patient had a CPT of <100 mg/g and Rutgeerts score of 2:i2, another patient had a comparable CPT value (137 mg/g) and Rutgeerts score of i2, and 2 patients had CPT >100 mg/g (187, 273 mg/g) and a Rutgeerts score of i1.

PCDAI (remission defined as PCDAI <10) 6 months after ICR was not in association with the Rutgeerts score (remission defined as Rutgeerts score <i2) (P ¼ 0.206).

We did not find any difference in the long-term disease recurrence between patients who received prior anti-TNF-α therapy and those who did not by using the Kaplan-Meier curve (Supplemental Digital Content 2, Fig., *http://links.lww.com/MPG/A854*).

DISCUSSION

To our knowledge, this is the first prospective study evaluating the endoscopic recurrence after ICR in pediatric population on prophylactic AZA monotherapy. Despite the small sample size, it seems that anti-TNF-α therapy before elective or acute ICR is not a strong negative predictive factor with respect to the subsequent endoscopic relapse of the disease in patients without residual disease. In the scientific literature, we did not identify any other pediatric study evaluating predictive value of preoperative anti-TNF-α treatment with respect to postoperative endoscopic recurrence. Thus, we could not compare our results with those of other studies. Based on our data, it, however, seems that in pediatric patients without residual disease after ICR, it is possible to continue prophylactic AZA monotherapy even in patients with unfavorable preoperative course of the disease requiring anti-TNF-α therapy before surgery. A potential limitation of our study is that we did not determine AZA metabolites to optimize the dosage and to control the patient compliance.

In our study, the endoscopic recurrence rate was 38% at the sixth month compared with 45% in high-risk adult patients treated with thiopurines 6 months after surgery (14). Bobanga et al reported a recurrence rate of 87% in patients younger than 16 years, who underwent endoscopy at various time points of long-term follow-up (mean ¼ 3 years). Criteria for endoscopy and treatment after ICR were, however, not clearly described (only 13/34 patients underwent endoscopy) (25). In a retrospective study by Hansen et al (26) in 115 pediatric patients, 26% of the patients developed clinical recurrence (evaluated only by the physician global assessment) within 6 months after ICR; however, not all patients were treated with AZA. Baldassano et al (27) collected retrospective data from 68 patients after bowel resection (54% of them underwent ICR) and 17% developed clinical recurrence at 1 year (defined by a PCDAI >30). Pediatric patients after ICR constitute a high-risk group requiring strict follow-up after surgery. In our study, the PCDAI was not in association with endoscopic remission or recurrence. This is questionable, whether only clinical evaluation (using physician global assessment or PCDAI) is sufficient to reveal the recurrence of the disease that requires continuation of therapy. PCDAI was not confirmed as a good marker of disease severity found upon endoscopy (28); however, the present study did not include patients who had already undergone ICR. In addition, Crohn disease activity index has been found to have the same limitations in adults as a predictor of relapse (29). In spite of the fact that there are no follow-up studies comparing the predictive value of clinical compared with endoscopic recurrence with respect to the long-term clinical outcomes in children, we believe that suggestions/decisions regarding treatment based on the endoscopic follow-up after ICR should be standardized for the pediatric population, similar to adult patients. Studies identifying the predictors of disease recurrence in children are needed to identify subgroups of patients that need close postoperative follow-up, especially with respect to indication of early endoscopy after surgery. Based on our data, there is no difference between patients who received prior anti-TNF-α therapy and those who did not in long-term follow-up (endoscopy 1 year after ICR or later).

In a retrospective study carried out by Baldassano et al (27), preoperative 6-mercaptopurine, PCDAI at the time of surgery, and colonic disease were identified as risk factors of clinical recurrence. In the present study, patients with colonic disease were considered as high-risk patients and did not match the inclusion criteria for AZA monotherapy. According to our data, albumin level at the time of surgery seems to be a potential predictor of endoscopic recurrence at the sixth month; however, results did not reach statistical significance after correction for multiple testing. Moreover, all patients in our study had normal levels of albumin, but cut-off levels for healthy population may not be relevant for the outcome of the present study. If differences in albumin levels within normal range for healthy population may be predictors of disease recurrence, it needs further evaluation and another cut-off for this specific clinical situation may be defined in the future. Therefore, the potential predictors should be evaluated in a larger prospective study. Other previously identified risk factors include smoking (14), previous resection (in our study, no patient had previous surgery), and a perforating disease (not confirmed by our data, P ¼ 0.66) (30). Nevertheless, we may have not been able to find relevant predictors of unfavorable outcome because of the fact that the high-risk patients were not included in our study and did not continue (or start) anti-TNF-α treatment after surgery. Using CPT alone to suggest the timing of endoscopy was not reliable in our study. Three

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patients had low CPT (<250 mg/g) of which 1 patient had CPT <100 mg/g in spite of having endoscopic disease recurrence. The number of patients included in our study is, however, limited (we were able to collect CPT samples in 67% of patients only). These findings are not in accordance with those of a previous meta-analysis that identified CPT as a highly sensitive marker for assessing endoscopic postoperative recurrence (31). CPT was previously successfully tested as a marker of postoperative recurrence in children (32).

CONCLUSIONS

Prior anti-TNF-α therapy does not seem to be a strong risk factor for endoscopic recurrence within 6 months after ICR and probably in long-term follow-up. Serum albumin level at the time of surgery could be a potential predictor of disease recurrence; however, studies with a larger sample size are needed to confirm these findings.

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ORIGINAL ARTICLE

Risk factors for dermatological complications of anti-TNF therapy in a cohort of children with Crohn's disease

Ondrej Hradsky[1] & Denis Kazeka[1] & Ivana Copova[1] & Tereza Lerchova[1] & Katarina Mitrova[1] & Kristyna Pospisilova[1] & Miroslava Sulovcova[1] & Kristyna Zarubova [1] & Jiri Bronsky[1]

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Abstract

Studies showing a substantial frequency of dermatologic complications in paediatric Crohn's disease (CD) patients on anti-tumour necrosis factor (TNF) therapy preferentially include patients treated with infliximab. We aimed to identify risk factors for the cumulative incidence of skin complications in a paediatric cohort receiving either adalimumab or infliximab and found an association between current skin complications and the patient's current clinical condition. This study retrospectively evaluated dermatologic complications in an inception cohort of 100 paediatric CD patients receiving the first anti-TNF (Motol PIBD cohort). Patient data were collected every 3 months. The lesions were classified as psoriatic, atopic dermatitis, or others. We used Cox regression to evaluate the association between predefined variables and the time to complication and a generalised linear mixed model to assess the association between the patient's current condition and the occurrence of complications. Among the 89 included children, 35 (39%) presented with dermatologic lesions. The only predictor associated with any complication was infliximab (versus adalimumab) therapy (hazard ratio [HR]: 2.07; 95% confidence interval [CI]: 1.03–4.17; $p = 0.04$). Infliximab therapy (HR: 5.5; 95%CI: 1.59–19.06; $p = 0.01$) and a family history of atopy (HR: 3.4; 95%CI 1.35–8.57, $p = 0.002$) were associated with early manifestation of atopic dermatitis. Lower C-reactive protein levels (odds ratio [OR], 0.947; 95% CI, − 0.898 to 0.998; $p = 0.046$) and infliximab (versus adalimumab) were associated with the occurrence of any dermatologic complications (OR, 5.93; 95% CI, 1.59–22.07; $p = 0.008$).

Conclusion: The frequency of skin complications seems high in paediatric CD patients treated with anti-TNF and is even higher in those treated with infliximab.

What is Known:
- *The dermatologic complications occur during treatment with anti-tumour necrosis factor.*
- *The frequency of skin complications in paediatric patients with Crohn's disease is high.*

What is New:
- *Infliximab (vs. adalimumab) was identified as a strong risk factor for the cumulative incidence of skin complications.*
- *Lower C-reactive protein levels were associated with the current occurrence of dermatologic complications.*

* Ondrej Hradsky
ondrej.hradsky@lfmotol.cuni.cz; ondrej.hradsky@gmail.com

Denis Kazeka
denkazeka@gmail.com

Ivana Copova
icopova.ic@gmail.com

Tereza Lerchova
terezadrskova@gmail.com

Katarina Mitrova
katarina.mitrova@gmail.com

Kristyna Pospisilova
kimmulka@gmail.com

Miroslava Sulovcova
mirkasluovcova@gmail.com

Kristyna Zarubova
zarubova.kristyna@gmail.com

Jiri Bronsky
jiri.bronsky@gmail.com

[1] Department of Pediatrics, 2nd Faculty of Medicine, Charles University in Prague and Motol University Hospital, V Uvalu 84, 150 06, Prague 5, Prague, Czech Republic

Keywords Eczema · Psoriasis · Skin complications · Adalimumab · Infliximab

Abbreviation

ADA	Adalimumab
Anti-TNF	Anti-tumour necrosis factor
CI	Confidence interval
CD	Crohn's disease
CRP	C-reactive protein
f-CPT	F-calprotectin
HR	Hazards ratio
IFX	Infliximab
IQR	Interquartile range
OR	Odds ratio
wPCDAI	Crohn's disease activity index

Introduction

Adalimumab (ADA) and infliximab (IFX) are the only two biologics licensed for the treatment of paediatric Crohn's disease (CD) in the European Union and North America. These two anti-tumour necrosis factor (TNF) antibodies have shown similar efficacy as induction and maintenance therapy for CD in randomised control trials [1, 2]. Based mainly on the data from the TISKids study [3], the current European Crohn's and Colitis Organisation and European Society for Paediatric Gastroenterology, Hepatology and Nutrition guidelines recommend anti-TNF as the primary therapy for induction and maintenance in children with a high risk of poor outcome [4]. The safety issues of these drugs are thus becoming even more important owing to their wide use. Several complications of anti-TNF therapy have been described in adults [5, 6]. Recent data have shown that dermatologic complications are the most frequent [7, 8]. Psoriatic lesions, atopic dermatitis, and infections are the most common skin complications in the paediatric population [9–11]. Although most paediatric studies have described these complications preferentially with IFX treatment, data comparing ADA and IFX are scarce. Several risk factors have been proposed, although mostly with conflicting results, and only a few have been described as a risk more than once [7–12].

The aim of this study was to identify risk factors for the cumulative incidence of skin complications in a cohort of paediatric patients treated with either of the anti-TNF drugs - ADA or IFX. We aimed to evaluate the risk factors for all skin complications and separately for psoriatic lesions and atopic dermatitis. Furthermore, we evaluated the association between current skin complications and the current clinical condition of the patient.

Materials and methods

Patient population and data collection

The study was designed as a retrospective evaluation of dermatologic complications in the inception cohort of 100 paediatric patients with CD treated with ADA or IFX as a first-line biological treatment (Motol PIBD cohort). The cohort primarily focused on evaluating the efficacy of anti-TNF treatment, and the follow-up ended when the patient reached the primary endpoint of the cohort (stopping the current anti-TNF therapy, intensification of anti-TNF therapy, abdominal surgery, and complications of the disease, such as newly diagnosed ileocecal stricture, abscess, or perianal disease). The minimum follow-up period without reaching the endpoint was 24 months. The first administration of anti-TNF was administered between 2013 and 2017. Patient data were collected from electronic medical charts using the REDCap [13]. The collected data included demographic characteristics (age, sex, ethnicity); baseline characteristics and classification of the disease (immunopathology, Paris classification, anti-Saccharomyces cerevisiae antibody, anti-neutrophil cytoplasmic antibody), personal and family medical history (dermatologic conditions and inflammatory bowel disease [IBD]); clinical characteristics and basic laboratory data from the time of diagnosis, from the time of first anti-TNF application, and in 2- to 3-month intervals during follow-up (height, weight, weighted paediatric Crohn's disease activity index [wPCDAI], erythrocyte sedimentation rate, C-reactive protein [CRP], albumin, F-calprotectin [f-CPT], simple endoscopic score, and radiological findings). We also collected data on IBD-related medications (induction treatment, immunosuppressants, dose and interval of anti-TNF therapy, and drug levels and drug antibodies). Exclusion criteria for the analysis were lost to follow-up in less than 2 years, patients' non-adherence to anti-TNF treatment, and missing data on the outcome.

Variables as predictors

Based on previously published literature and theoretical discretion, we pre-selected potential risk factors for dermatological complications: sex, upper gastrointestinal tract involvement, penetrating and structuring behaviour, perianal disease, smoking, Z-score of body mass index, age at first application of anti-TNF treatment, type of anti-TNF, concomitant immunopathology, anti-*Saccharomyces cerevisiae* antibody, anti-neutrophil cytoplasmic antibody, family and personal history

Table 1 Demographic and clinical characteristics of patients

	Patients without dermatologic complication N = 54	Patients with dermatologic complication N = 35	p value
Age at first anti-TNF, years	15.03 (12.1–16.86), NA = 0	15 (13.84–16.25), NA = 0	0.28
Sex, man	35 (0.65), NA = 0	20 (0.57), NA = 0	0.47
Smoking	2 (0.04), NA = 0	2 (0.06), NA = 0	0.66
Ethnicity (Caucasians)	51 (0.96), NA = 1	34 (0.97), NA = 0	0.81
Family history of IBD	10 (0.19), NA = 0	2 (0.06), NA = 0	0.07
Immunopathology	2 (0.04), NA = 0	4 (0.11), NA = 0	0.16
Localisation L1[1]	17 (0.31), NA = 0	10 (0.29), NA = 0	0.77
Localisation L2[1]	5 (0.09), NA = 0	1 (0.03), NA = 0	0.21
Localisation L3[1]	32 (0.59), NA = 0	24 (0.69), NA = 0	0.37
L4 (non L4a nor L4b) [1]	19 (0.35), NA = 0	14 (0.40), NA = 0	0.65
Behaviour B1[1]	36 (0.67), NA = 0	28 (0.80), NA = 0	0.17
Behaviour B2[1]	11 (0.20), NA = 0	5 (0.14), NA = 0	0.46
Behaviour B3[1]	4 (0.07), NA = 0	2 (0.06), NA = 0	0.75
Behaviour B2&B3[1]	3 (0.06), NA = 0	0 (NA), NA = 0	0.08
Perianal disease[1]	12 (0.22), NA = 0	8 (0.23), NA = 0	0.94
Growth retardation, G1[1]	10 (0.19), NA = 0	12 (0.35), NA = 1	0.08
ASCA, positivity in IgG or IgA	42 (0.88), NA = 6	26 (0.84), NA = 4	0.65
ANCA, positivity	7 (0.14), NA = 5	6 (0.20), NA = 5	0.51
Number of eosinophils	0.13 (0.07–0.36), NA = 13	0.11 (0.05–0.23), NA = 5	0.06
wPCDAI	22.5 (11.88–32.50), NA = 6	35 (9.38–40.00), NA = 9	0.18
Height, Z-score	− 1.4 (− 4.72 to 0.13), NA = 0	− 1.39 (− 2.90 to 0.04), NA=0	0.20
BMI, Z-score	− 1.07 (− 2.37 to 0.11), NA = 0	− 1.33 (− 1.94 to 0.41), NA=0	0.94
Personal history of atopy	32 (0.59), NA=0	24 (0.69), NA = 0	0.37
Family history of atopy	10 (0.19), NA=0	12 (0.34), NA = 0	0.09
Family history of psoriasis	2 (0.04), NA=0	2 (0.06), NA = 0	0.66
Anti-TNF, infliximab	27 (0.50), NA=0	23 (0.66), NA = 0	0.14
Anti-TNF, adalimumab	27 (0.50), NA=0	12 (0.34), NA = 0	0.14
Follow-up on anti-TNF	2.11 (0.63–3.10), NA=0	2.84 (1.92–3.69), NA = 0	0.02
Time to anti-TNF, years	0.78 (0.38–2.03), NA=0	0.89 (0.31–1.62), NA = 0	0.81
Percentage of time on immunomodulator[2]	1 (0.95–1.00), NA=0	1 (1.00–1.00), NA = 0	0.77

[1] According to the Paris classification, [2] 82 was exposed to azathioprine, one to 6-mercaptopurin and two to methotrexate

Continuous variables are presented as medians (interquartile ranges) and categorical variables as absolute frequencies and percentages, and NA number of missing data

TNF, tumour necrosis factor; ASCA, anti-Saccharomyces cerevisiae antibody; ANCA, anti-neutrophil cytoplasmic antibody; wPCDAI, weighted paediatric Crohn's disease activity index; BMI, body mass index

of atopy or psoriasis, and the number of eosinophils at the time of first administration of anti-TNF therapy. For the secondary outcomes, we tested the associations between current skin manifestations and current parameters of disease activity (wPCDAI, CRP, f-CPT), immunosuppressive therapy, and anti-TNF drug serum levels and antibodies.

Outcome

We collected data on the potential skin complications of anti-TNF therapy at each follow-up visit. All patients who manifested potential skin complications of anti-TNF therapy were instructed to visit a dermatologist. The type of dermatologic complication was assessed by a paediatric gastroenterologist or a paediatric dermatologist. If the skin lesion was determined by a paediatric dermatologist as uncertain, a biopsy was indicated. For the purpose of the incidence cohort, the lesions were classified as psoriatic (including psoriasis, psoriatic eczema, and pustulosis), atopic dermatitis, or others probably related to anti-TNF therapy. Significant worsening of atopic dermatitis was also considered a potential complication of anti-TNF therapy.

Statistical analysis

All data were analysed using R statistical software (version 3.6.0) [14]. Continuous variables were described as medians and interquartile ranges (IQR). Categorical variables were described as absolute frequencies and percentages. Missing data were not imputed. The pre-selected predictors were tested using unadjusted Cox regression for three outcomes (time to the first occurrence of any dermatological compilation, time to first psoriatic lesion, and time to first atopic dermatitis or time to first significant worsening of atopic dermatitis during anti-TNF treatment). To assess the importance of particular variables, we further tested the association of skin complications with the variables using multivariable Cox proportional-hazards models. As the secondary aim was to evaluate the association between current dermatological complications and current patient condition, we used a generalised linear mixed model with dermatological complications as an outcome. All these mixed models were adjusted for the follow-up time. The trough levels were tested as quartiles and antibodies as categorical variables. When the values were missing, we omitted the time points from the current analysis.

Ethical considerations

The study was approved by the local ethics committee, and informed consent was obtained from the parents of all patients.

Table 2 Frequency of dermatologic complications

	Number of patients
Any dermatologic complications	35 (0.39)
Psoriatic lesions	13 (0.15)*
- Psoriasis	4 (0.04)
- Psoriatic eczema	6 (0.07)
- Pustulosis	5 (0.06)
Atopic dermatitis	18 (0.20)
Other, probably associated with anti-TNF	11 (0.12)

Some patients exhibited more than one dermatologic complication. *TNF* tumour necrosis factor. * One patient had psoriatic eczema and pustulosis, and one patient had psoriasis and psoriatic eczema

Results

Among the 100 patients included in the cohort, 11 were excluded (Fig. 1). The characteristics of the 89 included patients are summarised in Table 1 and Supplementary Table S1. The median follow-up period for the patients was 2.49 (IQR: 0.90–3.47) years.

Frequency and cumulative incidence of dermatologic complications

Among the entire cohort, we found 35 (39%) patients with any recorded dermatologic manifestations. The most frequent

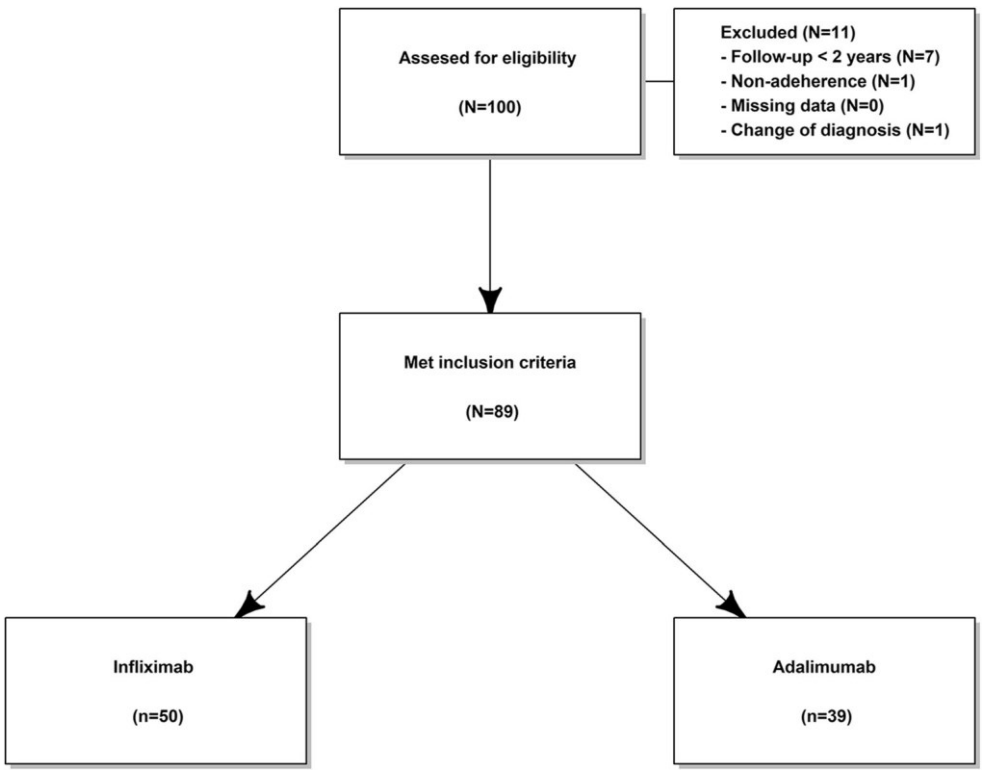

Fig. 1 Flowchart showing exclusions from the cohort

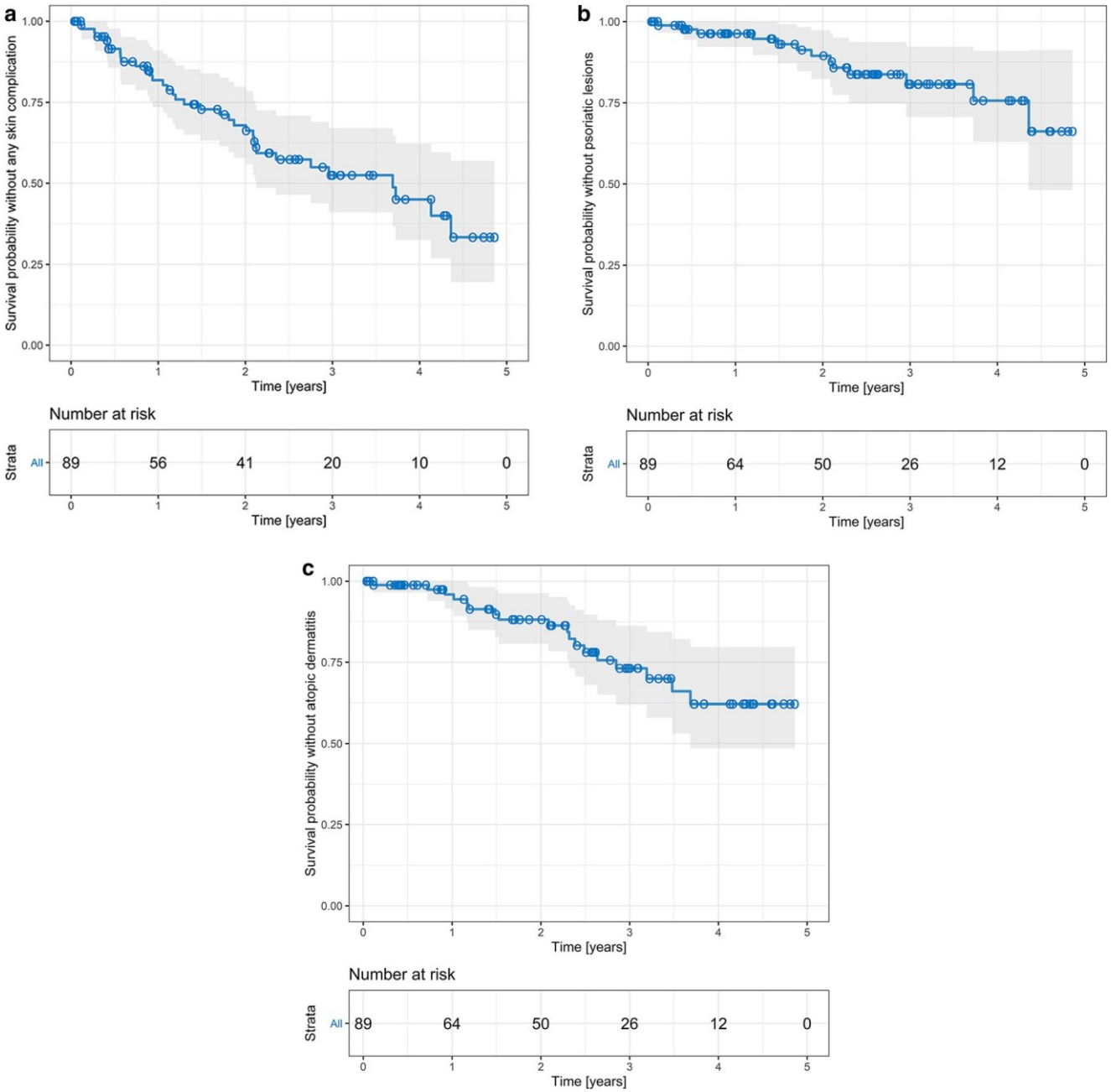

Fig. 2 Cumulative incidence of dermatological complications in paediatric Crohn's disease patients treated with anti-TNF therapy. a For any dermatologic complications, b for psoriatic complications, c for atopic dermatitis. o, censored

symptom was worsening or new-onset atopic dermatitis (n = 18, 20%), followed by psoriatic lesions (n =13, 15%). The occurrence of all skin complications is presented in Table 2. Some patients presented with more than one dermatological complication. Survival curves show the time to first skin complications (Fig. 2). After 2 years of follow-up on anti-TNF therapy, approximately 35% of patients developed at least one of the dermatological complications. During the follow-up, 34 patients stopped anti-TNF treatment; only one patient within the cohort stopped treatment with ADA because of severe psoriasis. All other dermatological complications were managed with topical treatment.

Risk factors for dermatologic complications

In the unadjusted Cox regression analysis with predefined variables (Supplementary Table S2, Fig. 3), the only predictor associated with any complication was IFX therapy (hazard ratio [HR]: 2.07; 95% confidence interval [CI]: 1.03–4.17; p = 0.04). No association was found between the time to the occurrence of psoriatic lesions and the tested predictors. IFX therapy (compared to ADA; HR: 5.5; 95% CI: 1.59–19.06; p = 0.01; Fig. S1A), and a family history of atopy (HR: 3.4; 95% CI: 1.35–8.57; p = 0.002; Fig. S1B) were associated with the

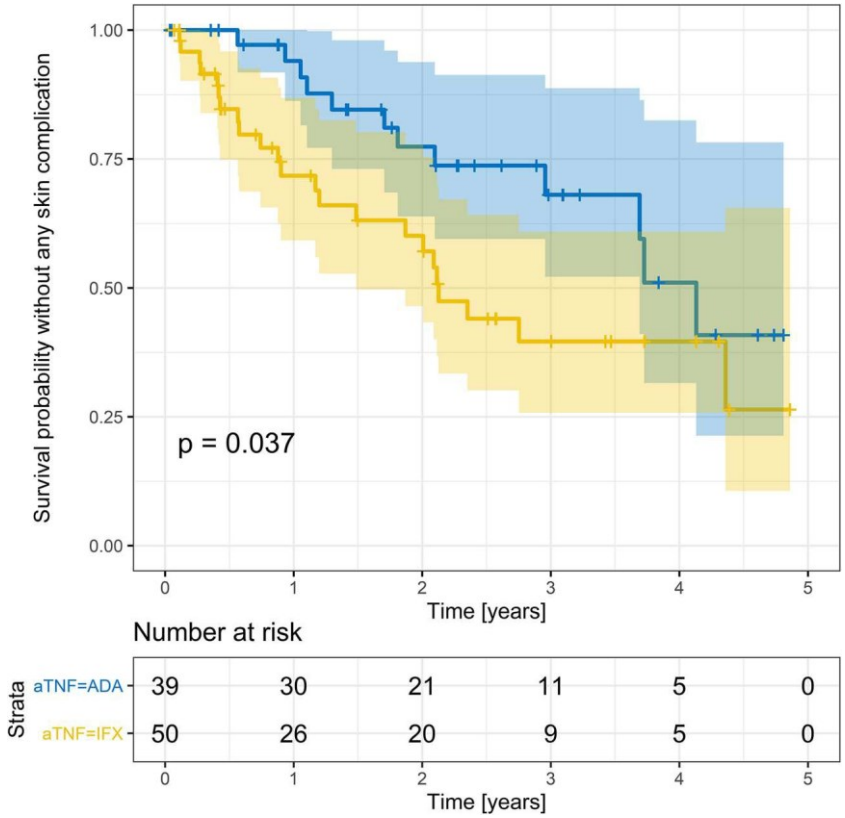

Fig. 3 Difference in cumulative incidence of dermatological complications in paediatric Crohn's disease patients treated with adalimumab or infliximab. o, censored

early manifestation of atopic dermatitis. When both were tested in the multiple Cox regression model, only the association with IFX remained significant (HR, 4.46; 95% CI, 1.26–15.85; $p = 0.021$).

Association between current dermatologic complication and patient's condition

Using a generalised linear mixed model adjusted for follow-up time, we found that lower CRP (odds ratio [OR], 0.947; 95% CI, 0.898–0.998; $p = 0.046$) and the type of anti-TNF were associated with the presence of any dermatologic complications (IFX versus ADA, OR: 5.93; 95% CI: 1.59–22.07; $p = 0.008$). Other factors that expressed patient disease activity (wPCDAI, f-CPT) were not associated with dermatological complications. The associations of both CRP level and type of anti-TNF remained significant in the multiple regression mixed model (Table S3). No association was found with trough levels or antibodies.

Discussion

In the cohort of paediatric CD patients receiving either of the two first-line anti-TNF drugs, after a median of 2.5 years, IFX therapy increased the risk of dermatological complications by two times (HR: 2.07; 95% CI: 1.03–4.17; $p = 0.04$) and by nearly six times when a flare-up of atopic dermatitis was considered the outcome (HR: 5.5; 95% CI: 1.59–19.06; $p = 0.01$).

The only paediatric study, including both drugs (ADA and IFX), found IFX to be a risk factor for psoriasiform lesions [9]. In contrast, large adult studies did not find differences in skin complications with regard to the type of anti-TNF therapy [7, 8, 15]. A recent meta-analysis showed a pooled incidence of eczema in approximately 5.5% (anti-TNF treated) patients in studies that combined 5526 adult patients [16]. Atopic dermatitis was observed in 20% of patients during a median follow-up of 2.5 years, which was slightly higher than that seen in other paediatric studies (12 %–14%) [9, 10]. The type of anti-TNF was strongly associated with atopic dermatitis in our cohort (nearly a six-fold increased risk for IFX) and remained independent of a presumptive association with a family history of atopy on multivariable analysis. Moreover, our data suggested that dermatological complications were associated with decreased systemic disease activity as measured with CRP. It could be hypothesised that effective blocking of TNF accompanied by low CD activity could shift the Th1/Th2 immune response towards Th2, which is the predominant response in atopic dermatitis [17].

The overall incidence of dermatological complications seems to be higher in children than in adults [7]. When prospectively collected, skin complications were found in nearly every other paediatric patient [10]. A total of 39% of non-infectious dermatological complications detected seemed to

be slightly higher than the frequency recognised by retrospective paediatric studies [9, 11].

The true clinical question, assuming the comparable efficacy and safety of these two drugs, is whether this slightly higher frequency of complications can lead to prioritising ADA in paediatric patients (or to give it priority, e.g. in a subgroup of patients). We should admit that even with these new findings, the current data are limited and do not allow us to make a firm generalisable conclusion. It should also be noted that in our cohort, dermatological complications led to anti-TNF withdrawal only in one patient treated with ADA. However, with a personalised approach, one could consider favouring ADA in paediatric patients with a family history of atopy.

This study has some limitations. First, although the cohort was prospectively captured, dermatological complications were collected retrospectively, and it is possible that skin lesions occurred in some patients without documentation in the medical records. Nevertheless, we believe that only a minority of lesions were missed, as the data were collected at intervals of less than 3 months. Moreover, the same technique of data collection was applied for patients treated with both IFX and ADA; thus, the difference should not be explained by that. Importantly, the cohort was homogenous (Table S1). Only the family history of atopy and follow-up period differed between the groups treated with ADA and IFX, and this was statistically managed by adjustment for these covariates in regression mixed models. Second, the cohort sample size was relatively small, and thus generalisation should be performed with caution. Third, although patients were instructed to visit a paediatric dermatologist, some were diagnosed (mainly with minor lesions) by a paediatric gastroenterologist. As the types of dermatologic lesions were not evaluated by one physician alone, lesions could be diagnosed differently.

Conclusion

In conclusion, we found a high frequency of skin complications, especially significant worsening or new-onset atopic dermatitis, in this cohort of paediatric patients with CD treated with anti-TNF therapy. IFX therapy appears to be a predictor of skin complications.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00431-021-04077-0.

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Author's contributions All authors have made substantial contributions to the published work. OH: study design and data analysis, writing up of the first draft of the paper. DK: review of the literature, patient recruitment, data collection. IC: patient recruitment, data collection. KM: patient recruitment, data collection. TL: patient recruitment, data collection. KP: patient recruitment, data collection. MS: patient recruitment, data collection. KZ: patient recruitment, data collection. JB: responsible for leading the project team and revised the work critically for important intellectual content.

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Data availability N/A

Code availability N/A

Declarations

Ethics approval The study was approved by the local ethics committee.

Consent to participate Informed consent was obtained from the parents of all patients.

Consent for publication N/A

Conflict of interest Ondrej Hradsky: Lectures/congress fees/consultancy (outside the submitted work)—MSD, AbbVie, Nutricia, Nestlé, Ferring, and Falk

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ORIGINAL ARTICLE

Seroprevalence of Epstein–Barr Virus, Cytomegalovirus, and Polyomaviruses in Children with Inflammatory Bowel Disease

Ondrej Hradsky[1] · Ivana Copova[1] · Kristyna Zarubova[1] · Marianna Durilova[1] · Jiri Nevoral[1] · Miroslav Maminak[1] · Petr Hubacek[2] · Jiri Bronsky[1]

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Abstract

Background Young age and thiopurine therapy are risk factors for lymphoproliferative disease among patients with inflammatory bowel disease (IBD).

Aims The aims of this study were to evaluate the prevalence of seropositivity for the Epstein–Barr virus (EBV) and human cytomegalovirus (CMV) among children and adolescents with IBD, to assess the viral load of EBV, CMV, and BK and JC polyomaviruses (BKV, JCV) in these patients, and to assess the influence of different therapeutic regimens on seroprevalence and viral load.

Methods Children who had been followed in our center were tested for EBV, CMV, BKV, and JCV in a cross-sectional study. One hundred and six children were included who had Crohn's disease (68 %), ulcerative colitis (29 %), and unclassified IBD (3 %).

Results We found that 64 % of patients were EBV seropositive. The proportion of EBV seropositive patients increased during childhood. Azathioprine therapy ($p = 0.003$) was associated with EBV seropositivity in a multiple logistic regression model, after adjusting for gender, age, and disease activity at determination. We found a significant association between the number of polymerase chain reaction copies and infliximab dose ($p = 0.023$). We did not find any significant association between CMV serology and CMV, BKV, or JCV viral load, or any other therapeutic regimen or clinical characteristics.

Conclusions Treatment with azathioprine appears to be a risk factor for early EBV seropositivity in children with IBD, and the infliximab dose was associated with a higher EBV viral load.

Keywords Epstein–Barr virus · Cytomegalovirus · Inflammatory bowel disease · Azathioprine · Infliximab · Immunosuppressive therapy

Introduction

Immunosuppressive therapy, which includes the use of thiopurines and biologic drugs, has improved outcomes associated with inflammatory bowel disease (IBD). However, this therapy is also associated with an increased risk of infectious and cancerous complications, such as lymphoproliferative disorders (LDs), which has become a major concern for clinicians managing patients with IBD [1]. Immunosuppressive therapy [2] and chronic inflammation [3] likely play major roles in the development of LDs, although it is difficult to distinguish the effect of immunosuppressive therapy from that of the inflammatory disorder itself. Recent data suggest that thiopurine therapy is associated with a three to fivefold increased risk of developing LD [4, 5]. Three lymphoproliferative conditions are reportedly related to immunosuppressive therapy [1]. The Epstein–Barr virus (EBV) plays a crucial role in the two most common conditions, which are post-transplant-like lymphoma with EBV reactivation and early post-mononucleosis lymphoproliferation in young EBV-seronegative males [4, 6]. However, hepatosplenic T cell

& Ondrej Hradsky
ondrej.hradsky@lfmotol.cuni.cz

[1] Department of Pediatrics, University Hospital Motol and Second Faculty of Medicine, Charles University in Prague, V Uvalu 84, Prague 5 150 06, Czech Republic

[2] Department of Microbiology, University Hospital Motol and Second Faculty of Medicine, Charles University in Prague, Prague, Czech Republic

lymphoma also develops in young male patients treated with thiopurine alone, or in association with anti-tumor necrosis factor (TNF)-a treatment, probably without relation to EBV [7–9]. Primary EBV infection has also been associated with hemophagocytic lymphohistiocytosis in children with IBD taking thiopurines [10]. Reported risk factors for developing EBV-related LDs are male gender, young age (under 35 years) [11], and treatment with thiopurines [4, 5]. Viral load has also been reported as a risk factor, although only in a post-transplant setting [12]. Thiopurines are often used in children, and thus this population appears to be more vulnerable to treatment-related complications. Very little data exists regarding EBV seroprevalence [13], and almost no data regarding viral load among children with IBD [14] has been published. Conflicting data exists regarding human cytomegalovirus (CMV) reactivation in adult patients who have been treated with anti-TNF therapy [15–17], and very little data have been published regarding CMV viral load, and no data regarding CMV seroprevalence, among children with IBD [14]. Similarly, very little data have been published regarding JC polyomavirus (JCV) in patients treated with immunosuppressive therapy [14, 18], which was previously associated with progressive multifocal leukoencephalopathy [19]. Reactivation of the closely related BK polyomavirus (BKV) has been described in immunocompromised hosts (kidney and hematopoietic stem cell transplant settings) [20], and thus BKV reactivation (as a trigger of T cell activity) in IBD might also be of interest.

The aims of the study were to evaluate the prevalence of EBV and CMV seropositivity among children and adolescents with IBD, to evaluate the viral load of EBV, CMV, BKV, and JCV in the peripheral blood of these patients, and to evaluate the influence of different therapeutic regimens on seroprevalence and viral load.

Methods

Subjects

Children with at least one visit to the University Hospital Motol between January and March 2014 were tested for EBV, CMV, BKV, and JCV and followed in this cross-sectional study. We included 106 children, with 72 (68 %) cases of Crohn's disease (CD), 31 (29 %) cases of ulcerative colitis (UC), and three (3 %) cases of unclassified IBD. The patients' demographic and clinical characteristics are listed in Table 1. In all patients treated with IFX, in order to standardize the calculated dose, the dose was calculated for their body weight and 8 weeks (the most common interval of IFX administration). Disease activity was expressed by clinical activity index scores for both CD and UC. Due to dissimilarities of PCDAI and PUCAI, we assigned the cutoff for severe activity of both diseases at 30 points or more.

EBV and CMV Serology, and the Detection of Viral Load

All patients were tested for the presence of antibodies against EBV proteins (antiviral capsid antigen [VCA] IgM and IgG, anti-early antigen [EA] IgG, and anti-Epstein–Barr nuclear antigen IgG) and against CMV (anti-CMV IgG and IgM). The patient's serum samples were tested by using a Liaison machine and appropriate detection kits (DiaSorin, Saluggia, Italy). Patients with antibodies against EBV VCA, Epstein–Barr nuclear antigen, EBV EA, or CMV were designated as seropositive.

Nucleic acid extraction from whole blood was performed using a DNA blood mini kit (Qiagen, Valencia, CA), following the manufacturer's instructions. Detection of the EBV, CMV, BKV, and JCV viral load was assessed by using real-time polymerase chain reaction (PCR) technology, according to published assays and was tested in all patients [21–23]. The viral load of EBV and CMV was normalized to 10,000 human genome equivalents, as assessed by quantification of albumin gene expression [24], while the viral load of JCV and BKV was expressed as copies per mL.

Statistical Analysis

All data analysis was performed using the R statistical software (version 3.0.3). Continuous variables were described as medians and interquartile ranges (IQR). Categorical variables were described as absolute frequencies and percentages. Welch's two-sample *t* test was used for comparing continuous variables between two groups. When testing our hypothesis regarding categorical data, the likelihood ratio test was used for odds ratio with 95 % confidence intervals (95 % CIs). Multivariate logistic regression was used to determine dependencies between the variables. In the multivariate analysis, the dependent variables were seropositivity, positive PCR results, and number of PCR copies. For a better description of the evolution of seropositivity during childhood with cross-sectional data, we used a nonparametric maximum likelihood estimate for the distribution from the interval censored data (R package Interval version 1.1–0.1) [25].

Ethical Considerations

The study was approved by the Ethics Committee of the authors' institution.

Table 1 Clinical characteristic of patients at diagnosis and at the time of testing

		CD $n = 72$		UC $n = 31$		IBD-U $n = 3$
Age at diagnosis (years)[a]		12.6 (10.9–14.6)		8 (4.9–11.6)		12.2 (10.9–13.6)
Age at EBV determination (years)[a]		15.2 (13.9–17)		12.9 (9.8–14.5)		15.9 (14.9–17.3)
Disease duration (years)[a]		2.2 (1.2–4)		2.7 (1.2–5.4)		3.9 (3.8–4.1)
Gender (male)		42 (58 %)		19 (61 %)		3 (100 %)
Localization/extension	L1	14 (19 %)	E1	2 (6 %)		
	L2	15 (21 %)	E2	3 (10 %)		
	L3	43 (60 %)	E3	26 (84 %)	E3	3 (100 %)
	L4	28 (39 %)			L4	1 (33 %)
Behavior/severity	B1	35 (49 %)	S1	5 (16 %)	S1	0
	B2	33 (46 %)	S2	14 (45 %)	S2	1 (33 %)
	B3	4 (6 %)	S3	11 (35 %)	S3	2 (67 %)
	p	11 (15 %)	S4	1 (3 %)	S4	0
PCDAI[a,b]		15 (10–20)				
PUCAI[a,b]				5 (0–5)		
ESR (mm/h)[a,b]		16 (10–26.2)		9 (5–21.5)		2 (1.5–5)
CRP (mg/L)[a,b]		1.8 (0.5–5.1)		1.1 (0.5–3.8)		0.8 (0.7–2.2)
CPT (lg/g)[a,b]		805 (149–1800)		191.5 (100–1783)		197 (148.5–374.5)
PLT (910^9/L)[a,b]		306 (261–386)		328 (254–408)		218 (199–220)

PCDAI Pediatric Crohn's Disease Activity Index, *PUCAI* Pediatric Ulcerative Colitis Activity Index, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *CPT* fecal calprotectin, *PLT* platelet count

[a] The values are expressed as median with interquartile range

[b] At the time of determination

Results

EBV Serology During Childhood

Among the 106 patients we tested, 68 (64 %) were EBV seropositive; the remainder of the patients' serological characteristics are listed in Table 2. We found a correlation between EBV seropositivity and age at serology determination (OR 1.26, 95 % CI 1.11–1.45, p \ 0.001). The proportion of seropositivity was lower at younger ages, as shown in Fig. 1. Seropositivity was 41 % (7/17) among patients aged \10 years, 55 % (23/42) among patients aged 10–15 years, and 81 % (38/47) among patients aged 15–19 years. We found 49 (68 %) patients with CD, three (100 %) patients with unclassified IBD, and 16 (52 %) patients with UC EBV seropositive.

Effect of Treatment on EBV Seropositivity Status During Childhood

Current treatment with azathioprine (AZA) (OR 6.03, 95 % CI 2.16–18.75, $p = 0.005$) was associated with EBV seropositivity in the multiple logistic regression model, after adjusting for gender, age, and severe activity of disease (expressed as PCADI or PUCAI higher or equal to 30 points) (Table 3). Treatment with 5-aminosalicylate and treatment with and combined AZA and IFX was associated with EBV seropositivity in the unadjusted model, although

Table 2 EBV serological characteristic of the patients among group of treatment regimen

	5-ASA	CS	AZA	IFX	IFX ? AZA	All
IgM-VCA?	1 (0.04)	0 (0)	6 (0.07)	4 (0.09)	5 (0.12)	6 (0.06)
IgG-VCA?	11 (0.41)	3 (0.38)	59 (0.69)	33 (0.72)	31 (0.78)	66 (0.62)
IgG-EA?	2 (0.07)	1 (0.12)	13 (0.15)	8 (0.17)	7 (0.18)	15 (0.14)
IgG-EBNA?	9 (0.33)	3 (0.38)	53 (0.62)	26 (0.57)	26 (0.65)	56 (0.53)

ASA, 5-aminosalicylates; CS, corticosteroids; AZA, azathioprine; IFX, infliximab; IFX ? AZA, infliximab in combination with azathioprine, all patients

Fig. 1 The prevalence of EBV seropositivity in age group. *Full circles* represent the mean for each numeric variable in each age category, and are presented with *error bars* (95 % CIs)

the association was not detected after adjusting for age, gender, and disease activity. No other medications were associated with EBV seropositivity (Table 3). Due to the cross-sectional study design, we also tested these associations using a nonparametric maximum likelihood estimate for the distribution from interval censored data. Using this model, AZA monotherapy ($Z = 3.519$, $p = 0.00043$) and combined treatment with AZA (Fig. 2) and IFX ($Z = 2.64$, $p = 0.0084$) were predictors for early EBV seropositivity during childhood. However, treatment with 5-aminosalicylates was negative predictor for early EBV seropositivity ($Z = -2.46$, $p = 0.014$).

Effect of Treatment on EBV Viral Load

EBV viral load was evaluated for all 106 patients included in this study, although only 15 (14 %) had detectable copies of EBV DNA. Among patients with positive PCR test results (EBV copies ⌈0), the median load was 0.87 copies (IQR 0.72–2.54) and the maximum load was 18.2. The patient with the highest EBV load was a 12-year-old girl with UC, who was being treated with AZA and IFX,

Table 3 Analysis of risk factor for EBV seropositivity

	Seropositive	Seronegative	All	*t* value or OR with 95 % CI	Unadjusted *p* value[a]	Adjusted *p* value[b]
Number	68	38	106			
Median age at determination (IQR) (years)	15.7 (13.8–17)	13.5 (10–14.9)	14.6 (13.1–16.7)	$t = -3.6$	0.001	
Gender (male proportion)	32 (0.47)	10 (0.26)	42 (0.4)	0.4 (0.16–0.93)	0.034	
Diagnosis (CD proportion)[c]	49 (0.72)	23 (0.61)	72 (0.68)	1.68 (0.72–3.9)	0.226	
Median platelet count (IQR)	298 (250–353)	341 (263–432)	308 (256–399)	$t = 1.9$	0.062	
Median PCDAI (IQR)[d]	15 (10–20)	15 (10–26.3)	15 (10–20)	$t = 1.4$	0.169	
Median PUCAI (IQR)[e]	0 (0–5)	0 (0–7.5)	2.5 (0–5)	$t = 1$	0.33	
PCDAI C 30 or PUCAI C 30[f]	2 (0.03)	8 (0.21)	10 (0.09)	0.11 (0.02–0.49)	0.003	
5-ASA	12 (0.18)	15 (0.39)	27 (0.26)	0.33 (0.13–0.8)	0.015	0.104
CS	3 (0.04)	5 (0.13)	8 (0.08)	0.3 (0.06–1.32)	0.111	0.687
AZA	61 (0.91)	24 (0.63)	85 (0.81)	6.03 (2.16–18.75)	\0.001	0.005[g]
IFX	34 (0.51)	12 (0.32)	46 (0.44)	2.17 (0.96–5.11)	0.064	0.460
ADA	2 (0.03)	2 (0.05)	4 (0.04)	0.55 (0.06–4.7)	0.555	0.795
IFX ? AZA	32 (0.48)	8 (0.21)	40 (0.38)	3.33 (1.38–8.76)	0.007	0.067
Untreated	0	2 (0.06)	2 (0.02)			

IQR, interquartile range; 5-ASA, 5-aminosalicylates; CS, corticosteroids; AZA, azathioprine; IFX, infliximab; ADA, adalimumab; IFX ? AZA, infliximab in combination with azathioprine

[a] Unadjusted *p* value

[b] Adjusted *p* value for gender, age and severe activity of disease at determination

[c] Data were analyzed using CD versus unclassified IBD and UC

[d] Calculated for patients with CD

[e] Calculated for patients with UC

[f] The severe activity of disease was expressed as PCDAI in patients with CD or PUCAI in patients with UC or unclassified IBD higher or equal to 30 points

[g] Using this model age, severe activity of disease and treatment with AZA were independent predictors of EBV seropositivity

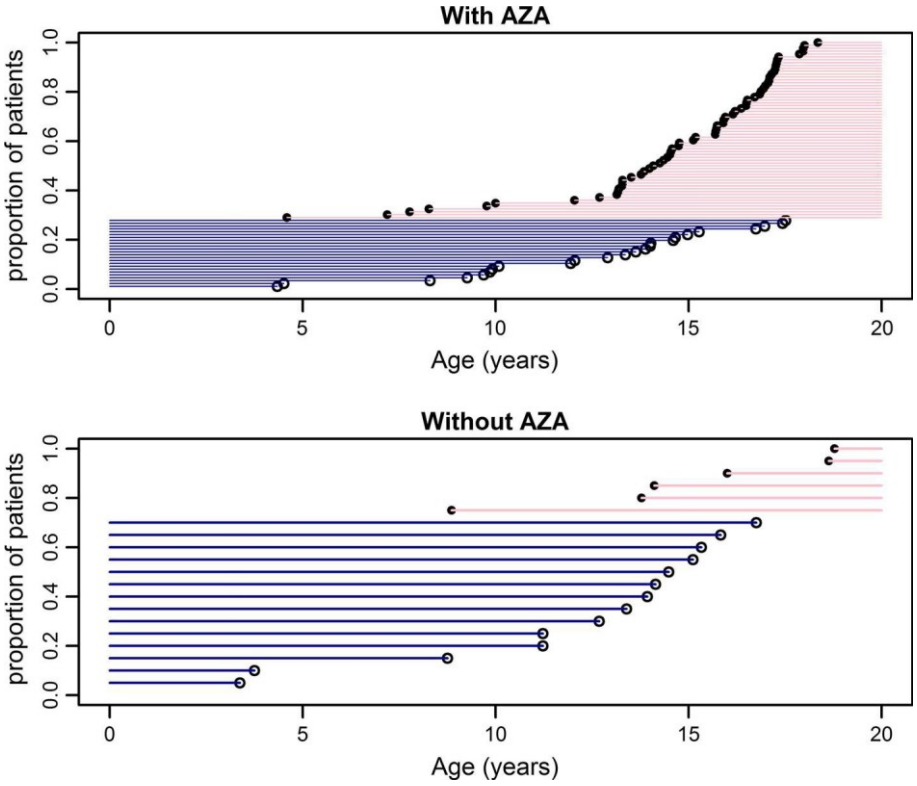

Fig. 2 The censored data model for EBV serology comparing patients with and without azathioprine. *Each line* represents one patient. *Full circles* represent positive serology test, *empty circles* negative test. For patients who had positive results the data were censured from *left*; for patients who had negative serology the data were censured from *right*. We found highly significant difference between patients with and without azathioprine. The asymptotic log-rank two-sample test (permutation form) p value was 0.00043

and was positive for VCA IgM, VCA IgG, and EA. In addition, her fecal calprotectin level was [1800 lg/g, and she had a Pediatric Ulcerative Colitis Activity Index score of 50. We did not detect any significant association between PCR positivity and any of the treatment regimens in both the unadjusted and adjusted models (Table 4). However, an association between number of PCR copies and IFX dose per kg and 8 weeks was detected (Kendall's tau = 0.19, p = 0.023). The association was even more profound after adjusting for age, gender, and disease activity (p = 0.003). No other associations between EBV PCR results and treatment regimens were observed.

CMV Detection

The proportion of patients in each group was unbalanced, and actual results of serological tests were as follows: 38 % (5/13) CMV seropositive among patients aged \10 years, 26 % (9/31) CMV seropositive among patients aged 10–15 years, and 22 % (9/36) CMV seropositive among patients aged 16–19 years. We did not find any association between age at determination and CMV seropositivity. The evolution of CMV seropositivity during childhood is shown in Fig. 3. We tested whether CMV seropositivity was associated with treatment modalities in a model with interval censored data, although no association was detected. In addition, CMV DNA was not detected in any patient.

BKV and JCV Detection

All patients' PCR tests were negative for JCV. However, PCR detected BKV in three patients (two males: 14.6, 17.3, and 18 years; 2.46, 0.87, and 4.14 copies/mL) who were receiving IFX and AZA treatment for CD. Owing to the low prevalence of BKV positivity, we did not test any associations.

Discussion

The European Crohn's and Colitis Organisation guidelines suggest that thiopurine treatment should be avoided in EBV-seronegative males under 35 years of age [1]. However, there are very little data regarding seroprevalence [13] EBV viral load among children with IBD [14]. The first report of the prevalence of EBV seropositivity (40 %) was in a letter by Love et al. [13] who described their work in a group of newly diagnosed IBD patients (median age, 13 years). However, our data indicate that the prevalence of EBV seropositivity increases during childhood among patients with established disease. The overall proportion of EBV seropositive patients was 64 % in the present study, although the median age at determination (14 years) was very similar to Love et al.'s report [13]. We can only speculate that this substantial difference in EBV seropositivity could be the result of the treatment used in our study

Table 4 Analysis of risk factors for EBV PCR positivity and PCR copies

	PCR positive	PCR negative	All	T value or OR with 95 % CI	Unadjusted *p* value[a]	Adjusted *p* value[b]	Correlation *p* value[c]
Number	15 (14 %)	91 (86 %)	106				
Median age at diagnosis (IQR)	14.5 (13.4–17.1)	14.6 (13–16.5)	14.6 (13.1–16.7)	$t = -0.6$	0.528		
Gender (male proportion)	8 (0.53)	34 (0.37)	42 (0.4)	0.52 (0.17–1.58)	0.246		
Diagnosis (CD proportion)[d]	10 (0.67)	62 (0.68)	72 (0.68)	0.94 (0.3–3.23)	0.911		
Median platelets count (IQR)	285 (266–321)	309 (250–400)	308 (254–396)	$t = 0.9$	0.355		
Median PCDAI (IQR)[e]	13.8 (12.5–16.9)	15 (10–20)	15 (10–20)	$t = 1.1$	0.266		
Median PUCAI (IQR)[f]	5 (5–10)	2.5 (0–5)	5 (0–5)	$t = -0.5$	0.645		
PCDAI C 30 or PUCAI C 30[g]	1 (0.07)	9 (0.1)	10 (0.09)	0.65 (0.03–3.88)	0.68		
5-ASA	2 (0.13)	25 (0.27)	27 (0.25)	0.41 (0.06–1.61)	0.217	0.289	0.123
CS	0 (0)	8 (0.09)	8 (0.08)				
AZA	14 (0.93)	72 (0.79)	86 (0.81)	3.69 (0.67–69.02)	0.15	0.219	0.173
IFX	9 (0.6)	37 (0.41)	46 (0.43)	2.19 (0.73–7.03)	0.163	0.228	0.003[h]
ADA	0 (0)	4 (0.04)	4 (0.04)				
IFX ? AZA	8 (0.53)	32 (0.35)	40 (0.38)	2.11 (0.7–6.53)	0.185	0.273	0.026
Untreated	0	2 (0.02)	2 (0.02)				

IQR, interquartile range; 5-ASA, 5-aminosalicylates; CS, corticosteroids; AZA, azathioprine; IFX, infliximab; ADA, adalimumab; IFX ? AZA, infliximab in combination with azathioprine

[a] Unadjusted *p* value

[b] Adjusted *p* value for gender, age, and severe activity of disease at determination

[c] Adjusted *p* value for gender, age, and severe activity of disease at determination for number of EBV PCR copies

[d] Data were analyzed using CD versus unclassified IBD and UC

[e] Calculated for patients with CD

[f] Calculated for patients with UC

[g] The severe activity of disease was expressed as PCDAI in patients with CD or PUCAI in patients with UC or unclassified IBD higher or equal to 30 points

[h] Using this model age, severe activity of disease and treatment with AZA were independent predictors of EBV seropositivity

group, mainly by AZA, which was noted in the current study as a risk factor for early EBV seropositivity. Although the specific mechanism is not yet understood, our results (OR 6.03, 95 % CI 2.16–18.75, $p \setminus 0.001$) confirm the previous observation [26] that AZA use is associated with EBV seropositivity. Moreover, we found a significant difference in the childhood EBV seropositivity of children treated with AZA and without AZA ($p = 0.00043$, Fig. 2). A triple mechanism to explain the immunosuppressive effect of AZA was proposed [27]. The induction of a very specific apoptotic pathway in the CD4? subset of CD28 co-stimulated lymphocytes. Second mechanism was the distortion in DNA, and the impairment in its repair system induces the activation of non-specific apoptotic pathways in proliferating lymphocytes and, finally, the inhibition of adenosine triphosphate and guanosine triphosphate de novo biosynthesis by methylmercaptopurine nucleotides. Further studies will be needed to clarify by which of the proposed mechanisms the immunosuppressive therapy may affect the seroprevalence of opportunistic viral infection. Moreover, whether patients diagnosed at an early age are primarily more immunosuppressed should be considered.

The regional epidemiological situation might be another explanation for the observed difference in seroprevalence between our results and Love et al.'s letter [13]. The prevalence of EBV seropositivity among patients aged 15–19 years was slightly higher (81 %) in the present study, compared to a prevalence of 71 % reported in another recent study of patients between 18 and 20 years of age [26]. Data on seroprevalence in the general population from our region are unfortunately not available. However, EBV serology has been studied recently in the general US population [28, 29]. The EBV seroprevalence differs significantly according to race or ethnicity. In non-Hispanic whites, seroprevalence by age group was as follows: 6–8 years: 43 %, 9–11 years: 40 %, 12–14 years: 53 %; 15–17 years, 64 %; and 18–19 years, 79 % [29]. Comparison with our study population will be very complicated

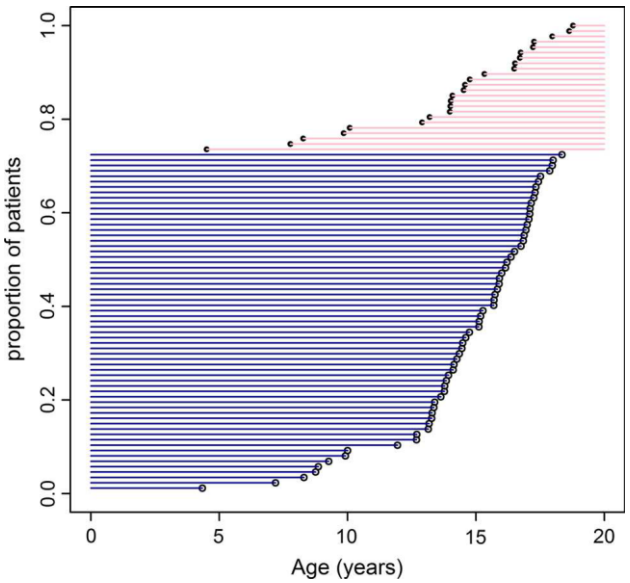

Fig. 3 The censored data model for CMV serology. *Each line* represents one patient. *Full circles* represent positive serology test, *empty circles* negative test. For patients who had positive results the data were censured from *left*; for patients who had negative serology the data were censured from *right*

mainly for cultural, socioeconomic, and geographical reasons. Serological testing has some limitations. It should be noted that the lack of IgM positivity may be a false negative in IBD young patients under immunosuppressive and/or immunomodulatory treatment as has been shown for CMV colitis [30, 31]. A further limitation in measuring serum antibodies is the fact that elevated levels of IgM can persist for up to 2 years after infection, and immunocompromised patients may not mount an IgM response [32]. So, it should be emphasized that quantitative RT-PCR assessment of the viral load directly on mucosal samples appears to be the most accurate test to determine viral infection.

Contrary to EBV, CMV seropositivity prevalence observed in children aged \10 years (33 %) was in accordance with the prevalence observed in a large study of healthy children (6–11 years: 36 %). On the other hand, the prevalence observed among children aged [10 years of age in our study was even lower (26 %) compared to that observed in healthy children (12–19 years: 58 %) in the mentioned study [33]. Further studies are needed to elucidate whether CMV seroconversion from seropositivity to seronegativity is possible in IBD patients treated with immunosuppressive drugs. Moreover, the possibility of seroconversion of CMV antibodies in these patients under immunosuppressive therapy, the possibility that they cannot mount an antibody response, should be also considered. We can speculate that another explanation for the insignificant decrease in seropositivity might be a birth cohort effect; recently younger patients have more often been exposed to CMV seropositive patients who were more frequently hospitalized with immunosuppressed children (e.g., after kidney, liver, or hematopoietic stem cells transplantation). Also the younger age of patients at diagnosis and the younger age at CMV serology determination could be another explanation.

The prevalence of EBV DNA positivity among children with IBD was lower in the present study compared to recently published data from the adult population (14 vs. 35 %) [34]. However, our results were similar to the prevalence in the control group (15 %) in that same study and also to the prevalence (12 %) observed in another study of patients before IFX treatment [17]. In the adult population, the prevalence of EBV DNA positivity among patients with IBD was significantly higher than that in the control group, independent of their therapeutic regimen [34]. The same study also reported that IFX, in monotherapy or combined with AZA, was associated with a higher prevalence of EBV DNA positivity [34]. In contrast, although the prevalence of EBV DNA was not significantly different in our study groups, we observed that the number of EBV DNA copies and the dose of IFX per kg and 8 weeks were significantly associated (adjusted $p = 0.0089$). Although the threshold for PCR prediction of EBV infection is unknown [35], viral load is known to be a predictor of lymphoma development in a post-transplant setting [12], Unfortunately, we could not confirm the previous observation that among adult patients age at determination was a risk factor for the presence and viral load of EBV [34].

We did not detect CMV DNA in our samples, as previously reported among adult IBD patients before and during IFX treatment [17]. Similarly, another report found that only one of 62 pediatric patients with an inflammatory condition (e.g., juvenile rheumatoid arthritis, CD, or UC) was CMV positive [14]. None of the children we tested, even those treated with a combination of IFX and AZA, were JCV PCR positive. Recently presented data regarding polyomavirus excretion in children with rheumatic diseases indicate there is an increased reactivation of polyomaviruses (as evidenced by viruria) in patients receiving biologic and non-biologic immunosuppressive therapies [36]. Unfortunately, we were unable to test the association with treatment regimens, given the low prevalence of BKV. However, BKV was detected only in blood samples from patients treated with a combination of AZA and IFX. Regarding polyomaviral load, biologic therapy appears to be more important than AZA, although it is not known whether this replication leads to specific complications.

Regarding the risk of EBV infection, further prospective studies are needed to determine whether AZA treatment is responsible for the higher prevalence of EBV seropositivity at younger ages, and whether there is a relationship between

AZA treatment and the risk of EBV-associated LDs. It is also curious that IFX, which leads to more extensive replication of the virus, does not appear to be a risk factor. The main limitation of the current study was its cross-sectional design, and therefore, we tested the associations in a model that should partially overcome this problem. As in many other pediatric studies, the number of patients included was small, and we were unable to include a control group, due to ethical difficulties; therefore, the aim of the study was not to compare the prevalence and load between IBD patients and healthy subjects. In addition, this study was conducted at a single tertiary center, and therefore, it is possible that our patients were not representative of the general IBD population, especially given the high proportion of very young IBD patients.

Conclusions

This is the first study to describe the relationships between viral load and serology of EBV, CMV, BK, and JC polyomaviruses, and therapeutic regimens among pediatric patients with IBD. Our results indicate how the prevalence of seropositivity changes during childhood. We also found that AZA was a risk factor for early EBV seropositivity in children with IBD, although the dose of IFX was associated with the number of PCR EBV copies. The relationship between these results and EBV-associated LDs should be elucidated in further studies.

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Conflict of interest None.

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ORIGINAL ARTICLE

Musculoskeletal system in children and adolescents with inflammatory bowel disease: normal muscle force, decreased trabecular bone mineral density and low prevalence of vertebral fractures

Klara Maratova[1] & Ondrej Hradsky [1] & Jana Matyskova[1] & Ivana Copova[1] & Ondrej Soucek[1] & Zdenek Sumnik [1] & Jiri Bronsky [1]

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Abstract Low bone mineral density (BMD) and an increased fracture incidence are two extraintestinal complications associated with inflammatory bowel disease (IBD). We aimed to evaluate musculoskeletal traits and assess vertebral fracture (VF) rate in children and adolescents with IBD. Seventy patients with IBD with a median age of 13.8 years were included. The BMD and geometric parameters of the non-dominant tibia were assessed using pQCT. Dynamic muscle functions were evaluated using jumping mechanography. VFs were assessed according to the semiquantitative standardized method by Genant. The muscle functions adjusted for the patients' weight did not differ from the reference population. A low trabecular BMD (Z-score − 1.6; $p < 0.001$) and cortical thickness (Z-score − 0.7; $p < 0.001$) were found in children and adolescents with IBD. Conversely, an increased cortical BMD (Z-score 1.1; $p < 0.001$) was noted. No significant association was found between the 25-OHD serum levels and the bone or muscle measurements. One patient with asymptomatic VF was identified.

Conclusion: IBD in childhood or adolescents affects bones but not muscles. Bone changes are independent of the 25-OHD serum level. A thoracolumbar spine X-ray should not be routinely recommended in children with IBD.

What is Known:
- *Low bone mineral density and an increased fracture rate arethe complications associated with IBD.*
- *Bone strength and structural development is strongly dependent on skeletal muscle stimulation.*

What is New:
- *Children with IBD have altered bone density and geometry but normal dynamic muscle functions.*
- *Thoracolumbar spine X-ray should be indicated on an individual basis in children with IBD.*

Keywords Inflammatory bowel disease · Bone strength · Muscle functions · Peripheral quantitative computed tomography · Mechanography · Vitamin D

Abbreviations

25-OHD	25-hydroxycholecalciferol
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
CRP	C-reactive protein

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* Ondrej Soucek
ondrej.soucek@lfmotol.cuni.cz

Klara Maratova
klara.maratova@fnmotol.cz

Ondrej Hradsky
ondrej.hradsky@lfmotol.cuni.cz

Jana Matyskova
jana.matyskova@seznam.cz

Ivana Copova
ivana.copova@fnmotol.cz

Zdenek Sumnik
zdenek.sumnik@lfmotol.cuni.cz

Jiri Bronsky
jiri.bronsky@fnmotol.cz

[1] Department of Pediatrics, Second Faculty of Medicine, Charles University and Motol University Hospital, V Uvalu 84, 150 06 Prague 5, Czech Republic

CSA	Cross-sectional area
DXA	Dual energy X-ray absorptiometry
F-calprotectin	Fecal calprotectin
IBD	Inflammatory bowel disease
M1LH	Multiple one-legged hopping
PCDAI	Pediatric Crohn's Disease Activity Index
pQCT	Peripheral quantitative computed tomography
PTH	Parathormone
PUCAI	Pediatric Ulcerative Colitis Activity Index
S2LJ	Single two-legged jump
SSI	Polar strength strain index
VF	Vertebral fracture

Introduction

Inflammatory bowel disease (IBD) is a chronic disease that negatively affects the mucosa of the gastrointestinal tract [1]. The optimal treatment outcome is to achieve mucosal healing and thus limit the development of secondary complications [2, 3]. In addition to malnutrition, growth retardation, delayed puberty [4, 5], osteoporosis, and sarcopenia have also been reported in patients with IBD [6].

Childhood and especially adolescence are critical periods for bone development. Approximately 40% of the total bone mineral content is acquired during puberty and adolescence in girls [7]. Therefore, an impairment in bone development due to inflammatory process in the bowel during this period could result in impaired bone strength and an increased fracture risk in adulthood. The etiopathogenesis of skeletal complications in IBD is multifactorial and has not been entirely elucidated to date. The main contributing factor of low bone mass accrual is most likely the overproduction of pro-inflammatory cytokines (TNF-α, IL-1 β, and IL-6) [8], which promote osteoclastogenesis, and corticosteroid (CS) therapy, which induces osteoblast and osteocyte apoptosis [9]. Malnutrition, delayed puberty, low physical activity, and 25-hydroxyvitamin D (25-OHD) deficiency have also been implicated in the literature [10–12].

Several studies have been conducted to ascertain the bone mineral density (BMD) in children and adolescents with IBD using dual energy X-ray absorptiometry (DXA) [4, 13–15] or peripheral quantitative computed tomography (pQCT) [6, 16]. The prevalence of low BMD in pediatric patients with IBD varies depending on the methodology used and patient selection; it has been reported in up to 27% of patients [17]. According to the official position of the International Society for Clinical Densitometry, osteoporosis in children should be diagnosed based on both a low BMD and a positive fracture history. Data on the incidence of fractures in the pediatric IBD population are scarce. Cases of vertebral fractures (VFs) have been reported [18–22], but the results of the largest epidemiological study showed a non-significant trend toward an increased risk of VF (OR 2.7, 95% CI 0.8–10.8) in adolescents with IBD, especially in patients with Crohn's disease (CD) (OR 5.4, 95% CI 0.5–60.0), compared to the healthy population [23]. This is in contrast to a number of studies in adults that have clearly reported an increased risk of VFs [24–26].

According to Frost's mechanostat theory [27], impaired bone strength may be caused by osteocyte apoptosis and subsequent bone resorption due to insufficient stimulation of the intraosseous space by skeletal muscles [28]. Surprisingly, limited knowledge is available concerning muscle functions and their roles in the occurrence of skeletal complications in patients with IBD. Interestingly, although no differences in physical activity have been found between patients with IBD and healthy controls [29, 30], several studies have described lean muscle mass deficit at time of diagnosis in these patients using DXA or pQCT [10, 16, 31, 32]. This deficit is evident not only in adults [33] but also in adolescents with CD in whom an up to 6% reduction in lean mass has been observed [32]. The impairment of skeletal muscles in IBD patients appears to be multifactorial. In addition to malnutrition and CS therapy, the inflammatory process itself may play a role. Rather than increased protein degradation, muscle loss appears to be caused by disruption of the proteosynthetic IGF1/Akt pathway [33]. High IL-6 levels, which have previously been noted in IBD patients [34], have been linked to a decrease in the expression of the pro-myogenic factor IGF-1 [35] and thus impairment of Akt activity and the anabolic GH/IGF-1 pathway. Increased oxidative stress may also play a role [33].

We hypothesized that the musculoskeletal system, especially bone strength and muscle functions, was impaired in children with IBD. The aims of this study were as follows: (1) to characterize the muscle-bone unit in children and adolescents with IBD using pQCT and jumping mechanography; (2) to assess the prevalence of VFs using lateral thoracolumbar spine X-ray; (3) to determine the vitamin D serum levels; and (4) to determine the clinical and laboratory predictors of key muscle and bone parameters.

Participants and methods

Participants

This cross-sectional study included 70 Caucasian children and adolescents (39 boys) with IBD (53 CD and 17 UC), all of whom were regularly monitored at a tertiary referral IBD center. Between 2011 and 2013, 82 patients eligible for inclusion in this study were invited to participate. The inclusion criteria were as follows: (1) age between 6 and 19 years and (2) a confirmed diagnosis of IBD based on the Porto criteria [1]. The exclusion criteria were as follows: (1) any disease

affecting bone metabolism, with the exception of long-term controlled autoimmune thyroiditis; (2) pregnancy; (3) a history of extensive surgery of the small bowel; (4) total parenteral nutrition; (5) vitamin D or calcium supplementation 2 months prior to enrollment in the study; and (6) growth hormone therapy. Out of the 82 screened patients, 12 were excluded from the study (4 underwent small bowel resection of more than 30 cm long, 2 required total parenteral nutrition, and 6 patients did not consent with the inclusion in the study). Out of the 12 excluded patients, 2 were diagnosed with UC, and 10 with CD. Although the excluded patients were significantly older than the patients included in the study (median age 16.8 and 13.8 years, respectively, $p = 0.002$), we found no difference in *Z*-scores for height (median *Z*-score − 0.38 and − 0.78, respectively, $p = 0.4$), weight (median *Z*-score − 0.28 and − 0.53, respectively, $p = 1.0$) or BMI (median *Z*-score − 0.19 and − 0.27, respectively, $p = 0.5$) between the groups. Disease activity index (PCDAI or PUCAI) at the time of the screening was available in 7 excluded patients with no difference when compared to the scores of the included patients (median 5.0 and 7.5, respectively, $p = 0.5$).

The study was approved by the Ethics Committee of the Motol University Hospital and was in compliance with the Declaration of Helsinki. Written informed consent was obtained from all participating patients and their parents or legal guardians.

Anthropometry

Body height was measured to the nearest 1 mm using an anthropometer (A-226 manufactured by Trystom in Olomouc, Czech Republic). Body weight was measured in light clothing or underwear using an electronic scale to the nearest 0.1 kg (TH200, manufactured by Tonava in Upice, Czech Republic). The body mass index (BMI) was calculated using the formula: body weight [kg]/body height^2 [m]. Sex- and age-specific *Z*-scores were calculated using Czech national reference data [36].

Dynamic muscle function assessment

The assessment of dynamic muscle functions was conducted by jumping mechanography (Leonardo Mechanograph® Ground Reaction Force Platform, manufactured by Novotec Medical GmbH in Pforzheim, Germany). The mechanograph consists of two symmetrical force plates that are both equipped with eight force sensors. The force sensors record the vertical ground reaction force exerted on the platform at a frequency of 800 Hz. The signal was analyzed using the software provided by the manufacturer (Leonardo Mechanography GRFP version 4.2).

The single two-legged jump (S2LJ) and multiple one-legged hopping (M1LH) tests were used for the dynamic muscle function assessment as described elsewhere [37, 38]. Maximum muscle power (P_{max}, *W*) assessed with the S2LJ test and maximum muscle force (F_{max}, *N*) assessed with the M1LH test were normalized to subject's body mass ($P_{max/mass}$, W/kg) or weight ($F_{max/BW}$, no unit), respectively, which is the standard procedure within the software that aims to ameliorate the dependence of the measures on body size [38]. The tests were repeated three times, and only the highest value was selected for further analysis. Because one of the complications of IBD in children is a short stature, sex- and height-specific *Z*-scores were calculated using our own previously published reference data [38]. The body mass and weight were calculated in the resting position prior to the start of the test using the integrated software.

Bone strength assessment

An XCT 2000 scanner (Stratec Medizintechnik, Pforzheim, Germany) was used for bone assessment at the non-dominant tibia. The length of the tibia was measured from the medial malleolus to the superior margin of the medial condyle of the tibia to the nearest 1 mm. A scout scan was taken to determine the position of the reference line, followed by two single CT scans with a 0.4-mm voxel size and a 2.0-mm slice thickness. The scans were taken at distances corresponding to 4 and 66% of the measured bone length. The trabecular BMD was measured at the distal tibia (4% site). The cortical BMD and thickness, the total and cortical bone cross-sectional areas (total bone CSA and cortical bone CSA), and the polar strength strain index (SSI) were measured at the proximal tibia (66% site). The images were analyzed, and numerical values were calculated using the integrated XCT software, version 6.20 C. Only good quality scans without movement artifacts were analyzed. To ensure measurement accuracy, calibration of the scanner was performed once a week using a phantom provided by the manufacturer. Sex- and height-specific *Z*-scores were calculated using published reference data [39].

Fracture assessment

During the personal interview, information regarding long bone and VF history was collected using a structured questionnaire. To ascertain the prevalence of asymptomatic VFs, lateral thoracolumbar spine X-rays were taken in 65 out of 70 patients. The five patients who did not consent to the lateral thoracolumbar spine X-ray were not included in the fracture assessment. The image evaluation was performed by an experienced observer using the semiquantitative standardized assessment of VFs by Genant [40].

Biochemical analysis

The following markers were tested as predictors of the musculoskeletal status: serum levels of 25-hydroxyvitamin D (25-OHD, using a chemiluminescent microparticle immunoassay); C-reactive protein (CRP, using an immunoturbidimetric assay) and fecal calprotectin (F-calprotectin, analyzed via the immunochromatographic ELISA Quantum Blue assay). The serum levels of parathormone (PTH) were determined using an electrochemiluminescence immunoassay. The erythrocyte sedimentation rate (ESR) was measured in the first hour using the Westergren method. Blood and fecal samples were collected during the patient's regular follow-up visit. The muscle and bone assessment were performed on the same day. The disease activity was assessed using the Pediatric Crohn's Disease Activity Index (PCDAI) [41] and the Pediatric Ulcerative Colitis Activity Index (PUCAI) [42]. The serum levels of 25-OHD were evaluated based on the most recent global consensus guidelines [43].

Statistical analysis

All data were analyzed using the *R* statistical software (version 3.2.3) [44]. Continuous variables were described as the medians and interquartile ranges (IQRs). Categorical variables were described as the absolute frequencies and percentages. The *Z*-scores were tested for differences from zero using the one-sample *t* test. A two-sample *t* test with Welch's approximation was used to test differences in numerical variables. A likelihood-ratio test (binomial logistic regression) was used to test differences in categorical variables. The association between two numerical variables was measured using multiple linear regression.

Results

Clinical data

The clinical and biochemical characteristics of the study participants according to sex and diagnosis are shown in Table 1. The median age of the participants was 13.8 years (IQR 11.9–15.5 years), and the median age at the diagnosis of IBD was 11.2 years (IQR 8.6–13.8 years). The patients with CD were older and were diagnosed at a later age than patients with UC ($p = 0.024$, $p = 0.008$, respectively). The study participants showed significantly lower median *Z*-scores for height and weight compared to the reference data. Additionally, the patients with CD were shorter and weighted less than those with UC. Six patients (8.6% of all) had a BMI < 10th percentile, 3 patients (4.3%) were underweight with a BMI < 3rd percentile, and 5 patients (7.1%) had a BMI > 97th percentile. No difference was found in the *Z*-scores of the anthropometric measurements between the two sexes.

The cumulative dose of CS therapy was monitored 12 months prior to enrollment in the study. A low dose (the median cumulative dose was 31.28 mg/kg/year) was prescribed in the steroid-treated patients ($N = 16$).

There were no significant differences between the CD and UC groups for the F-calprotectin, ESR, PTH, and 25-OHD on the day of the musculoskeletal examination. Nine out of 55 (16.4%) patients were vitamin D deficient (< 30 nmol/l), 11 (20%) patients were insufficient (30-50 nmol/l) and 35 (63.6%) patients had 25-OHD levels within the normal range (> 50 nmol/l). Significantly higher serum levels of ALP were noted in the boys and the UC patients compared to the girls and the patients with CD, respectively. When compared to the age- and sex-adjusted laboratory reference ranges, the PTH and ALP serum levels were normal in all but 8 (11%) patients, whose ALP serum levels were increased.

Dynamic muscle functions and bone density and strength assessment

The height-specific *Z*-scores for the key parameters of dynamic muscle function ($P_{max/mass}$ and $F_{max/BW}$) showed no significant differences in the children and adolescents with IBD compared with the reference data (Fig. 1). P_{max} was slightly decreased ($p = 0.032$) but was normal after normalization to body mass.

Significant changes were observed in the BMD and bone geometry (Fig. 2). In particular, our study participants had a decreased trabecular BMD ($p < 0.001$) and cortical thickness ($p < 0.001$). Interestingly, these changes occurred while the parameters of bone strength index SSI ($p = 0.01$) and cortical BMD ($p < 0.001$) were significantly increased compared with their healthy peers.

Lower *Z*-scores for $P_{max/mass}$ ($p = 0.005$), $F_{max/BW}$ ($p = 0.038$), and trabecular BMD ($p = 0.002$) were noted in the girls, when compared to the boys.

Predictors of muscle function and bone density

The associations between selected biochemical markers and the *Z*-scores of the muscle function and bone density and strength variables are listed in Table 2. Neither the serum 25-OHD levels nor serum PTH levels were associated with the muscle functions or bone density or strength parameters.

Long bone and vertebral fracture assessment

Of 70 patients, 5 patients (7.1%) had a positive long bone fracture history. The only reported fractures occurred in either the radius or ulna. A history of long bone fractures was not

Table 1 Clinical and biochemical characteristics of IBD patients, stratified by sex and diagnosis

	Total ($N = 70$)	Boys ($N = 39$)	Girls ($N = 31$)	P_A	CD ($N = 53$)	UC ($N = 17$)	P_B
Age (year)	13.8 (11.9–15.5)	13.4 (11.6–12.8)	14.3 (12.5–16.3)	0.375	14.2 (12.7–16.1)	12.3 (9.0–14.5)	0.024
Age at diagnosis	11.2 (8.6–13.8)	11.0 (8.5–12.8)	12.6 (8.9–15.0)	0.171	12.6 (9.7–14.1)	9.0 (5.0–11.1)	0.008
Height (*Z*-score)	− 0.8 (− 1.6–0.0)***	− 0.7 (−1.9–0.0)***	−1 (− 1.5–0.0)***	0.790	− 1.2 (− 1.9–0.3)***	−0.1 (−0.9–0.4)	0.005
Weight (*Z*-score)	− 0.5 (− 1.1–0.3)***	− 0.6 (−1.3–0.3)**	−0.5 (− 1.1–0.3)*	0.720	− 0.8 (− 1.4–0.1)***	0.3 (−0.5–0.7)	0.039
BMI (*Z*-score)	− 0.3 (− 0.9–0.5)	− 0.4 (− 1.2–0.5)	− 0.2 (− 0.6–0.4)	0.584	− 0.3 (− 0.9–0.4)	0.2 (−0.4–0.7)	0.508
Extraintestinal manifestation (%)	65 (93)	36 (92)	29 (94)	0.841	49 (92)	16 (94)	0.813
Anti-TNF therapy (%)	44 (63)	25 (64)	19 (61)	0.809	29 (55)	15 (88)	0.008
Patients with CS in last 12 m (%)	16 (23)	11 (28)	5 (16)	0.226	8 (15)	8 (47)	0.009
PCDAI/PUCAI	7.5 (0.0–12.5)	5.0 (0.0–12.5)	7.5 (0.0–15.6)	–	10.0 (5.0–15.6)	0.0 (0.0–5.0)	–
CRP [mg/l]	0.8 (0.5–2.8)	0.9 (0.5–3.7)	0.7 (0.5–2.5)	0.539	1.4 (0.5–3.9)	0.5 (0.5–1.5)	0.009
F-calprotectin [μg/g]	319 (100–1662)	319 (100–1503)	544 (114–1662)	0.522	434 (100–1800)	242 (100–604)	0.252
ESR [mm/h]	15.5 (9.2–22.8)	14.0 (6.0–24.8)	17.5 (13.0–22.0)	0.684	17.0 (12.2–22.8)	8.5 (5.0–16.8)	0.086
PTH [pmol/l]	3.8 (2.9–5.4)	4.1 (2.9–5.8)	3.6 (3.1–4.5)	0.616	3.6 (2.8–5.4)	3.8 (3.5–5.5)	0.573
25-OHD [nmol/l]	56.5 (42.5–74.1)	57.5 (42.5–76.0)	56.5 (42.8–67.4)	0.834	56.1 (42.2–67.4)	60.2 (43.6–90.1)	0.302

Data are expressed as the medians (interquartile ranges). The *Z*-scores were tested for differences from zero using the one-sample *t* test. Statistically significant differences are marked with an asterisk: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ vs. healthy children. *P* value^A shows differences between the sexes. *P* value^B shows differences between the CD and UC patients. Two-sample *t* test with Welch's approximation was used to test differences in numerical variables. A likelihood-ratio test (binomial logistic regression) was used to test the differences in categorical variables

IBD inflammatory bowel disease, *CD* Crohn's disease, *UC* ulcerative colitis, *CS* corticosterone, *TNF* tumor necrosis factor, *PUCAI* Pediatric Ulcerative Colitis Activity Index, *PCDAI* Pediatric Crohn's Disease Activity Index, *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate, *PTH* parathormone, *25-OHD* 25-hydroxycholecalciferol

associated with an impaired trabecular BMD ($p = 0.059$) or SSI ($p = 0.269$). According to Genant's semiquantitative assessment, a VF (grade 1) was found only in 1 (1.5%) of the evaluated patients ($N = 65$). However, a vertebral height reduction between 10 and 20% was noted in 18 (27.7%)

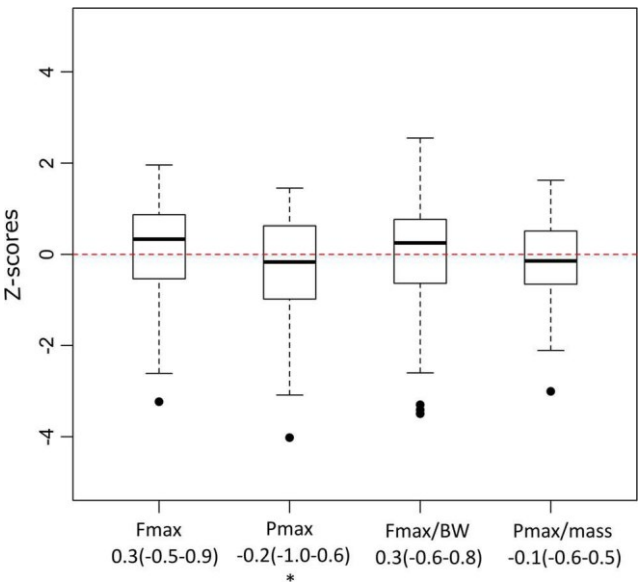

Fig. 1 Dynamic muscle function parameters expressed as sex- and height-specific *Z*-scores. The *box plots* represent the *Z*-scores distribution. The median and interquartile range (IQR) values are provided below each plot. The *Z*-scores were tested for difference from zero using the one-sample *t* test. Statistically significant differences are marked with an asterisk: $*p < 0.05$

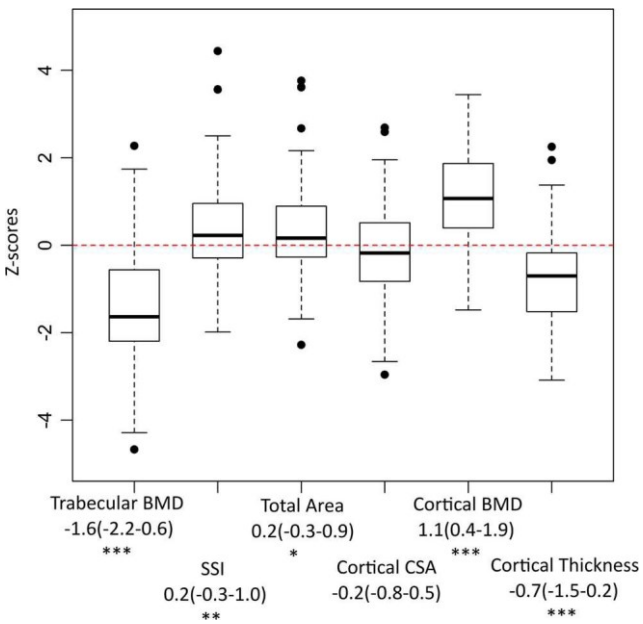

Fig. 2 Bone mineral density and geometry parameters expressed as sex- and height-specific *Z*-scores. The *box plots* represent the *Z*-score distribution. The median and interquartile range (IQR) values are provided below each plot. The *Z*-scores were tested for difference from zero using the one-sample *t* test. Statistically significant differences are marked with an asterisk: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

Table 2 Association between selected biochemical parameters and *Z*-scores for the mechanography and pQCT variables in children and adolescents with IBD

	$F_{max/BW}$	$P_{max/mass}$	Trabecular BMD	SSI
PTH	− 0.40 (− 1.03 to 0.23)	− 0.03 (− 0.19 to 0.14)	− 0.28 (− 0.57 to 0.01)	− 0.10 (− 0.30 to 0.10)
25-OHD	− 0.02 (− 0.06 to 0.02)	0.00 (− 0.02 to 0.01)	− 0.01 (− 0.03 to 0.01)	− 0.01 (− 0.02 to 0.00)

The data are presented as mean estimates (95% confidence intervals) of multiple linear regression. The dependent variables were the *Z*-scores of the individual mechanography and pQCT parameters listed in the upper row. The explanatory variables were age, sex, disease duration, and the biochemical parameters shown in the first column

PTH parathormone, *25-OHD* 25-hydroxycholecalciferol

patients. The vertebral height reduction was not associated with trabecular BMD ($p = 0.838$) or SSI ($p = 0.4918$).

Discussion

In the present study, we showed that dynamic muscle functions in children and adolescents with IBD were within the normal reference range, whereas the trabecular BMD and cortical thickness were decreased. This suggests that impaired bone density and altered bone geometry are not secondary to insufficient skeletal muscle function but rather caused by the disease itself.

Our patients presented with no significant differences in dynamic muscle functions compared with the reference data. To the best of our knowledge, only three studies have investigated muscle strength in children and adolescents with IBD, all of which used a hand dynamometer [6, 30, 45]. In contrast to our results, the isometric grip strength in these studies was significantly decreased compared to the healthy population. Hand dynamometry provides an assessment of the maximal isometric grip strength of the non-weight-bearing muscles of the upper extremity, and does not mirror the functional status of the muscle system in general. Jumping mechanography assesses muscle coordination and contraction velocity (i.e., muscle performance parameters) [46] and represents a simple, reliable, well reproducible, and widely used method for the analysis of muscle functions in children and adults [38, 47]. Moreover, a strong correlation between maximum voluntary muscle force assessed by jumping mechanography and BMC assessed by pQCT at the tibia was found in the healthy population [48] or in girls with Turner syndrome [47]. Therefore, we attribute the contrary results between the present study and the previous studies to the use of different methodological approach.

We observed decreased trabecular BMD and cortical thickness, whereas the cortical BMD and bone strength index SSI were increased in patients with IBD. Several studies were performed previously to ascertain the BMD in children and adolescents with IBD, most of which used DXA [4, 13–15]. The results of these studies showed decreased BMD compared to the healthy population [14, 15]. However, in contrast to pQCT, DXA does not take into account the bone size in the measured patient. Therefore, data obtained using this method must be interpreted in relation to the patient's height. Furthermore, DXA is limited in its inability to differentiate bone compartments and to assess bone geometry. In children with IBD, BMD measured by pQCT was performed at either the tibia [13, 16, 49] or the radius [6]. The results of these studies showed, in agreement with our study, significantly decreased trabecular BMD [6, 13, 16, 49] and normal [16] or increased [6] cortical BMD.

Bechtold et al. reported altered bone geometry [6] characterized by decreased total and cortical CSA in children and adolescents with IBD, and Griffin et al. [49] observed decreased periosteal and increased endocortical circumference. Both observations were in accordance with our results, which revealed a significantly decreased cortical thickness. A decreased cortical thickness, low trabecular, and high cortical BMD in children and adolescents with IBD suggest alterations in bone turnover (i.e., low-intracortical remodeling resulting in decreased intracortical porosity). This may indicate primary bone disease caused by inflammation, rather than insufficient mechanical stimuli caused by decreased muscle function parameters.

The serum 25-OHD levels were not associated with changes in the bone density and geometry or with dynamic muscle functions. The question of whether vitamin D insufficient patients with IBD should be supplemented and the recommended dosage are still subjects of debate. In our recent study [50], we showed that the administration of 2000 IU of cholecalciferol was associated with an increase in the trabecular BMD and muscle power in adolescents with IBD. Because this effect was independent of the serum 25-OHD concentration at the start of the study, routine supplementation with vitamin D thus seems to be a reasonable approach for bone strength improvement in these children, regardless of their vitamin D status. However, no randomized controlled study has been published to confirm this observation.

How and to what extent the BMD changes in patients with IBD influence bone strength and the fracture risk are not entirely clear because the calculated bone strength indices give

contradictory results. Decreased bone strength measured by pQCT using the cortical section modulus Zp at the time of diagnosis and 12 months after the diagnosis was noted by Dubner et al. [16]. In contrast, we observed a high SSI in children with IBD compared to the reference. This discrepancy may be due to the different formulas used to calculate the bone strength indices in the two studies. However, although osteoporosis is a systemic disease, distinct skeletal sites may be affected differently. Clinically, vertebral fractures are a concern in patients evaluated for secondary osteoporosis. The prevalence of VF in adult patients with IBD is 4.03 times higher than the prevalence in healthy controls (OR, 4.03; 95% CI, 1.652–9.847) [51]. A similar finding was not confirmed in children; nevertheless, a trend toward an increased risk (OR 2.7, 95% CI 0.8–10.8) was observed [23]. Despite a significantly decreased trabecular BMD, only one of the patients who participated in the present study was diagnosed with asymptomatic VF (Grade 1) using Genant's semiquantitative method [40]. Another 27.7% of our patients presented with < 20% decrease in vertebral height. In accordance with the results in adult IBD patients [51], we found no correlation between the vertebral height reduction and the trabecular BMD *Z*-score. This may suggest that a low trabecular BMD is not the only risk factor for an increased risk of vertebral fractures in patients with IBD or that a further decrease may be expected with age, with more VFs occurring later in adulthood. We conclude that the inclusion of a single spinal radiograph in regular diagnostic procedure is not indicated in asymptomatic children with IBD.

Limitations

As a limitation of our study, we consider the use of already published references [39] for the bone density and bone geometry analyses due to a lack of a control population of our own. The use of previously published references was necessary because concerns were raised by the ethics committee over unnecessary radiation exposure despite the very low dose. Furthermore, serial lateral thoracolumbar spine X-rays would be required to accurately distinguish VFs from physiological variants in the vertebrae. However, we did not anticipate an increase in the VF rate because only one patient exhibited asymptomatic VF, and the group was stable with regard to disease activity.

The results of this study could be influenced by low activity of IBD in the studied population. All but one patient included in the study were either in remission or exhibited mild disease activity. The observed musculoskeletal changes may therefore be more pronounced in more severe cases of IBD. However, as IBD control is improving due to the use of modern therapeutics [2] and because we excluded only small part of our IBD patients with a comparable disease activity index, we are convinced that this group represents today's children with IBD.

Due to the cross-sectional design of this study, we cannot comment on the potential benefits of vitamin D supplementation on the musculoskeletal system in children with IBD. However, this issue was the aim of our interventional study, which showed a positive effect of 2000 IU of cholecalciferol [50].

Conclusion

Children and adolescents with IBD have decreased trabecular BMD, but normal muscle functions. Bone impairment is most likely a direct effect of IBD, independent either on 25-OHD-deficiency or decreased muscle power or force. Thoracolumbar spine X-ray should be indicated on an individual basis and not routinely implemented as a screening approach in children with IBD.

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Authors' Contributions

Klara Maratova and Jana Matyskova performed the pQCT and mechanography assessment. Ondrej Hradsky provided the statistical analyses. Ivana Copova, Ondrej Hradsky, and Jiri Bronsky recruited the patients. Ondrej Soucek, Zdenek Sumnik, and Klara Maratova were responsible for interpretation of the data and drafted the paper. All co-authors revised the final version of the manuscript and approved it for publication.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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Supplementation with 2000 IU of Cholecalciferol Is Associated with Improvement of Trabecular Bone Mineral Density and Muscle Power in Pediatric Patients with IBD

Ondrej Hradsky, MD, PhD, Ondrej Soucek, MD, PhD, Klara Maratova, MS, Jana Matyskova, MS, Ivana Copova, MD, Kristyna Zarubova, MD, Jiri Bronsky, MD, PhD, and Zdenek Sumnik, MD, PhD

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Background: Inflammatory bowel diseases (IBD) are associated with altered bone health and increased risk for fractures. Vitamin D deficiency is frequently found in IBD; however, the effect of vitamin D supplementation on bone health of children with IBD is poorly understood. We aimed to observe the changes in volumetric bone density and dynamic muscle functions after vitamin D substitution in a cohort of pediatric patients with IBD.

Methods: This was a prospective observational study of 55 patients (aged 5–19 years) with IBD. Bone quality was assessed using peripheral quantitative computed tomography and muscle functions by jumping mechanography at baseline and after a median of 13.8 (interquartile range, 12.0–16.0) months of daily substitution of 2000 IU of cholecalciferol.

Results: Median serum levels of 25-hydroxyvitamin D increased from 58 nmol/L at the baseline visit to 85 nmol/L at the last follow-up visit (P , 0.001); no signs of overdose were reported. The Z-scores of trabecular bone mineral density, cortical bone cross-sectional area, and maximal muscle power improved significantly during the follow-up period (+0.5, P ¼ 0.001, +0.3, P ¼ 0.002 and +0.5, P ¼ 0.002, respectively). Cholecalciferol substitution was positively associated with trabecular bone mineral density and maximal muscle power (estimates 0.26, 95% confidence interval 0.14–0.37, P , 0.0001 and 0.60, 95% confidence interval 0.32–0.85, P , 0.0001, respectively) but not with the Strength–Strain Index or maximal muscle force (F_{max}).

Conclusions: We observed an improvement in bone and muscle parameters after cholecalciferol substitution in pediatric patients with IBD. Therefore, vitamin D substitution can be considered in such patients.

(Inflamm Bowel Dis 2017;23:514–523)

Key Words: vitamin D, inflammatory bowel disease, bone mineral density, muscle strength

Inflammatory bowel diseases (IBD) are chronic relapsing disorders associated with altered bone health,[1–8] increased risk for bone fractures,[9–12] and, probably, impaired muscle function.[6,7] The possible risk factors associated with bone fragility include disease activity, prolonged treatment with corticosteroids, malabsorption, vitamin D deficiency, impaired muscle function, compromised linear growth, and delayed puberty.[13]

Vitamin D is an important factor for bone health.[14] Vitamin D deficiency seems to be highly prevalent among pediatric patients with IBD,[15,16] and some (but not all[17]) studies have shown that patients with IBD present with lower 25-hydroxyvitamin D (25OHD) serum levels compared with those observed in the healthy population.[18] Winter season, dark skin, involvement of the upper gastrointestinal tract (in Crohn's disease [CD]), more severe disease course, low serum albumin level, low body mass index, and low weight Z-score were identified as risk factors for lower 25OHD in pediatric patients with IBD.[15,16,19,20] In contrast to adults,[21] 25OHD levels were not correlated with bone mineral content in cross-sectional studies in children with IBD.[2,15]

Although different administration regimes of vitamin D have been tested in pediatric patients with IBD,[22–24] the appropriate mode and dosage of supplementation is unclear. Based on a recent randomized controlled trial,[24] daily oral administration of 2000 IU of cholecalciferol seems to be safe; however, it is still challenging to achieve a 25OHD concentration above 80 nmol/L during the whole year, which was the primary endpoint of this study.

The rationale for cholecalciferol supplementation in chronic diseases is based on the established effect of vitamin D on bone mineralization mediated by calcium absorption from the gut. Little is known about the effect of vitamin D on skeletal muscles; however, animal studies have shown its positive role in the regulation of myogenesis.[25]

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From the Department of Pediatrics, 2nd Faculty of Medicine, Charles University in Prague and Motol University Hospital, Prague, Czech Republic.

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Address correspondence to: Ondrej Soucek, MD, PhD, Department of Pediatrics, 2nd Faculty of Medicine, Charles University in Prague and Motol University Hospital, V Uvalu 84, Prague 5, 150 06, Czech Republic (e-mail: ondrej.soucek@lfmotol.cuni.cz).

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Owing to the insufficient data, a recent meta-analysis of randomized controlled trials (RCTs) on the efficacy of vitamin D supplementation on bone mineral density (BMD) in patients with IBD failed to reach a conclusion.[26] A pediatric study by Benchimol et al[27] failed to show a difference in the improvement of BMD between patients treated with 50,000 IU of 25OHD once per month and patients supplemented with calcium alone. Although some RCTs showed a positive effect of vitamin D supplementation on bone quality in healthy children,[28–31] other studies failed to find a significant impact on BMD.[32,33] Similarly, the impact of vitamin D supplementation on muscle function is still unclear. While a study showed that parenteral vitamin D supplementation did not significantly improve muscle function in healthy adolescent girls,[34] another recent association study showed significantly higher muscle strength in boys with serum levels of 25OHD in the highest tertile compared with those with serum levels of 25OHD in the lowest tertile.[35]

Primary and Secondary Aims

The primary aim of our study was to observe the changes in bone quality parameters measured by peripheral quantitative computed tomography (pQCT) and dynamic muscle functions assessed by jumping mechanography after daily substitution of 2000 IU of cholecalciferol for 12 months in a cohort of pediatric patients with IBD. Secondarily, we assessed the changes in 25OHD serum levels and tried to find potential predictors (baseline values of bone and muscle characteristics, anthropometric parameters, and 25OHD levels) to determine patients suitable for vitamin D substitution.

MATERIALS AND METHODS

Design and Subjects

The study was designed as a longitudinal prospective observational study. Between 2011 and 2013, we screened all pediatric patients with IBD followed at our tertiary referral center, for eligibility (Fig. 1) to enter this study. Patients aged between 5 and 19 years, those who were diagnosed with IBD based on Porto criteria[36,37] and those who showed a willingness to participate in the study were included. Patients who had other disease that has a known impact on the muscle–bone unit (patients with compensated autoimmune thyroiditis were not excluded), those who showed noncompliance to treatment, pregnant women, patients who had undergone extensive small bowel surgery, and those on total parenteral nutrition and growth hormone therapy were excluded. Among the 70 patients who were enrolled, 55 (79%) completed the study.

The blood sampling, bone density and strength assessment, and muscle function analyses were performed at the initiation (baseline visit) and then between 12 and 24 months (median 13.8 months, interquartile range 12.0–16.0) of vitamin D supplementation (last follow-up visit). Regular clinical follow-up of the patients was conducted between these visits, at intervals of 2 to 3 months. We had retrospectively collected the basic characteristics of the patients and applied the Montreal classification at the time of diagnosis. The cumulative dose of corticosteroids over 12 months before initiation of vitamin D supplementation was calculated and the percentage of patients treated with corticosteroids was noted. The information about treatment was collected at every visit.

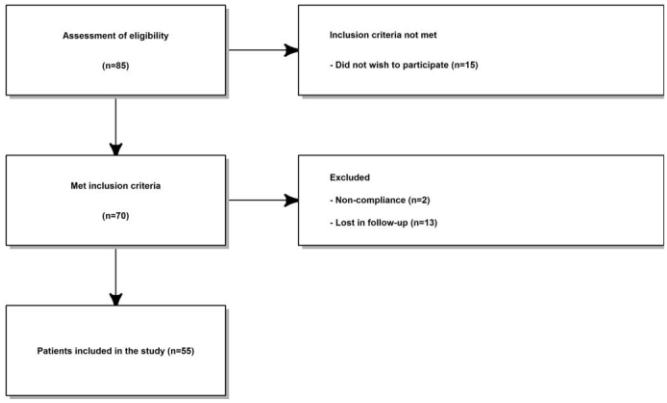

FIGURE 1. Flowchart of pediatric patients with IBD.

Vitamin D Supplementation

No patient received any regular substitution of vitamin D or calcium at the screening visit. Enteral nutrition was not considered as supplementation. Three months after the screening visit, all patients were prescribed 2000 IU of cholecalciferol (oral drops) (Vigantol; Merck KGaA, Darmstadt, Germany) daily. The levels of 25OHD were tested every 2 to 3 months, during the regular follow-up.

Disease Activity and Laboratory Parameters

We obtained blood samples and assessed disease activity on the day of the muscle and bone assessment (baseline and last follow-up) and during interim outpatient clinic visits. Activity of the disease was assessed using the pediatric ulcerative colitis activity index (PUCAI) in patients with ulcerative colitis (UC) and using pediatric Crohn's disease activity index (PCDAI) in combination with C-reactive protein in patients with Crohn's disease (CD). The remission was defined as pediatric ulcerative colitis activity index ⩽10 points in patients with UC and pediatric Crohn's disease activity index ⩽10 points in combination with C-reactive protein ⩽5 mg/L in patients with CD. Simultaneously, we tested the erythrocyte sedimentation rate, the levels of serum albumin, parathormone (electrochemiluminescence immunoassay), serum calcium, and full blood count. The levels of 25OHD were determined using chemiluminescent microparticle immunoassay.

Anthropometry

The body height was measured to the nearest 1 mm, using the anthropometer A-226 (Trystom, Olomouc, Czech Republic). Body weight was measured to the nearest 0.1 kg (with the patients

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wearing just their underwear), using an electronic scale (TH200; Tonava, Upice, Czech Republic). The body mass index was calculated using the formula: body weight (kg)/body height^2 (m). Gender- and age-specific Z-scores were calculated using Czech national reference data.[38]

Dynamic Muscle Function Assessment

Dynamic muscle functions were assessed using jumping mechanography (Leonardo Mechanograph Ground Reaction Force Plate; Novotec Medical GmbH, Pforzheim, Germany), as described elsewhere.[39] The mechanograph consists of two symmetrical force plates, each equipped with eight force sensors. The force sensors record the vertical ground reaction force exerted on the platform at the frequency of 800 Hz.[40] The signal was analyzed using the software provided by the manufacturer (Leonardo Mechanography GRFP version 4.2). Single two-leg jumping and multiple one-leg hopping tests were used to assess the dynamic muscle functions. Maximal muscle power (P_{max}, W) and maximal power adjusted for the body mass (P_{max}/mass, W/kg) of the patients were assessed using the single two-legged jumping test. Maximal muscle force (F_{max}, N) and maximal force adjusted for the body weight (F_{max}/body weight, no unit) of the patients were assessed using the multiple one-leg hopping test. The tests were repeated three times and only the highest value recorded was selected for further analysis. Gender- and height-specific Z-scores were calculated using previously published reference data.[39] Body mass and body weight were calculated in the resting position before starting the test, using the integrated software.

Bone Strength Assessment

In this study, pQCT scanner XCT 2000 (Stratec Medizintechnik, Pforzheim, Germany) was used for bone analyses at the nondominant tibia. The length of the tibia was measured to the nearest 1 mm, from the medial malleolus to the superior margin of the medial condyle of the tibia. To determine the position of the distal tibial growth plate and to adjust the reference lines, a scout scan was performed, followed by two single scans with 0.4-mm voxel size and 2.0-mm slice thickness. These scans were performed at a distances corresponding to the 4% and 66% of the measured bone length. At the distal tibia (4% site), trabecular BMD was measured. At the proximal tibia (66% site), the cortical BMD and thickness, total and cortical bone cross-sectional area (total cross-sectional area (CSA), cortical CSA), and the polar Strength–Strain Index were measured. The images were analyzed and numerical values were calculated using the integrated XCT software, version 6.20 C. Only good-quality scans without movement artifacts were used for further analysis. Gender- and height-specific Z-scores were calculated using the reference data published by Moyer-Mileur et al.[41] To insure the accuracy of measurement, the scanner was calibrated once a week using a phantom provided by the manufacturer.

Statistical Analysis

All data were analyzed using R statistical software (version 3.2.3).[42] Continuous variables were described as median and interquartile range. Categorical variables were described as absolute frequencies and percentages. The Z-scores were tested for difference from zero using the one sample *t*-test. A linear mixed model (R package "lme4"[43] and "lmerTest"[44]) was used to test the effect of cholecalciferol substitution on 25OHD levels during the study period and on the anthropometric, mechanographic, and pQCT characteristics at the baseline and last visit. The selection of the variables included in the regression models was based on the Akaike information criterion, using automatic backward elimination of all effects of the linear mixed effects model. To test whether the changes of bone and muscle characteristics truly depend on cholecalciferol substitution, we constructed the linear mixed model where trabecular BMD, Strength–Strain Index, P_{max}, P_{max}/mass, F_{max}, or F_{max}/body weight Z-scores were the outcomes, and cholecalciferol substitution, the duration of cholecalciferol substitution, body weight, the diagnosis (CD or UC), disease activity (remission), age at baseline visit, the duration of disease treatment at baseline visit, the proportion of patients on corticosteroids during the last 12 months and, in the case when bone parameters were used as outcomes, the Z-scores of P_{max}, P_{max}/mass, F_{max}, and F_{max}/body weight and the other bone measure (Strength–Strain Index or trabecular BMD Z-scores) and, in case when muscle parameters were used as outcomes, the Z-scores of trabecular BMD and Strength–Strain Index were used as predictors.

To determine the predictors of improvement of trabecular BMD Z-score and P_{max}/mass Z-score, we used linear regression modeling. The models testing each predictor (baseline outcome value, e.g., trabecular BMD Z-score at baseline or P_{max}/mass Z-score at baseline, 25OHD levels at baseline, corticosteroids administered last year as categorical variables, and remission at baseline) were adjusted for age, sex, duration of substitution, and disease (CD versus UC). These predictors and covariables were selected according to the clinical consideration. *P* values less than 0.05 were considered statistically significant. All *P* values were two sided.

ETHICAL CONSIDERATIONS

The study was approved by the ethics committee of the hospital, and informed consent was obtained from the parents of all patients.

RESULTS

Baseline Patient Characteristics

Patient characteristics and Montreal classification[45] are summarized in Table 1. Complete assessment of dynamic muscle functions by jumping mechanography could not be performed in only one patient because of her acute physical condition.

Changes in Anthropometric Characteristics

Our patients were slightly shorter and lighter than healthy subjects of the same age (P , 0.001 for both). Anthropometric

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TABLE 1. Demographic Characteristics of Patients and Montreal Classification of IBD

	CD	UC	All
No. of patients (%)	38 (69)	17 (31)	55
Age at diagnosis, yr	12.3 (10.1–13.4)	9 (5.0–11.1)	11.2 (8.4–12.9)
Age at baseline visit, yr	14.3 (12.8–15.7)	12.3 (9.0–14.5)	13.5 (12.0–15.4)
Study follow-up, yr	1.2 (1.0–1.4)	1.1 (1.0–1.3)	1.1 (1.0–1.3)
Sex, male (%)	19 (50)	11 (65)	30 (55)
Localization			
L1	9 (24)		
L2	11 (29)		
L3	18 (47)		
Upper GI involvement	16 (42)		
Behavior			
B1	26 (68)		
B2	12 (32)		
B3	0		
Perianal disease	6 (16)		
Extension			
E1		1 (6)	
E2		4 (24)	
E3		12 (71)	
Severity			
S1		2 (12)	
S2		10 (59)	
S3		5 (29)	
S4		0	
Extraintestinal manifestation	3 (8)	1 (6)	4 (7)

The medians and interquartile ranges are shown for continuous variables, whereas categorical variables are described by the absolute frequencies and percentages.
CD, Crohn's disease; GI, gastrointestinal; UC, ulcerative colitis.

parameters were not different between the baseline and last follow-up visit (Table 2).

Changes in Laboratory Parameters

During the study period, 226 measurements of serum 25OHD levels from 55 patients were obtained. The median number of measurements per patient was 5 (range 4–6). We found an association between daily oral cholecalciferol substitution and 25OHD serum levels, using the linear mixed model (P , 0.001). Among all observations of 25OHD levels after substitution had commenced, 76% of the participants had 25OHD levels permanently ≥50 nmol/L and 98% subjects had levels ≥30 nmol/L. The median serum 25OHD levels increased from 58 nmol/L at the baseline visit to 85 nmol/L at the last follow-up visit (Table 2). Figure 2 shows the percentage of patients with 25OHD levels above the defined cutoffs at the baseline and last follow-up visits, in paired observations.

Changes in Bone Quality

The Z-scores of trabecular BMD, cortical bone CSA, and cortical thickness were lower at the baseline compared with reference (P , 0.001, P¼0.035, and P , 0.001, respectively), and they significantly improved at the last visit (P , 0.001, P , 0.001, and P , 0.001, respectively). Baseline Z-scores for total bone CSA and Strength–Strain Index did not differ from the reference and improved at the last visit (P¼ 0.001 and 0.002, respectively). Only the cortical BMD Z-score was higher at the baseline (P , 0.001) and remained unchanged at the last visit (Table 2).

Changes in Muscle Functions

Among all the tested baseline parameters, only P_{max} Z-score was lower than that in healthy subjects (P ¼ 0.018). Although F_{max} and F_{max}/body weight Z-scores remained unchanged between the baseline and last visit, P_{max} and P_{max}/mass increased significantly (P , 0.001 and P¼0.002, respectively; Table 2).

Predictors of Bone Quality

Using the described statistical methods, we constructed a model (Table 3) to determine the independent predictors of

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TABLE 2. The Anthropometric, pQCT, and Mechanographic Characteristics and Activity of the Disease at Baseline and Last Follow-up Visits

	Baseline	Last Visit	Beta/OR	95% CI	*P*	Missing Values
Anthropometry						
Height Z-score	20.95 (21.59 to 0.07)[a]	20.91 (21.42 to 20.01)	0.09	20.02 to 0.20	0.11	
Weight Z-score	20.7 (21.14 to 0.21) [a]	20.67 (21.48 to 0.1)	0.021	20.113 to 0.155	0.76	
BMI Z-score	20.3 (20.85 to 0.33)	20.53 (21.15 to 0.24)	20.035	20.201 to 0.132	0.68	
Laboratory parameters						
25OHD levels (nmol/L)	57.63 (42.79 to 78.5)	85.2 (61.05 to 95.65)	21.75	11.636 to 31.736	¸0.001	10/8
Parathormone levels (pmol/L) (normal values: 1.30–7.60)	3.79 (2.99 to 5.76)	3.54 (3.09 to 4.48)	20.297	20.821 to 0.226	0.27	12/12
pQCT						
Trabecular BMD Z-score	21.72 (22.25 to 20.42)[a]	21.19 (22.2 to 20.26)	0.26	0.137 to 0.38	¸0.001	
Cortical BMD Z-score	1.07 (0.45 to 1.93)[b]	1.4 (0.57 to 1.9)	0.12	20.102 to 0.334	0.30	
Total bone CSA Z-score	0.15 (20.33 to 0.67)	0.35 (20.31 to 1.34)	0.32	0.146 to 0.484	0.001	
Cortical bone CSA Z-score	20.3 (21.01 to 0.25)[a]	0.04 (20.69 to 0.91)	0.55	0.384 to 0.705	¸0.001	
Cortical thickness Z-score	20.91 (21.61 to 20.27)[a]	20.8 (21.35 to 0.05)	0.23	0.144 to 0.453	¸0.001	
Strength–Strain Index Z-score	0.18 (20.32 to 0.99)	0.22 (20.45 to 1.63)	0.36	0.135 to 0.574	0.002	
Mechonography						
P_{max} Z-score	20.24 (20.96 to 0.44)[a]	0.33 (20.07 to 1.19)	0.79	0.542 to 1.035	¸0.001	1/1
F_{max} Z-score	0 (20.53 to 0.82)	0.07 (20.57 to 0.85)	20.016	20.172 to 0.141	0.85	1/2
P_{max}/mass Z-score	20.06 (20.65 to 0.51)	0.24 (20.54 to 0.69)	0.28	0.11 to 0.459	0.002	1/1
F_{max}/body weight Z-score	0.22 (20.63 to 0.8)	20.05 (20.49 to 0.82)	0.002	20.206 to 0.209	0.99	1/2
Disease activity						
Patients in remission	28 (56)	31 (67)	3.76	0.84 to 16.847	0.084	5/9
PCDAI/PUCAI (score)	5 (0 to 10)	5 (0 to 10)	21.63	24.315 to 1.053	0.24	8/10
CRP (mg/L)	0.65 (0.5 to 2.35)	0.5 (0.5 to 1.8)	20.913	22.58 to 0.755	0.28	1/2
Treatment						
Corticosteroids within last 12 months	14 (25)[c]	5 (9)[a,d]	0	0 to 0.008	¸0.0001	
Anti-TNF therapy	35 (64)	28 (51)	0.16	0.017 to 1.52	0.11	

Medians and interquartile ranges (IQR) are shown for continuous variables, total numbers and percentages describe the categorical data. Coefficients are displayed as estimates with 95% confidence intervals (95% CI) and odds ratios (ORs) with 95% CI for continuous and categorical data, respectively. Missing values are shown separately for baseline (before slash) and follow-up (following slash) visits. Disease remission was defined as PCDAI ¸10 and CRP ¸5 mg/L in Crohn's disease patients and PUCAI ¸10 in patients with ulcerative colitis.

[a]Baseline parameter was significantly lower than in healthy subjects.

[b]Baseline parameter was significantly higher than in healthy subjects.

[c]The median prednisolone cumulative dose for these subjects was 31.3 (IQR 27.1–43.9) mgkgy . [21]

[d]The median prednisolone cumulative dose for these subjects was 3.9 (IQR 2.9–47.2) mg$kg $y. [21]

25OHD, 25-hydroxycholecalciferol; BMD, bone mineral density; BMI, body mass index; CSA, cross-sectional area; PCDAI, pediatric Crohn disease activity index; PUCAI, pediatric ulcerative colitis activity index; TNF, tumor necrosis factor.

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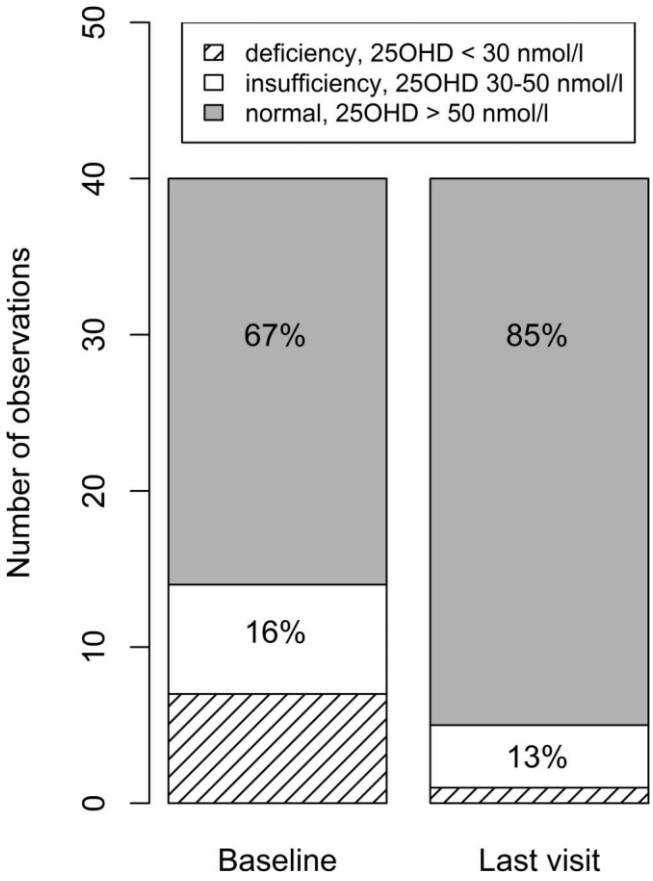

FIGURE 2. Difference between the proportion of patients with normal, insufficient, and deficient serum 25-hydroxyvitamin D (25OHD) levels at the baseline visit and after daily supplementation with 2000 IU of cholecalciferol (only paired observations included, n ¼ 40). Approximately, 17% of patients had 25OHD serum levels , 30 nmol/L before commencement of the substitution, whereas only 2% of them showed insufficient levels after substitution.

bone health. Trabecular BMD Z-score was positively associated with cholecalciferol substitution (beta 0.26, 95% confidence interval [CI] 0.14–0.37, P , 0.0001); conversely, Strength–Strain Index Z-score was not significantly associated. The association between trabecular BMD Z-score and cholecalciferol substitution was independent of the serum levels of 25OHD (data not shown).

Predictors of Muscle Function

The models for predicting muscle characteristics are described in Table 4. We found that P_{max} and P_{max}/mass Z-scores were associated with cholecalciferol substitution (beta 0.60, 95% CI 0.32–0.85, P , 0.0001 and beta 0.20, 95% CI 0.029–0.37, P ¼ 0.043, respectively). On the contrary, F_{max} and F_{max}/body weight Z-scores were not significantly associated with cholecalciferol substitution. The association between P_{max} and P_{max}/mass Z-scores, and cholecalciferol substitution was independent of serum levels of 25OHD (data not shown).

TABLE 3. The Predictors of Bone Strength Measures

	Estimate	95% CI	*P*
Trabecular BMD Z-score			
Cholecalciferol substitution (yes)	0.26	0.14 to 0.37	, 0.0001
Strength–Strain Index Z-score			
F_{max}/body weight Z-score	0.29	0.10 to 0.45	0.0013
P_{max} Z-score	0.57	0.36 to 0.78	, 0.0001
P_{max}/mass Z-score	20.42	20.76 to 0.10	0.016
Age at baseline visit	20.21	20.29 to 0.11	, 0.0001

The predictors were selected based on the Akaike information criterion, using automatic backward elimination of all effects of the linear mixed effects model. The age at baseline visit, the duration of disease treatment at baseline visit, the diagnosis (CD or UC), corticosteroid treatment within last 12 months, cholecalciferol substitution status (yes/no), the duration of cholecalciferol substitution, disease remission (defined as PUCAI , 10 points in UC patients and PCDAI , 10 points in combination with CRP , 5 mg/L in CD patients), muscle functions (P_{max}, P_{max}/mass, F_{max} and F_{max}/body weight Z-scores) and Strength–Strain Index and trabecular BMD Z-scores, respectively, were used as predictors in the initial multiple regression mix models (before the selection of explanatory variables was performed).

BMD, bone mineral density; F_{max}, maximal muscle force; F_{max}/body weight, maximal muscle force adjusted for subject's body weight; P_{max}, maximal muscle power; P_{max}/mass, maximal muscle power adjusted for subject's body mass.

Prediction of Patients Who Would Benefit from Substitution

Trabecular BMD Z-scores improved in 40 (73%) patients and P_{max}/mass Z-score improved in 37 (69%) patients. Only three out of the 55 patients (5%) failed to show improvements in trabecular BMD or P_{max}/mass Z-scores. Hence, the possibility of determining the predictors for identification of these three patients was very low in our cohort. Therefore, we focused on the predictors that can identify the patients with the most profound changes in bone and muscle measures.

Among the tested baseline predictors (bone quality, muscle function and anthropometry parameters, and 25OHD levels), we found that only baseline Z-score of trabecular BMD was a negative predictor of changes in trabecular BMD Z-score in the model adjusted for sex, age, type of the disease (CD versus UC), and duration of cholecalciferol substitution (beta 20.10, 95% CI 20.19 to 20.01, P ¼ 0.031). Similarly, only low P_{max}/mass Z-score at the baseline (beta 20.31, 95% CI 20.52 to 20.01, P ¼ 0.005) was negatively associated with changes in P_{max}/mass Z-score at the last follow-up, in the adjusted model.

DISCUSSION

In this study, we found that daily oral administration of 2000 IU of cholecalciferol was associated with improvement in trabecular BMD at the tibia and muscle power in pediatric patients with IBD. On the contrary, other important muscle–bone unit parameters such as the Strength2Strain Index and maximum muscle force remained unchanged.

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TABLE 4. The Predictors of Dynamic Muscle Functions

	Estimate	95% CI	P
P_{max} Z-score			
Cholecalciferol substitution (yes)	0.60	0.32–0.85	< 0.0001
Disease remission (yes)	0.64	0.23–1.02	0.0025
Strength–Strain Index Z-score	0.37	0.19–0.55	0.0001
P_{max}/mass Z-score			
Cholecalciferol substitution (yes)	0.20	0.029–0.37	0.043
Disease remission (yes)	0.55	0.22–0.87	0.0007
Strength–Strain Index Z-score	0.16	0.0093–0.29	0.030
F_{max} Z-score			
Trabecular BMD Z-score	0.28	0.16–0.41	0.0001
Strength–Strain Index Z-score	0.16	0.019–0.30	0.035
Age at baseline visit	0.093	0.014–0.16	0.021
F_{max}/body weight Z-score			
Strength–Strain Index Z-score	0.26	0.097–0.44	0.0065
Age at baseline visit	0.19	0.081–0.30	0.0014

The predictors were selected based on the Akaike information criterion, using automatic backward elimination of all effects of the linear mixed effects model. The age at baseline visit, the duration of disease treatment at baseline visit, the diagnosis (CD or UC), corticosteroid treatment within last 12 months, cholecalciferol substitution status (yes/no), the duration of cholecalciferol substitution, disease remission (defined as PUCAI < 10 points in UC patients and PCDAI < 10 points in combination with CRP < 5 mg/L in CD patients), trabecular BMD and Strength–Strain Index Z-scores were used as predictors in the initial multiple regression mix models (before the selection of explanatory variables was performed).

BMD, bone mineral density; F_{max}, maximal muscle force; F_{max}/body weight, maximal muscle power adjusted for subject's body weight; P_{max}, maximal muscle power; P_{max}/mass, maximal muscle power adjusted for subject's body mass.

Baseline Bone and Muscle Measures

Baseline data showed that volumetric bone density and bone geometry at the tibia were negatively altered in patients with IBD, when compared with the healthy population. This finding is in agreement with previously published studies.[6,7] On the contrary, mechanography parameters were comparable with those in healthy children at the baseline, with the exception of P_{max} Z-score, which was decreased. During follow-up and vitamin D supplementation, we observed a significant positive change in both bone health parameters and P_{max} in the majority of patients. In a study by Benchimol et al,[27] patients with lumbar spine area BMD < 21 were substituted with 50,000 IU of 25OHD monthly for 6 months in one arm and with 1000 mg of elemental calcium daily in the second arm. No significant difference was found between these two small groups or between these patients and those with normal BMD in the lumbar spine area at the baseline, without any intervention during follow-up. BMD was evaluated using dual-energy X-ray absorptiometry (DXA). In contrast to pQCT, dual-energy X-ray absorptiometry does not enable distinction between trabecular and cortical bone density, and is unable to assess bone geometry; thus, the changes that we observed with pQCT might be missed with dual-energy X-ray absorptiometry. To the best of our knowledge, no studies have described the effect of cholecalciferol substitution on muscle function in pediatric patients with IBD thus far.

Improvement in Muscle–Bone Unit with Cholecalciferol Substitution

We did not prove any substantial effect of disease activity on the bone–muscle unit in our patients with IBD. In contrast, it has been shown that bone quality could improve in patients with IBD who receive standard therapy without any other intervention, especially in the first few months after manifestation, owing to decreased inflammation activity.[6,7] In an inception cohort study, Dubner *et al*[6] showed an improvement of trabecular BMD during the first 6 months after diagnosis of IBD; however, no change was found during the second half of the first year of treatment. Similarly, in a cross-sectional study, patients assessed shortly after diagnosis had more impaired bone–muscle unit, compared with that in patients assessed longer after the diagnosis.[7] The discrepancy between our study and previous studies could be explained by the fact that the median follow-up period from diagnosis till the baseline in our cohort was 1.8 years (interquartile range 1–4 years), which was significantly longer than the follow-up period in previous studies. Thus, we assume that the observed changes in bone and muscle parameters in our patients were not caused by the initiation of IBD therapy, which can be further supported by the similar proportions of patients in remission at the baseline and last follow-up visits (56% versus 67%, respectively). Moreover, the median disease activity indices (pediatric Crohn's disease activity index or pediatric ulcerative colitis activity index) and the levels of C-reactive protein remained stable (Table 2) at both time points of assessment. Meanwhile, we cannot exclude the possibility that treatment of IBD induces changes in the muscle–bone unit that could be apparent later after diagnosis, when the disease itself is already under control.

Role of Steroids and Antitumor Necrosis Factor Treatment

Studies on the role of glucocorticoids in the bone health of children with IBD show conflicting results[6,7,46]; however, some studies showed a negative effect of glucocorticoids on BMD.[47–50] The models in our study did not identify corticotherapy as a negative predictive factor for bone and muscle parameters; however, prolonged treatment with corticosteroids was rarely provided to our patients. Among children who were treated using corticosteroids, the cumulative dose of prednisone was 29 mgkgyr (interquartile range 20–39 mgkgyr). Moreover, these low doses were taken only by 25% of patients within 12 months before the baseline visit and by 9% of patients within 12 months before the second assessment. Our study was neither focused nor powered enough to show the influence of corticotherapy on the muscle–bone unit. Therefore, the study does not allow for definite conclusion about the impact of corticoid treatment history on the development of musculoskeletal measures.

Although recent studies have suggested a protective role of antitumor necrosis factor treatment in the bone health of patients

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with IBD who do not receive vitamin D substitution,[51,52] we did not find this modality to impact the effect of substitution. Moreover, antitumor necrosis factor treatment was not predictive in selecting patients suitable for supplementation (data not shown).

Improvement of Serum 25OHD Levels with Cholecalciferol Substitution

Baseline serum levels of 25OHD in our cohort were comparable with previously published pediatric data.[15,53] A significant proportion of patients showed improved vitamin D status after cholecalciferol supplementation. Our approach to supplement with 2000 IU of cholecalciferol was safe, and we did not experience any laboratory or clinical signals of overdosing. The 25OHD serum level of 80 nmol/L, which has been suggested as an appropriate target level for children with IBD,[24] was achieved in more than one-half of our patients. Our study design does not allow us to discuss the vitamin D supplementation strategy in patients with IBD in general, but as the vast majority of our patients achieved vitamin D sufficiency[14] at the last visit and did not show increased parathyroid hormone levels, we cannot assume that an even higher dosage could result in a better biological action.

Prediction of Patients Who Would Benefit from Cholecalciferol Substitution

The finding of the current study that cholecalciferol substitution improves the muscle–bone unit in pediatric patients with IBD at least to some extent raises a question about the patients who are more likely to benefit from the supplementation. Although we found that low baseline Z-scores of trabecular BMD and P_{max}/mass predicted improvement of the respective parameters, the associations were relatively weak, and we were unable to construct a sufficiently robust model to select patients suitable for supplementation. Importantly, we did not find baseline levels of 25OHD predictive for trabecular BMD or for P_{max}/mass Z-scores improvement. Based on our findings, we conclude that serum 25OHD levels are not valid markers of sufficiency and, thus, could account for the previous observations showing no relation between vitamin D status, and bone density[2,15] and muscle CSA in children with IBD.[7] Only 5% of patients showed no improvement in trabecular BMD or P_{max}/mass Z-score; thus, it is clinically irrelevant to identify predictors for selecting these 5% of patients. Until better predictors are found, cholecalciferol substitution seems to be reasonable for all pediatric patients with IBD.

Clinical Relevance of Data

Although previously published studies show that decreased volumetric bone density is associated with an increased risk for fracture in healthy children,[54] owing to insufficient data, we cannot conclude whether improvement in bone and muscle measures due to vitamin D substitution actually decreases the risk for fractures in pediatric population with IBD. Moreover, inconsistent data exist on differences in fracture prevalence between healthy children and children with IBD.[11,12] Further, the need for screening of asymptomatic vertebral fractures has been discussed.[10,55] To clearly answer this question, a placebo-controlled randomized controlled trial assessing the influence of vitamin D substitution on the frequency of fractures during several years of follow-up in patients with IBD is required.

Limitations of the Study

The study had some limitations. First, it was not an interventional study and no control population was recruited. This was due to insufficient number of patients to be randomly distributed into treatment or placebo intervention groups. Moreover, relatively substantial amount of missing values, mainly of 25OHD serum levels, affected the reliability of the conclusion with regard to the secondary outcome. In particular, we decided to use repetitive measures of 25OHD from individual patients to test the association between cholecalciferol substitution and the change in 25OHD serum levels. However, we have adjusted for the random effect of individual patients by using the mixed model, to overcome the potential impact of unequal number of repetitive measurements. Although the variation in the follow-up period between baseline and the last visit was not a significant predictor in the regression mixed model and did not influence the significance of the other predictors of trabecular BMD Z-score in the regression model, this could also be considered as a limitation. Nevertheless, after creating an arbitrary threshold at 18 months of follow-up to divide the study participants into two groups (i.e., follow up , 18 months; N ¼ 43, and 18 months or more, N ¼ 12, respectively), neither trabecular vBMD Z-scores (0.29 6 0.47 versus 0.15 6 0.41, P ¼ 0.32) nor P_{max}/mass Z-scores (0.23 6 0.67 versus 0.46 6 0.55, P ¼ 0.25) were different between the groups.

CONCLUSION

In conclusion, we demonstrated the association between daily supplementation with 2000 IU of oral cholecalciferol, and improvement in trabecular BMD and maximal muscle power in children with IBD. Substitution of 2000 IU of cholecalciferol helped the vast majority of our patients to achieve vitamin D sufficiency, and was safe. Although we were unable to find a sufficiently strong predictor for identifying patients suitable for substitution, and despite the observational design of our study and the short-term follow-up, we conclude that it seems reasonable to consider vitamin D substitution in all pediatric patients with IBD.

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Postvaccination Immunogenicity of BNT162b2 SARS-CoV-2 Vaccine and Its Predictors in Pediatric Inflammatory Bowel Disease

*Jiri Bronsky, MD, PhD, *Ivana Copova, MD, *Marianna Durilova, MD, PhD, *Denis Kazeka, MD, *Michal Kubat, MD, *Tereza Lerchova, MD, PhD, *Eva Vlckova, MD, *†Katarina Mitrova, MD, PhD, ‡Michal Rataj, ‡Adam Klocperk, MD, PhD, ‡Anna Sediva, MD, PhD, and *Ondrej Hradsky, MD, PhD

ABSTRACT

Objectives: We prospectively compared the postvaccination immunity to messenger ribonucleic acid BNT162b2 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine of our pediatric patients over 12 years old with inflammatory bowel disease (IBD) to that of healthy controls and looked for predictors of its robustness.

Methods: Anti-receptor binding domain, anti-spike S2, and anti-nucleocapsid immunoglobin-G (IgG) and immunoglobin-A levels were measured in 139 pediatric patients with IBD [65 fully vaccinated (2 doses), median age 16.3, interquartile range (IQR) 15.2–17.8 years, median time from vaccination (IQR) 61.0 (42.0–80.0) days] and 1744 controls (46, 37–57 years) using microblot array.

Results: All IBD and control patients developed positive anti-receptor binding domain IgG antibodies at comparable titers. The proportion of observations with positive anti-spike S2 IgG was higher in patients with IBD than in controls [63% vs 21%, odds ratio 2.99 (1.51–5.90)], as was its titer [median (IQR) 485 (92–922) vs 79 [33–180] IU/mL]. Anti-receptor binding domain and anti-spike S2 IgG levels were associated with IBD status. We found an association between anti-spike S2 IgG levels and time since vaccination (β −4.85, 95% CI −7.14 to 2.71, $P = 0.0001$), history of SARS-CoV-2 polymerase chain reaction positivity (206.76, 95% CI 39.93–374.05, $P = 0.0213$), and anti-tumor necrosis factor treatment (−239.68, 95% CI −396.44–83.55, $P = 0.0047$). Forty-three percent of patients reported vaccination side effects (mostly mild). Forty-six percent of observations with positive anti-nucleocapsid IgG had a history of SARS-CoV-2 infection.

Conclusions: Patients with IBD produced higher levels of postvaccination anti-spike S2 antibodies than controls. Previous SARS-CoV-2 infection is associated with higher production of postvaccination antibodies and anti-tumor necrosis factor treatment with lower production.

Key Words: COVID-19, nucleocapsid antigen, receptor binding domain, spike protein, tumor necrosis factor

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What Is Known

- Patients with inflammatory bowel disease (IBD) were excluded from registration trials of BNT162b2 messenger ribonucleic acid vaccine.
- There is scarce data on the efficacy and safety in patients with IBD, especially children.

What Is New

- Pediatric IBD patients produced higher levels of anti-spike S2 antibodies than controls.
- Previous severe acute respiratory syndrome coronavirus 2 infection was associated with higher production of postvaccination antibodies and anti-tumor necrosis factor treatment with lower production.

Vaccination against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in patients with inflammatory bowel disease (IBD) is recommended by many professional societies (1–3). However, patients with IBD were excluded from registration trials of vaccines; thus, there is scarce data on the efficacy and safety in patients with IBD, especially children. Vaccination by messenger ribonucleic acid (mRNA) vaccine BNT162b2 (Comirnaty—Pfizer/BioNTech) of children 12 years and older was approved by the European Medicine Agency in spring 2021, and in the Czech Republic, it commenced on July 1, 2021. A clinical study in healthy individuals has shown that immune responses in the age group of 12–15 year-olds were comparable with those in the age group of 16–25 year-olds, and the side effects were mild (4). Based on the

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From the *Department of Paediatrics, 2nd Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic, the †Clinical and Research Centre for Inflammatory Bowel Disease ISCARE and First Faculty of Medicine, Charles University, Prague, Czech Republic, and the ‡Department of Immunology, 2nd Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic.

Address correspondence and reprint requests to Jiri Bronsky, MD, PhD, Head, Gastroenterology and Nutrition Unit, Department of Paediatrics, University Hospital Motol, V Uvalu 84, 15006, Prague, Czech Republic (e-mail: jiri.bronsky@lfmotol.cuni.cz).

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Ethical Approval: This study was approved by the ethics committee of the Motol University Hospital. All patients or their legal guardians signed an informed consent form prior to inclusion in the study.

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recommendation of the Czech Working Group for Pediatric Gastroenterology, children with IBD in our country were not prioritized for vaccination against SARS-CoV-2, and vaccination in this group started along with other healthy children after vaccine approval in July 2021. According to a position paper by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition Porto IBD working group, surveillance of postvaccination antibodies in patients with pediatric IBD (pIBD) are recommended (5). Based on this recommendation, we initiated a prospective follow-up of patients with pIBD.

Information about postvaccination SARS-CoV-2 immunity in patients with pIBD is scarce (6–8). This is the first prospective study focused primarily on patients with pIBD (12–18 years) vaccinated against SARS-CoV-2 using an mRNA vaccine.

SARS-CoV-2 virus contains various epitopes. Antibodies against some of them are used for testing of postinfection and/or postvaccination status. Anti-receptor binding domain of S1 subunit of spike protein (anti-RBD) antibodies and anti-spike S2 antibodies are against antigens on the viral surface and are considered markers of postvaccination or postinfection status. On the contrary, anti-nucleocapsid antibodies are formed against an antigen inside of the viral particle and are thus considered as markers of postinfection status only.

Our primary aim was to compare postvaccination immunity (production of anti-RBD immunoglobin-G [IgG], and anti-spike S2 IgG) in patients with pIBD and healthy controls (HCs). We also focused on predictors of postvaccination immunity status and the role of history of SARS-CoV-2 infection, IBD treatment used, and disease activity at the time of observation.

METHODS

Study Subjects

After the approval of SARS-CoV-2 vaccination for children 12 years and older in the Czech Republic in July 2021, we started to prospectively recruit patients with pIBD irrespective of treatment at our IBD center. Participation in the study was offered to all ambulatory and hospitalized patients with IBD during the period of September 8, 2021 to December 17, 2021, irrespective of the time since SARS-CoV-2 infection or vaccination. Altogether, 195 observations [median age 15.6, interquartile range (IQR) 13.6–17.1] of 139 patients were obtained. Of these, 83 observations were from 65 patients [median age 16.4, IQR 14.5–17.9, 40 patients with Crohn's disease (CD), 21 with ulcerative colitis, and 4 with IBD unclassified] fully vaccinated against SARS-CoV-2 [received 2 doses of mRNA vaccine BNT162b2 (Comirnaty—Pfizer/BioNTech); median time from vaccination (IQR) was 61.0 (42.0–80.0) days]. The basic characteristics of vaccinated patients with pIBD are listed inTables 1A and 1B.

Forty-four of the patients had previously tested positive for SARS-CoV-2 by polymerase chain reaction (PCR) (67 observations, 34 in the fully vaccinated group and 33 in the nonvaccinated group). Seventy-nine observations were neither vaccinated nor had positive SARS-CoV-2 PCR. We also included 1744 adult HCs (1460 female, median age 46, IQR 37–57 years) who were previously fully vaccinated against SARS-CoV-2 [received 2 doses of mRNA vaccine BNT162b2 (Comirnaty—Pfizer/BioNTech); the median time from vaccination was 97 (70–124) days]. HCs consisted of healthcare workers of our University Hospital who voluntarily agreed to participate in testing of postvaccination antibodies between April and June 2021.

Collection of Clinical Data and Laboratory Samples

Clinical data (patient characteristics, disease phenotype, disease activity, clinical indices, laboratory markers, IBD treatment, history of SARS-CoV-2 infection or vaccination, and potential side effects of vaccination) were prospectively recorded in the database. Information on SARS-CoV-2 infection had been collected prospectively into our database since the outbreak of the SARS-CoV-2 pandemic in the population in spring 2020. The data underlying this article will be shared upon reasonable request by the corresponding author. Two mL of blood was drawn from each patient to measure postinfection and postvaccination antibodies. Blood was allowed to clot and then centrifuged, and the separated serum was frozen at −20°C until analysis.

Laboratory Methods

Antibodies were measured in all patients using the Microblot-Array COVID-19 IgG/IgA (TestLine Clinical Diagnostics, Brno, Czech Republic) according to the manufacturer's instructions. We measured anti-RBD IgG and IgA, anti-spike S2 IgG and IgA, and anti-nucleocapsid IgG and IgA. Antibody titers were quantified in U/mL based on the intra-assay calibration curve. Titers >210 U/mL were considered positive, and titers 185–210 U/mL were considered borderline positive. The assay validity was verified using an internal assay and conjugate controls.

Definitions of Postvaccination and Postinfection Status

For the purpose of this study, we considered history of SARS-CoV-2 PCR positivity as positive postinfection status. Anti-RBD antibodies and anti-spike S2 antibodies were considered markers of postvaccination or postinfection status. Anti-nucleocapsid antibodies were considered markers of postinfection status but were analyzed independently of the history of SARS-CoV-2 PCR positivity.

Evaluation of IBD Treatment Used and Disease Activity

IBD treatments of any length received at the time of observation were evaluated in this study. Disease activity was evaluated using clinical indices [weighted Pediatric Crohn's Disease Activity Index (wPCDAI) and Pediatric Ulcerative Colitis Activity Index (PUCAI)] (9,10), C-reactive protein (CRP), and fecal calprotectin (F-CPT) levels.

Aims of the Study

The primary aim [tested in 83 observations in fully vaccinated pIBD patients (N = 65) and fully vaccinated HCs (N = 1744)] was to compare the immunogenicity of the mRNA SARS-CoV-2 BNT162b2 vaccine (defined as the production of anti-RBD IgG and anti-spike S2 IgG) in patients with pIBD and HCs.

Nestlé, Ferring, and Falk. The remaining authors report no conflicts of interest.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (*www.jpgn.org*).

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TABLE 1A. Basic characteristics of the patients

No. of patients	CD (N = 40)	IBDU (N = 4)	UC (N = 21)	Overall (N = 65)
General characteristics				
Female sex	19 (47.5%)	0 (0%)	7 (33.3%)	26 (40.0%)
Age at diagnosis (median, IQR), y	10.7 (9.49–13.0)	6.73 (6.73–6.73)	11.1 (8.23–13.2)	10.8 (9.29–12.9)
Age at observation (median, IQR), y	16.4 (15.2–18)	16.1 (16–16.1)	16.3 (15.2–17)	16.3 (15.2–17.8)
Paris classification				
A1a: <10 y	16 (40.0%)	2 (50.0%)	6 (28.6%)	24 (36.9%)
A1b: 10–16 y	22 (55.0%)	2 (50.0%)	14 (66.7%)	38 (58.5%)
A2: 17–40 y	2 (5.0%)	0 (0%)	1 (4.7%)	3 (4.6%)
L1: ileal	10 (25.0%)	NA	NA	NA
L2: colonic	9 (22.5%)	NA	NA	NA
L3: ileocolonic	21 (52.5%)	NA	NA	NA
L4: upper GI	19 (47.5%)	NA	NA	NA
B1: nonstricturing, nonpenetrating	34 (85.0%)	NA	NA	NA
B2: stricturing	4 (10.0%)	NA	NA	NA
B2B3: stricturing + penetrating	1 (2.5%)	NA	NA	NA
B3: penetrating	1 (2.5%)	NA	NA	NA
Perianal disease	6 (15.0%)	NA	NA	NA
G0: no history of growth retardation	31 (77.5%)	2 (50.0%)	18 (85.7%)	51 (78.5%)
G1: history of growth retardation	9 (22.5%)	2 (50.0%)	3 (14.3%)	14 (21.5%)
E1: proctitis	NA	1 (0%)	1 (4.8%)	NA
E2: left sided	NA	1 (25.0%)	4 (19.0%)	NA
E3: extensive	NA	0 (0%)	2 (9.5%)	NA
E4: pancolitis	NA	2 (25.0%)	14 (66.7%)	NA
S0: never severe	NA	3 (50.0%)	18 (85.7%)	NA
S1: ever severe	NA	1 (0%)	3 (14.3%)	NA

CD = Crohn's disease; IBDU =inflammatory bowel disease unclassified; IQR = interquartile range; NA = not applicable; UC = ulcerative colitis.

TABLE 1B. Characteristics of the patients at the time of observation

	CD (N = 49)	IBDU (N = 5)	UC (N = 29)	Overall (N = 83)
Treatment				
Adalimumab	21 (42.9%)	0 (0%)	1 (3.4%)	22 (26.5%)
Infliximab	16 (32.7%)	0 (0%)	14 (48.3%)	30 (36.1%)
Ustekinumab	1 (2.0%)	0 (0%)	0 (0%)	1 (1.2%)
Vedolizumab	7 (14.3%)	2 (40.0%)	2 (6.9%)	11 (13.3%)
Immunomodulator	26 (53.1%)	3 (60.0%)	17 (58.6%)	46 (55.4%)
Combo therapy	30 (61.2%)	5 (100%)	22 (75.9%)	57 (68.7%)
5-Aminosalicylates	0 (0%)	2 (40.0%)	25 (86.2%)	27 (32.5%)
Disease activity				
wPCDAI (median, IQR)	0 (0–7.50)	NA	NA	0 (0–7.50)
PUCAI (median, IQR)	NA	0 (0–0)	0 (0–0)	0 (0–0)
CRP (median, IQR) mg/l	0.50 (0.50–2.80)	0.50 (0.50–0.50)	0.50 (0.50–1.20)	0.50 (0.50–2.30)
Fecal calprotectin (median, IQR), µg/g	79.0 (25.0–519)	224 (42.0–273)	28.0 (9.0–229)	55.0 (17.5–398)

CD = Crohn's disease; Combo therapy = combination of biological therapy and immunomodulator; CRP = C-reactive protein; IBDU = inflammatory bowel disease unclassified; IQR = interquartile range; PUCAI = Paediatric Colitis Activity Index; UC = ulcerative colitis; wPCDAI = weighted Paediatric Crohn's Disease Activity Index; NA = not applicable.

The secondary aims [tested in 83 observations in fully vaccinated pIBD patients (N = 65)] were to describe:

1. the association between postvaccination antibody titers in patients with pIBD and postinfection status, IBD treatment used, and disease activity at the time of observation;
2. the association of postinfection antibody titers in patients with pIBD and postinfection status, IBD treatment, and disease activity at the time of observation; and
3. side effects of SARS-CoV-2 vaccination in patients with pIBD.

Other aims [tested in 195 observations in all pIBD patients (N = 139)–both fully vaccinated (N = 65) and nonvaccinated (N = 74)] were to compare:

1. IgG antibody levels in vaccinated and nonvaccinated patients with pIBD; and
2. history of SARS-CoV-2 PCR positivity and anti-nucleocapsid IgG positivity in patients with pIBD.

IgA Antibodies

Data on anti-RBD, anti-spike S2, and anti-nucleocapsid IgA antibodies were analyzed separately and are presented in the Supplemental Digital Content, *http://links.lww.com/MPG/C992*.

Statistical Analysis

All data were analyzed using R statistical software version 4.0.4 (R Core Team, 2021, https://www.R-project.org/). Continuous variables are described as medians and IQR. Categorical variables are described as absolute frequencies and percentages. Missing data were not imputed. Three different data sets were established: (1) vaccinated patients with pIBD and vaccinated HCs; (2) fully vaccinated patients with pIBD; and (3) fully vaccinated and not fully vaccinated patients with pIBD. To avoid pseudoreplication, we used mixed models for all analyses; for categorical outcomes (eg, positivity for SARS-CoV-2 antibodies), we used a generalized linear mixed model; for linear outcomes (eg, level of SARS-CoV-2 antibodies), we used a linear mixed model. Only fixed effects of the predictors were presented. Due to the assumption that antibodies levels decline over time, we added the variable "time since vaccination" to all models with RBD and Spike S2 as outcomes. We also adjusted models for "history of SARS-CoV-2 PCR positivity." When appropriate, we added interaction effects to our model. We constructed a final prediction model based on the clinical selection of the predictors.

RESULTS

Primary Aim—Postvaccination Immunity in pIBD and HCs

We compared 65 fully vaccinated patients with pIBD (83 observations) with 1744 fully vaccinated HCs. All patients with pIBD and HCs developed positive anti-RBD IgG antibodies, and levels were similar in both groups [median (IQR) 971 (960–987) vs 972 (781–1030 IU/mL); Figure 1a, Supplemental Digital Content, *http://links.lww.com/MPG/C992*]. In an adjusted linear-mixed model, anti-RBD IgG levels were associated with pIBD status (Fig. 1A).

The proportion of observations with positive anti-spike S2 IgG was higher in patients with pIBD than in HCs [63% vs 21%, OR 2.99 (1.51–5.90), $P = 0.02$; Figure 1b, Supplemental Digital Content, *http://links.lww.com/MPG/C992*], as were anti-spike S2 IgG titers [median (IQR) 485 (92–922) vs 79 (33–180) IU/mL, Figure 1c, Supplemental Digital Content, *http://links.lww.com/MPG/C992*]. In adjusted linear-mixed model, anti-spike S2 IgG levels were associated with pIBD status (Fig. 1B).

Secondary Aims

In this part of the study, 65 fully vaccinated patients with pIBD (83 observations) were included.

Predictors of Postvaccination Immunity in Patients with pIBD

In the linear-mixed model adjusted for time from vaccination, no association was found between numerical values of anti-RBD IgG levels and history of SARS-CoV-2 PCR positivity (Figure 2, Supplemental Digital Content, *http://links.lww.com/MPG/C992*). We found no association between the history of SARS-CoV-2 PCR positivity and the presence of anti-spike S2 IgG as a categorical variable, however in linear-mixed model adjusted for time from vaccination, numerical values of anti-spike S2 IgG were positively associated with a history of SARS-CoV-2 PCR positivity (Fig. 2).

The association between IBD treatment and postvaccination IgG antibody levels is shown in Table 2. In linear regression mixed model adjusted for time interval from vaccination and history of SARS-CoV-2 PCR positivity, anti-spike S2 IgG levels were lower in observations treated with anti-TNF (tumor necrosis factor) (β −215, 95% CI −379 to −53, $P = 0.01$, Figure 3, Supplemental Digital Content, *http://links.lww.com/MPG/C992*).

As most of our patients were in clinical remission (Table 1B), we did not test the association between clinical indices of disease activity (wPCDAI and PUCAI) and postvaccination antibodies. In linear mixed models adjusted for time since vaccination and history of SARS-CoV-2 positivity, CRP, but not F-CPT, was associated with postvaccination production of anti-RBD and anti-spike S2 antibodies. Thus, CRP was added to the final mixed model to predict the anti-RBD IgG and anti-spike S2 IgG status.

We made final mixed models containing time interval from vaccination (β −6.57, 95% CI −12.92 to −0.28, $P = 0.0496$), history of SARS-CoV-2 PCR positivity (β 9.41, 95% CI −43.98 to 63.34, $P = 0.7372$), anti-TNF (β −9.53, 95% CI −59.71 to 40.59, $P = 0.72$), immunomodulatory treatment (β −1.56, −34.34 to 30.63, $P = 0.93$), and CRP (β −6.57, −12.92 to −0.28, $P = 0.0496$). We found an association between CRP levels and anti-RBD IgG levels (Table 1a, Supplemental Digital Content, *http://links.lww.com/MPG/C992*). We also found association between anti-spike S2 IgG levels and time since vaccination (β −4.85, 95% CI −7.14 to 2.71, $P = 0.0001$), history of SARS-CoV-2 PCR positivity (β −4.85, 95% CI −7.14 to 2.71, $P = 0.0001$), and anti-TNF treatment (β −239.68, 95% CI −396.44 to −83.55, $P = 0.0047$), but not with immunomodulatory treatment (β −105.48, −242.93 to 33.70, $P = 0.15$) or CRP (β −3.71, 95% CI −25.25 to 17.82, $P = 0.74$; Table 1b, Supplemental Digital Content, *http://links.lww.com/MPG/C992*). The levels of anti-RBD and anti-spike S2 IgG stratified according to various types of biological therapy are shown in Figures 4a and b, Supplemental Digital Content, *http://links.lww.com/MPG/C992*.

Predictors of Postinfection Immunity in Vaccinated Patients with pIBD

In linear regression mixed model adjusted for time interval from vaccination and history of SARS-CoV-2 PCR positivity, anti-nucleocapsid IgG levels were lower in observations treated with anti-TNF (β −220, 95% CI −345 to −96, $P < 0.001$) and with immunomodulators (IMM) (β −119, 95% CI −229 to 9.49, $P = 0.04$; Table 2). In categorical model, lower proportion of observations

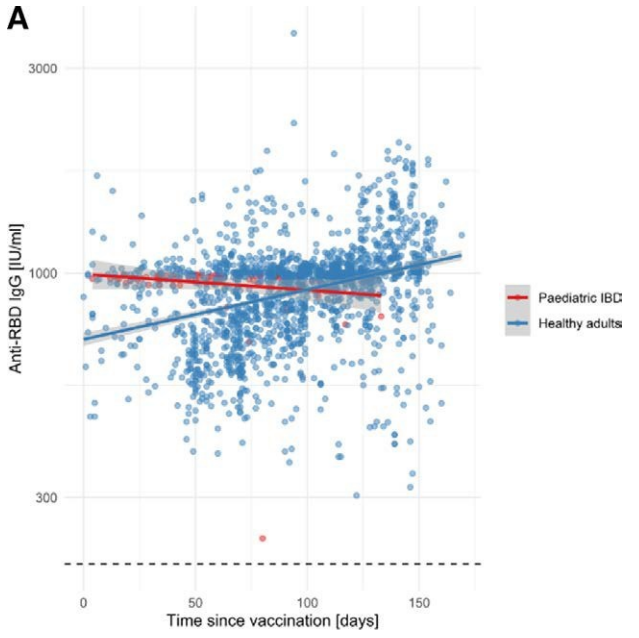

FIGURE 1a. Association of anti-RBD IgG antibodies and time since vaccination in pIBD patients and healthy adults. Linear mixed model adjusted for time from vaccination, age, and interaction between time from vaccination and case/control variable; IgG=immunoglobulin G; pIBD=paediatric inflammatory bowel disease; CI95 = 95% confidence interval.

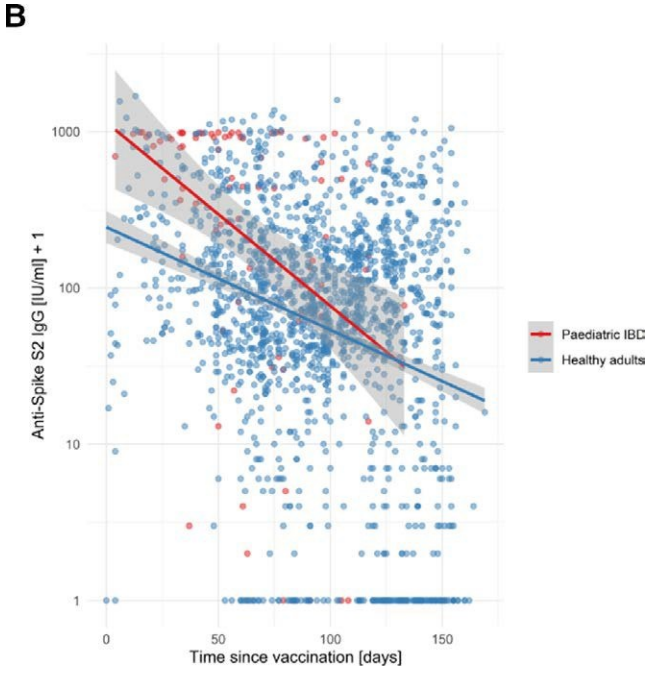

FIGURE 1b. Association of anti-spike S2 IgG antibodies and time since vaccination in pIBD patients and healthy adults. Linear mixed model adjusted to time from vaccination, age and interaction between time from vaccination and case/control variable; IgG=immunoglobulin G; pIBD=paediatric inflammatory bowel disease; CI95 = 95% confidence interval

with anti-TNF developed positive anti-nucleocapsid IgG (OR 0.137, 95% CI 0.025–0.762, $P = 0.023$). Levels of anti-nucleocapsid IgG stratified according to various types of biological therapy are shown in Figure 4c, Supplemental Digital Content, *http://links.lww.com/MPG/C992*. As most of our patients were in clinical remission (Table 1B), we did not test the association between clinical indices of disease activity (wPCDAI and PUCAI) and postinfection antibodies. We did not find an association between anti-nucleocapsid IgG levels and CRP or F-CPT levels.

Side Effects of Vaccination in Patients with pIBD

Out of 65 vaccinated patients with pIBD, 28 (43%) reported side effects following the first dose of vaccination (2 with fever, 26 with mild complaints—pain, edema, or erythema in the injection site, fatigue, headache, or slightly elevated body temperature). Twenty-eight patients (43%) reported side effects after the second dose (3 with fever, 1 with vomiting with subsequent proven SARS CoV-2 PCR positivity, 1 with worsening of pustulous skin lesions, and 23 with mild complaints). In all patients, the side effects were short-lasting and self-limiting.

Other Aims

In this part of the study, 139 patients with pIBD (195 observations) were included, of which 65 were fully vaccinated (83 observations) and 67 observations (34 observations from the vaccinated group and 33 observations from the nonvaccinated group) in 44 patients had a history of SARS-CoV-2 PCR positivity.

Comparison of Vaccinated and Nonvaccinated Patients with pIBD

Observations in fully vaccinated pIBD had higher levels of all investigated IgG antibodies than nonvaccinated patients with pIBD (anti-RBD: β 630, 95% CI 537–722, $P < 0.0001$; anti-spike S2: 258, 95% CI 157–359, $P < 0.0001$; anti-nucleocapsid: 133, 95% CI 64–202, $P = 0.0002$), even after adjustment for history of SARS-CoV-2 PCR positivity. Both anti-spike S2 IgG and anti-nucleocapsid IgG were also associated with a history of SARS-CoV-2 PCR positivity in linear regression mixed model adjusted to postvaccination status (β 292, 95% CI 182–402, $P < 0.0001$ and 119, 95% CI 41–197, $P = 0.0033$, respectively; Table 2 and Figure 5, Supplemental Digital Content, *http://links.lww.com/MPG/C992*).

Comparison of History of SARS-CoV-2 PCR Positivity and Anti-Nucleocapsid IgG Positivity in Patients with pIBD

Among observations with positive anti-nucleocapsid IgG antibodies (N = 46), 25 had a history of SARS-CoV-2 PCR positivity (54%). Among observations with insufficient anti-nucleocapsid IgG antibodies (N = 149), 42 had a history of SARS-CoV-2 PCR positivity (28%; Table 3, Supplemental Digital Content, *http://links.lww.com/MPG/C992*).

Postvaccination and Postinfection IgA Antibodies

In linear mixed model, anti-RBD IgA antibodies were associated with time from vaccination and age but not with pIBD status. Anti-spike S2 IgA antibodies were associated with pIBD status, time from vaccination, and the interaction between these 2 variables (Figure 6a and b, Supplemental Digital Content, *http://links.lww.com/MPG/C992*).

Observations in fully vaccinated patients with pIBD had higher levels of anti-RBD IgA antibodies than nonvaccinated

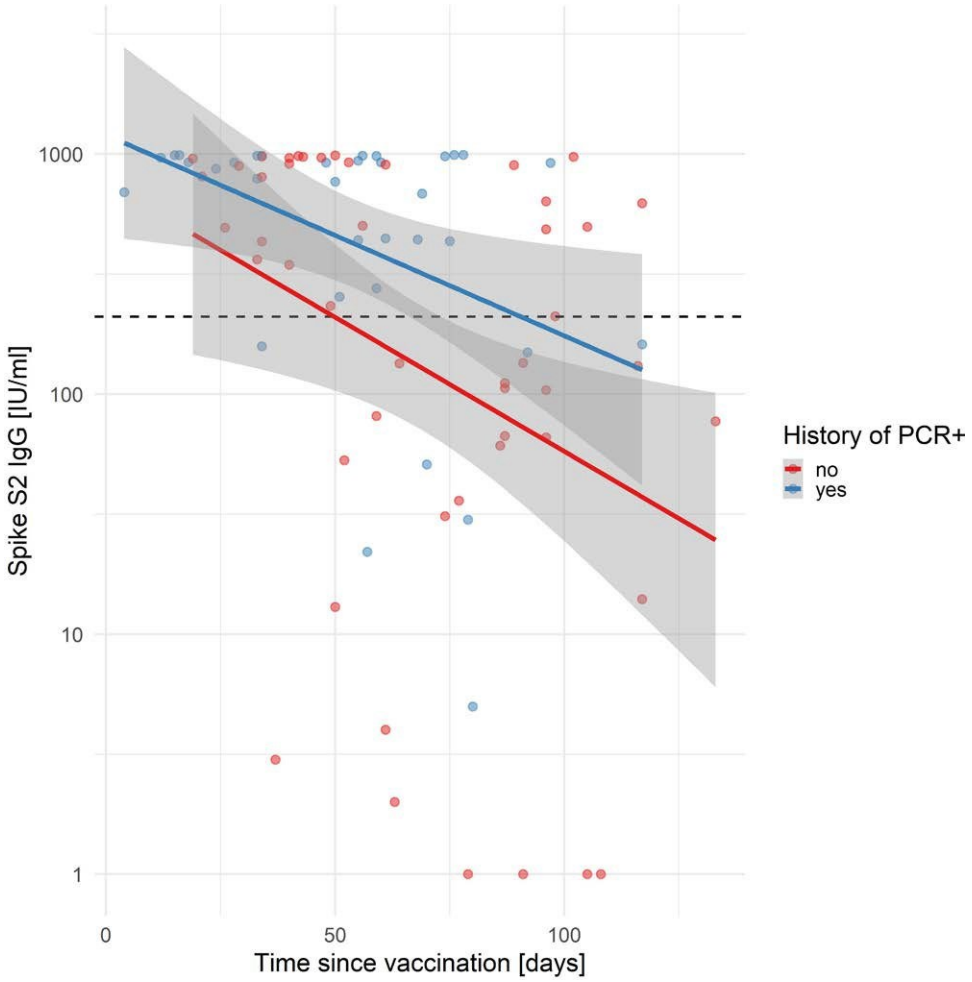

FIGURE 2. Association of anti-spike S2 IgG antibodies and history of SARS-CoV-2 PCR positivity in pIBD patients. IgG = immunoglobin-G; PCR = polymerase chain reaction; pIBD = pediatric inflammatory bowel disease; SARS-CoV-2 = severe acute respiratory syndrome-associated coronavirus 2.

patients with pIBD (β 169, 95% CI 82–255, $P = 0.0001$), even after adjustment for history of SARS-CoV-2 PCR positivity (Figure 7a, Supplemental Digital Content, *http://links.lww.com/MPG/C992*). No association was found between anti-spike S2 IgA or anti-nucleocapsid IgA levels and vaccination status or history of SARS-CoV-2 positivity (Figure 7b and c, Supplemental Digital Content, *http://links.lww.com/MPG/C992*).

Based on visual evaluation of the data in vaccinated patients with pIBD, anti-spike S2, but not anti-RBD IgA antibodies, was higher in patients with a history of SARS-CoV-2 PCR positivity, and both antibody types decreased over time after vaccination (Figures 8a and b, Supplemental Digital Content, *http://links.lww.com/MPG/C992*).

Based on visual evaluation of the data in vaccinated patients with pIBD, anti-RBD, but not anti-spike S2 and anti-nucleocapsid IgA was lower in those with anti-TNF and combination treatments (Figure 9, Supplemental Digital Content, *http://links.lww.com/MPG/C992*).

DISCUSSION

We have shown the excellent postvaccination status of patients with pIBD when evaluated using anti-RBD IgG antibodies. These were positive in both patients with pIBD and HCs, which is in line with data published so far in adult patients with IBD (11–14). However, higher anti-RBD and anti-spike S2 IgG levels were independently associated with pIBD status, suggesting a better response to vaccination in our pIBD population than in adult HCs. In contrast, most studies show lower seroconversion rates in adult patients with IBD than that in HCs (11,15–17); however, the difference is small, and the rates are still better than in patients with other immune-mediated disorders (12). The pooled relative risk of breakthrough infections in vaccinated patients with IBD is similar to that of vaccinated controls (11,13). Our data suggest that pediatric patients with IBD may have a better response to the SARS-CoV 2 mRNA vaccine than adult patients with IBD; however, studies comparing these 2 cohorts are lacking. There were differences in the production of postvaccination antibodies, especially anti-spike S2 IgG. Unlike anti-RBD, anti-spike S2 has also been associated with anti-TNF treatment. This suggests that anti-spike S2 may be a more sensitive marker of postvaccination status than anti-RBD.

History of SARS-CoV-2 PCR positivity is strongly associated with higher levels of anti-spike S2 IgG antibodies. This suggests that previous infection may increase the production of antibodies after vaccination, even in patients with pIBD. This phenomenon is independent of treatment with immune-mediating drugs and points

TABLE 2. Association of postvaccination antibodies and IBD treatment—summary of regression models adjusted to time from vaccination and history of SARS-CoV-2 PCR positivity

Postvaccination antibody	Treatment modality	Yes (IQR or %)	No (IQR or %)	Beta (95% CI)	*P* value
Anti-RBD IgG	aTNF	978 (960.75–990)	966 (959.5–984)	−15.32 (−65.99 to 35.22)	0.56
Anti-spike S2 IgG	**aTNF**	**253.5 (58–829)**	**892 (354–974)**	**−214.86 (−378.65 to 52.77)**	**0.01**
Anti-nucleocapsid IgG	**aTNF**	**90 (12–247)**	**379 (109–619)**	**−220.18 (−345.37 to 95.8)**	**<0.001**
Anti-spike S2 IgG categorical	aTNF	27 (0.52)	25 (0.48)	0.171 (0.029 to 1.011)	0.051
Anti-nucleocapsid IgG categorical	**aTNF**	**14 (0.27)**	**38 (0.73)**	**0.082 (0.008 to 0.848)**	**0.036**
Anti-RBD IgG	IFX	976.5 (946.5–990)	971 (960–985)	2.51 (−43.32 to 48.34)	0.92
Anti-spike S2 IgG	IFX	253.5 (58–889.75)	502 (105–962)	−44.47 (−218.2 to 130.42)	0.62
Anti-nucleocapsid IgG	IFX	78 (14.25–243.5)	195 (58–437)	−93.11 (−228.91 to 43.38)	0.19
Anti-spike S2 IgG categorical	IFX	16 (0.53)	14 (0.47)	0.557 (0.156 to 1.986)	0.367
Anti-nucleocapsid IgG categorical	IFX	8 (0.27)	22 (0.73)	0.313 (0.082 to 1.205)	0.091
Anti-RBD IgG	combo	969 (960–986)	979 (961.5–989.25)	1.91 (−32.72 to 37.22)	0.92
Anti-spike S2 IgG	combo	493 (80–962)	464.5 (103.5–914)	−28.22 (−191.48 to 135)	0.73
Anti-nucleocapsid IgG	combo	160 (45–435)	121.5 (16–312.5)	−1.49 (−127.33 to 125.26)	0.98
Anti-spike S2 IgG categorical	combo	37 (0.65)	20 (0.35)	1.047 (0.292 to 3.759)	0.943
Anti-nucleocapsid IgG categorical	combo	24 (0.42)	33 (0.58)	1.193 (0.317 to 4.488)	0.794
Anti-RBD IgG	IMM	969 (959.25–985.75)	978 (960–988)	−4.39 (−37.4 to 28.82)	0.8
Anti-spike S2 IgG	IMM	435 (77–895)	622 (105–938)	−131.14 (−277.79 to 15.63)	0.08
Anti-nucleocapsid IgG	**IMM**	**120.5 (29.5–356.5)**	**170 (45–477)**	**−119.37 (−229.08 to 9.49)**	**0.04**
Anti-spike S2 IgG categorical	IMM	28 (0.61)	18 (0.39)	0.673 (0.176 to 2.572)	0.563
Anti-nucleocapsid IgG categorical	IMM	16 (0.35)	30 (0.65)	0.452 (0.116 to 1.762)	0.253
Anti-RBD IgG	5-ASA	976 (960.5–986.5)	970.5 (958.75–986.25)	19.33 (−27.41 to 65.72)	0.42
Anti-spike S2 IgG	5-ASA	439 (105–918.5)	497.5 (96.25–922)	−28.01 (−204.85 to 144.6)	0.75
Anti-nucleocapsid IgG	5-ASA	139 (50.5–380)	149 (13.5–394.25)	−13.61 (−172.78 to 129.4)	0.85
Anti-spike S2 IgG categorical	5-ASA	17 (0.63)	10 (0.37)	0.908 (0.265 to 3.115)	0.878
Anti-nucleocapsid IgG categorical	5-ASA	11 (0.41)	16 (0.59)	1.323 (0.316 to 5.545)	0.701

Significant results are highlighted in bold. 5-ASA = 5-aminosalicylic acid; aTNF = anti-tumor necrosis factor alpha therapy; "categorical" = IgG levels of antibodies above the cut-off of the assay; combo = combination therapy (aTNF + immunomodulator); IBD = inflammatory bowel diseases; IFX = infliximab; IMM = immunomodulator; "No" = median levels (IU/mL)/no. of postvaccination antibodies in subjects not receiving the treatment modality; PCR = polymerase chain reaction; RBD = receptor binding domain of S1 subunit of spike protein; SARS-CoV-2 = severe acute respiratory syndrome-associated coronavirus 2; "Yes" = median levels (IU/mL)/no. of postvaccination antibodies in subjects receiving the treatment modality.

to the potentiation of postinfection and postvaccination status of the immune system in healthy individuals. Importantly, this mechanism was not disrupted in individuals with immune-suppressed pIBD.

Anti-TNF treatment was independently and strongly associated with lower anti-spike S2 IgG levels. The negative effect of anti-TNF treatment (especially infliximab, IFX) on postvaccination status has been observed in many studies in adult patients with IBD and thoroughly discussed by the scientific community (11,12,15,16,18–30). However, other studies failed to find any association (31,32) and even large systematic reviews and meta-analyses have shown conflicting results, especially when combination therapy with IMM is considered (11–13). In our study, neither IFX alone nor combination therapy was associated with postvaccination IgG antibody levels. The use of IMM had a slight association with lower anti-spike S2 levels; however, the difference was not statistically significant ($P = 0.08$). Although anti-TNF might attenuate postvaccination serological response, it seems that the rate of SARS-CoV-2 infection does not increase in vaccinated patients with IBD on anti-TNF therapy (32,33). Given the lack of evidence that anti-TNF increases the risk of severe COVID-19, it is unclear whether subjects treated with anti-TNF require either an accelerated booster vaccine or postvaccination serological monitoring (34). Based on our data (Figure 3, Supplemental Digital Content, *http://links.lww.com/MPG/C992*), anti-TNF treatment was associated with a faster decrease in anti-spike S2 titers.

Adverse side effects in our study were described in 43% of patients both after the first and second doses of the mRNA vaccine. The vast majority of cases were mild and self-limiting. This is in line with data showing consistently good tolerance in adult patients with IBD and the safety of SARS-CoV-2 vaccines (35–39).

Postinfection immunity (represented by positive anti-nucleocapsid IgG levels) was augmented by BNT162b2 vaccination, as expected. This is in accordance with the above-mentioned potentiation of postinfection and postvaccination immunity, even in immune-suppressed pIBD individuals. Moreover, lower anti-nucleocapsid IgG levels were independently associated with anti-TNF and IMM treatment. These results correspond to previ- ously published large-scale therapeutic drug monitoring studies in adult patients with IBD, showing impaired SARS-CoV-2 antibody responses in patients treated with IFX or adalimumab (ADA) (40,41). Only 54% of anti-nucleocapsid IgG positive patients with pIBD had a history of positive SARS-CoV-2 PCR, even though we had collected this information prospectively since the outbreak of the COVID-19 pandemic. Thus, in approximately half of the

patients with pIBD, the infection went undetected as it had a fully asymptomatic course or no epidemiological history.

None of the above-mentioned studies specifically reported patients with pIBD, and data on this population are scarce. In a prospective longitudinal study of patients with pIBD treated with IFX or vedolizumab (VEDO) [N = 436, mean age 17 years (2–26)], 44 patients (10%) were positive for postinfection SARS-CoV-2 anti-RBD IgG antibodies (6). The titers of these antibodies were significantly lower than those of adults (N = 23) and pediatric (N = 11) non-IBD controls and, until 6 months postinfection, were undetectable in most patients. There was no difference in the seropositivity between patients treated with IFX and VEDO (P = 0.164). None of the patients were treated with azathioprine. In contrast, postvaccination antibodies tested in a subgroup of 33 patients (age not specified in the paper) reached titers up to 15 times higher. The authors concluded that postvaccination immunity is substantially higher than postinfection immunity in patients with pIBD treated with biologic medication (6).

The above-mentioned CLARITY IBD study focused on postvaccination immunity in patients with IBD (eligibility criteria over 5 years of age) treated with biologic medication; however, most of the patients were adults [mean age 43.8 years (IQR 32.8–57.6)], and the number of pediatric patients was not listed. The authors suggested significantly lower production of postvaccination antibodies in patients treated with IFX (especially in combination with IMM) than in patients treated with VEDO (24). Another large population-based Israeli study included 20 patients with pIBD (0.4%), but no sub-analysis was presented (33). A recent large-scale prospective observational cohort study (PREVENT-COVID) measured anti-RBD IgG levels in 1909 patients with IBD (1815 of whom received mRNA vaccines) (7). A subgroup of 45 children 12–18 years old was included. Forty-four patients developed detectable postvaccination antibodies. No further sub-analyses are presented. The study has shown that older age, anti-TNF, and IMM therapy reduced the postvaccination response (7).

In a retrospective study of patients with IBD aged <21 years (N = 340), SARS-CoV-2 anti-spike IgG antibodies were measured. Fifty-eight patients (17%) had previously experienced confirmed, probable, or suspected COVID-19 infection. Fifty-four patients (16%) had a history of close contact with SARS-CoV-2 but no clinical symptoms. Two hundred and eight patients (61%) had no history of contact or symptoms. Only 20 patients [6%; mean age, 18 years (IQR 17–20)] were vaccinated against SARS-CoV-2. Nineteen of these patients (95%) received biological therapy (IFX = 7, ADA = 2, ustekinumab = 10), and 2 received tofacitinib. Seroconversion was detected in all the vaccinated patients. All patients who received the mRNA vaccine had high postvaccination titers. No association was found between postvaccination titers and the type of biological therapy. Patients vaccinated with mRNA-1273 (NIH-Moderna) vaccine had significantly higher titers than those vaccinated with BNT162b2 (Pfizer/BioNTech) and JNJ-78436735 (Johnson and Johnson) vaccines (P = 0.005) (8).

CONCLUSIONS

In conclusion, patients with pIBD in our study showed an excellent postvaccination response that might be attenuated by anti-TNF therapy. The main strengths of this study are the prospective collection of data both on vaccination status and previous SARS-CoV-2 PCR positivity (with a primary focus on the pediatric population with IBD), use of a unified vaccination strategy (full vaccination scheme only by BNT162b2 mRNA vaccine), the inclusion of a robust control group with a comparable time frame from the second dose, and a reliable microblot array assay to detect large-scale antibodies. A potential limitation for the interpretation of antibody titers is the inclusion of non-age-matched adults as a control cohort, which we attempted to manage mathematically in our models. Also, we did not collect information on comorbidities in the HCs group. Moreover, with respect to the course of pandemic, adult patients had slightly lower rate of history of SARS-CoV-2 infection when compared to the pIBD patients (28% vs 41%), but adding anti-nucleocapsid IgG levels to the mixed models neither affected the results of anti-RBD nor anti-spike S2 analysis. Despite the negative asso- ciation between anti-TNF and postvaccination antibodies, it is questionable whether anti-TNF-treated patients should be prioritized for booster vaccines. They are probably not at risk of a severe course of COVID-19, however recently more frequent breakthrough infections were described in vaccinated patients treated with IFX compared to VEDO (42). Moreover, serology is a poor marker of vaccination-induced T-cell response, which might play a crucial role but has rarely been studied in patients with IBD so far (43–45).

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