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Article

A New Ultrasound Method to Study the Relations Between Ileocecal Valve Incontinence and Inflammation in Metabolic Associated Steatotic Liver Disease

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Abstract

Background: Gut dysbiosis may trigger inflammation and steatohepatitis (SH) in subjects with metabolic associated steatotic liver disease (MASLD). Both incidence and impact of ileocecal valve (ICV) dysfunction as cause of small intestine bacterial overgrowth in MASLD remain unknown because of the unmet need of non-radiology dependent diagnostic procedures. **Methods:** Exploiting water as contrast medium and a hydro colon machine (Clean Colon Srl, Monza, Italy) for automated retrograde gentle colonic irrigation, we developed a new abdominal ultrasound procedure for diagnosis of ICV incontinence and we present the results of a pilot feasibility study for the assessment of its relationship with triggering of inflammation in steatotic liver disease. **Results:** Consecutive patients (32) performing colonic irrigation in colonoscopy preparation underwent concomitant abdominal ultrasound examination. Patients were predominantly males (59%) with body mass index (BMI) $26,6 \pm 2,6$ kg/m² and liver steatosis was present in half of the patients. Liver stiffness was $26,6 \pm 2,6$ kPa, while serum transaminases ALT and AST were $33,1 \pm 9,5$ and $25,9 \pm 8,4$ U.I., respectively. The procedure that lasted a mean of 27min (range 20-35 min) was well tolerated in all, but 2 males with large prostate hypertrophy. Three ICV continence patterns (A - Early reflux, already at small water volume without evident cecum distension; B - Late reflux, after evident cecum distension; C - No reflux, either early or late after overall water irrigation and significant cecum distension) were observed during water irrigation (4266 ml mean, range 2000-8000 ml). ICV continence was significantly associated with IBS, steatosis, and liver stiffness (all $p \leq 0.0001$). Furthermore, IBS (OR 3.97), steatosis (OR 3.30), and liver stiffness (OR 2.43 per unit increase) were also independently associated with dichotomization of impaired ICV continence. A 35-year-old male with SIBO and LS = 8.7 kPa underwent a liver biopsy showing histologically-determined high level of steatosis (S3), hepatocyte ballooning, lobular inflammation (Grade 6/8) in absence of fibrosis (Stage 0/4 F0). **Conclusion:** This proof-of-concept study indicates that an easy and quick mini-invasive diagnosis of ICV incontinence is feasible using water as contrast medium during abdominal ultrasound examination. Preliminary results suggest that this new technique is worth of being validated in prospective studies investigating the impact ICV incontinence as possible co-factor of steatohepatitis in patients with MAFLD.

Keywords: steatotic liver disease (SLD); metabolic associated steatotic liver disease (MASLD), steatohepatitis; liver stiffness; ileocecal valve; small intestine bacterial overgrowth (SIBO); irritable bowel syndrome (IBS)

1. Introduction

Steatotic liver disease (SLD) is a major global health problem affecting approximately 30% of the general population in Western Countries and the most common clinical condition, namely hepatic steatosis associated with metabolic dysfunction (MASLD) is expected to increase significantly in the next years [1–3]. MASLD may progress to liver related complications (such as cirrhosis and hepatocellular carcinoma). In addition, type 2 diabetes mellitus (T2DM) that strongly associated with greater MASLD prevalence and severity is one of the most important risk factors for cardiovascular disease, leading cause of death in patients with MASLD [3,4]. Inflammation acts as an aggravating co-factor pressing on the steatosis “pedal” to trigger steatohepatitis, major “throttle” of progression for both liver and systemic diseases [5]. Major trigger of inflammation in both visceral and liver fat is gut dysbiosis caused by small intestine bacterial overgrowth (SIBO) and leading to leaky gut [5–8]. SIBO and ileocecal valve (ICV) incontinence syndrome are closely related, as a dysfunctional ICV leads to SIBO [9,10]. SIBO is more prevalent in patients with non-alcoholic-steatohepatitis (NASH) and its severity including fibrosis and inflammation, is associated with a higher incidence of SIBO [5–8,11]. This pathogenetic connection is linked to an increased intestinal permeability to bacterial toxins that contribute to liver inflammation and injury [5,11]. Unfortunately, both incidence and impact of ICV dysfunction in MASLD remain unknown because of the unmet need of non-radiology dependent diagnostic procedures. In recent years safe and suitable electromechanical hydro colon therapy machines for rectal colon cleansing have become a more widely used for pre-colonoscopy preparation [12–16]. Exploiting such a new colonic irrigation device and water as contrast medium [17], we combined hydro colon irrigation with abdominal ultrasound examination to develop a reliable non radiologic diagnostic procedure for diagnosis of ICV incontinence. We present here the results of a pilot feasibility study performed in a consecutive series of patients who underwent colonic irrigation and liver ultrasound.

2. Materials and Methods

2.1. Subjects

32 consecutive subjects who underwent rectal colon cleansing for pre-colonoscopy preparation were enrolled. All of them underwent a gastroenterology visit and clinical features suggestive of Irritable Bowel Syndrome (IBS) were evaluated using the Rome IV IBS questionnaire. Demographic data (sex, age), body mass index (BMI), serum transaminases (ALT, AST) and platelet counts were registered. Presence of steatosis and liver stiffness (LS) were measured by ultrasound. The study design and conduct, as well as data collection of this observational study were performed according to the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines [18].

2.2. Abdominal Hydro Colon Ultrasound

Abdominal ultrasound examination was performed with Samsung Samsung RS 85 Prestige instrument during colon cleansing (Hydro-Colon-US) with the 004RA equipment (Clean Colon Srl, Monza, Italy). The device is certified as class II B, according to Annex III of the Directive 93/42 CEE on medical devices implemented by Legislative Decree n. 46 of 24/02/1997. Some specific features of the device include: powered with rechargeable battery, automated water temperature control with electronic safety system with audible alarm, water pressure control with pressure switch, safety valve and audible alarm, discharge system control with automatic anti-clogging valve, hydraulics composed of 5 anti-return valves, pall filter application with a membrane system that filters up to

0.2 μ , blocking the passage of legionella, pseudomonas and fungi, disposable speculum with safety belt with Patent n. 01318041. Briefly, B-mode ultrasound images of the intestine were acquired during retrograde gentle introduction of water according to standard procedures [19].LS was measured by Shear Wave Elastography (SWE) during trans-abdominal ultrasound examination before initiation of hydro colon using the same Samsung RS 85 Prestige instrument. The US examination was conducted with the patient in the supine position with the right arm extended overhead in order to optimize the intercostal acoustic window. A 2D SWE region of interest (ROI) was placed in a clear area of the right hepatic parenchyma, avoiding large vessels and biliary ducts. For each participant, ten valid measurements were collected, and the median value (expressed in kPa) was recorded as the final result. All US scans were performed by an experienced radiologist (B. L.) blinded to the patients' clinical data.

2.3. Statistical Analysis

Descriptive statistics were performed for all variables. The categorical data are presented as counts and percentages, while the mean and standard deviation are reported for continuous normally distributed data. Associations between multiclass and binary variables were assessed using Pearson's chi-square test. For comparisons between multiclass variables and continuous ones, distributional assumptions were evaluated and non-parametric methods were applied. Differences across the groups were tested using the Kruskal–Wallis test. When the global test was significant, pairwise Mann–Whitney U tests were conducted for post-hoc comparisons, with Bonferroni correction applied to adjust for multiple testing. After the initial analyses, the ICV continence multiclass variable was dichotomized into a binary outcome. To assess the association between this new binary variable and the selected predictors, a multivariable penalized logistic regression model with L2 (ridge) regularization was fitted. The L2 penalty was applied to reduce overfitting and improve model stability in the presence of potential multicollinearity. Results were reported as penalized odds ratios with corresponding 95% confidence intervals. Correlations were considered significant with $R \geq 0.300$ and $p < 0.05$. All statistical analyses were performed using R (version 4.5.1; R Foundation for Statistical Computing, Vienna, Austria) and IBM SPSS Statistics (version 27; IBM Corp., Armonk, NY, USA)

3. Results

The characteristics of the study population are presented in **Table 1**

Table 1. Clinic-Pathologic Characteristics of 32 Subjects who underwent ultrasound hydro colon examination.

	All population (N = 32)
Age, years	57 \pm 19
Men, counts (%)	19 (59)
BMI, kg/m ²	26,6 \pm 2,6
Overall water, ml	4266 \pm 1122
Procedure time, minutes	27 \pm 4
ALT, U.I.	33,1 \pm 9,5
AST, U.I.	25,9 \pm 8,4
Platelet, count/ul	271.125 \pm 29.679

Steatosis, counts (%)	16 (50)
LS, kPa	5,6±8,4
IBS, counts (%)	19 (59)

Hydro Colon Abdominal Ultrasound

The abdominal ultrasound investigation of the intestine lasted a median of 27 minutes (range 20-35 minutes). The procedure was well tolerated in all, but 2 subjects who were males with large prostate hypertrophy that hampered the colon irrigation with reduced total water volume (< 2500ml) and caused some perineal discomfort

Ileocecal Valve Continence

During water irrigation and after about 3500 ml irrigation, the water front reached the cecum and at that time three types of patterns were observed and reported according to the ICV continence: A) Early reflux (already at small water volume without evident cecum distension); B) Late reflux (after evident cecum distension);C) No reflux (either early or late after overall water irrigation and significant cecum distension).The three-level ICV continence variable showed significant associations with both binary predictors. The relationship between ICV continence and IBS was statistically significant ($\chi^2 = 21.895$, $p < 0.0001$), as was the association between ICV continence and steatosis ($\chi^2 = 18.164$, $p = 0.0001$). Global chi-square tests indicated that the distribution of IBS and steatosis differed across the three ICV continence categories. Post-hoc pairwise comparisons confirmed that these differences were consistently significant across individual level contrasts for both IBS and steatosis. The continuous variable LS differed significantly across the three ICV continence categories (A, B, and C), as indicated by the Kruskal–Wallis test ($H = 18.139$, $p = 0.0001$). Post-hoc pairwise Mann–Whitney comparisons showed significant differences between A and B ($p = 0.0358$), A and C ($p = 0.0001$), and B and C ($p = 0.0336$), confirming that LS values varied meaningfully across all levels (**Figure 1**).After dichotomizing the ICV continence variable by merging categories A and B, the resulting binary outcome was analyzed using an L2-penalized multivariable logistic regression model including IBS, steatosis, and LS as predictors. All three variables were independently associated with the dichotomized outcome. IBS showed a positive association (coefficient = 1.378), corresponding to an odds ratio (OR) of 3.97. Steatosis was likewise associated with higher odds of the outcome (coefficient = 1.195; OR = 3.30). LS also demonstrated a significant positive association, with a coefficient of 0.887, indicating that the odds increased by a factor of 2.43 for each unit increase in LS

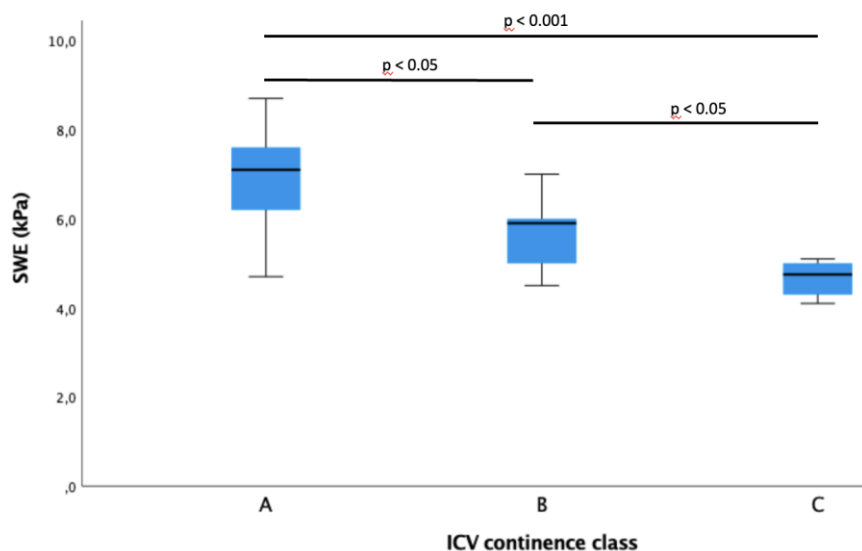


Figure 1. Distribution of liver stiffness values assessed by shear wave elastography (SWE) according to ileocecal valve (ICV) continence class. Patients were stratified into Grade A (early reflux), Grade B (late reflux), and Grade C (no reflux). Box plots show median and interquartile range, with whiskers indicating minimum and maximum values. SWE values were significantly higher in patients with ICV incontinence (Grades A and B) compared with continent subjects (Grade C), with a progressive decrease in liver stiffness across ICV continence classes. Statistical significance between groups is indicated ($p < 0.05$, $p < 0.001$).

Clinic Case Pathologic Characterization

One of the subjects was an obese (BMI 35.5 kg/m² and abdominal circumference: 122 cm) 35-year-old male patient with grade II obesity. The patient has several co-morbidities, such as obstructive sleep apnea syndrome (OSAS), irritable bowel syndrome with mixed habit (IBS-M) and SIBO, diagnosed on glucose Hydrogen Breath Test showing a rise in breath hydrogen by 18 ppm above baseline. Lactose intolerance was excluded by Breath Test. He had 1st evidence of ultrasound hepatic steatosis at 12 yrs of age and insulin resistance since the age of 13, treated with physical exercise and for 1 year at age 19 with metformin. The patient underwent blood analyses with the following results: blood counts: RBC 5.01 $10^6/\mu\text{L}$, Hb 14.8 g/dl, WBC 4600 $10^3/\mu\text{L}$, Platelets 236,000 $10^3/\mu\text{L}$; biochemical tests: AST/ALT 30/51 U/l, GGT 16 U/l, ALP 59 U/l, Albumin 4.6 g/dl, Total bilirubin 0.7 mg/dl, INR 1.05; Glucose 93 mg/dl (5.17mmol/L), insulin 8.34 mUI/L, HOMA index 1.91, Total cholesterol 160 mg/dl (4.14 mmol/L), HDL 46 mg/dl (1.19 mmol/L), LDL 101 mg/dl (2.61 mmol/L), Triglycerides 62 mg/dl (0.70 mmol/L), TSH 1.5 mUI/L, Creatinine 0.98 mg/dL and Ferritin 236 $\mu\text{g/L}$. FIB-4 (age \times AST/(platelet count \times $\sqrt{\text{ALT}}$) index was 0.62. Indirect measures of liver stiffness and steatosis were obtained by transient elastography (TE) and attenuation rate parameters (CAP or UAP) measured with both Fibroscan (Touch 502, Echosens, France) and FT900 (Hisky Medical, China) instruments: results water is observed from cecum into terminal ileum hampering a normal distention of the cecum itself (**Figure 2, a-f**). This evidence is pathognomonic of an incontinent ICV. A percutaneous liver biopsy was performed with 16-gauge needle providing 2.5 cm. liver specimen in day hospital service at the Hepatology Unit and Reference Centre of the Tuscany Region for Chronic Liver Disease and Cancer of the University Hospital of Pisa (AOUP), Pisa, Italy. At the microscopic examination liver parenchyma with preserved architecture show portal tracts with a mild inflammatory infiltrate confined within the limiting plate, consisting of lymphocytes, some neutrophils, and some histiocytes with ceroid pigment; CK7 and CK19 staining shows a modest and diffuse ductular reaction at the portal lobular interface. Lobular area with diffuse micro- and macro vesicular steatosis affecting approximately 90% of hepatocytes; numerous hepatocytes with hyper-glycogenotic nuclei, some with ballooning degeneration. Some necro-inflammatory foci are associated with lymphocytes (2 at 20x magnification), and some lipid-granulomas. Iron staining negative (**Figure 3, a-b**). Histologic diagnosis was: Non-Alcoholic Steatohepatitis (NASH). Grade 6/8: steatosis 3, hepatocytes with ballooning degeneration 1, lobular inflammation 2. Stage 0/4 fibrosis absent. According to histology, serology and imaging results the final clinic-pathologic diagnosis was: Metabolic Dysfunction-Associated Steatohepatitis (MASH) in subject with Small Intestinal Bacteria Overgrowth secondary to ICV incontinence



Figure 2. a-e: the ultrasound hydro-colon examination performed using Clean Colon 004RA and Samsung RS 85 Prestige equipments. **a)** Bottom of the cecal colon and last ileal loop (UA) in basal conditions with regular thickness of the intestinal wall; **b)** progressive distension of the cecal colon by retrograde colon wash with instant reflux of water within the last ileal loop; **c)** concomitant and progressive distension of cecal colon and last ileal loop (UA); **d)** distension of cecal colon and last ileal loop (UA); **e)** distension of the terminal ileum at the end of the retrograde water flow.

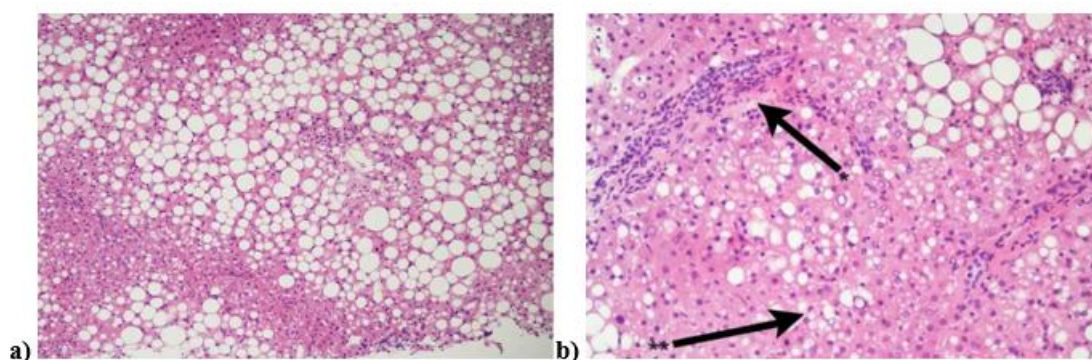


Figure 3. a-b: histologically diagnosis of Metabolic Dysfunction-Associated Steatohepatitis (MASH). **a)** Micro- and macrovesicle steatosis affecting approximately 90% of hepatocytes; **b)** Portal tracts with a mild inflammatory infiltrate confined within the limiting plate; ** hepatocytes with hyper-glycogen nuclei, some with ballooning degeneration.

4. Discussion

This pilot study presents a novel non-X-rays procedure for an easy and quick mininvasive diagnosis of ileocecal valve incontinence using water as contrast medium via colon irrigation by mean of a hydro colon automated equipment. The new diagnostic ultrasound technique appears safe and well tolerated and properly suited to diagnose ileocecal valve incontinence. Given the narrative nature of this feasibility study does not allow any specific observation on the potential clinical applications of the new diagnostic method. However, some interesting speculations are prompted by the interesting results, namely the differential clinic pathologic characteristics of the patients with and without ileocecal continence. Provided the bias of selection of subjects undergoing colonoscopy who had a high incidence of symptoms of IBS nevertheless the high rate of ICV incontinence associated with steatosis was rather surprising. Furthermore, and very interestingly all subjects with

steatosis and ICV incontinences in spite of normal FIB-4 index showed border line or slightly higher values of liver stiffness. It is well known that LS is influenced by three major physical vectors, inflammation, sinusoidal congestion and fibrosis and clinically useful cut-offs so far proposed are instrument and technique specific [20]. However, the results are consistent with previous evidence that LS values below 8 kPa are associated frequently with intrahepatic inflammation without significant fibrosis [21]. Indeed, this was the case of the patient with MASLD, ICV incontinence and SIBO who underwent a liver biopsy and in spite of a slightly elevated LS by SWE and TE (8.5 kPa and 8.7 kPa, respectively) and mildly elevated AST/ALT (30/51 UI/ml), showed normal platelet counts and FIB-4 score of 0.62 and steatohepatitis at histology (Grade 6/8) in absence of fibrosis (Stage 0/4). Similarly early forms of steatohepatitis were detected by TE and reported in IBS patients with leaky gut [22–24] where dysbiosis associated with SIBO that may occur as consequence of incontinence of ileocecal valve [10,25] as documented in our case. European guidelines for the clinical management of patients with MASLD propose an algorithm aimed at identifying patients at higher risk of progressive liver disease based on non-invasive tests [26,27]. Specifically, the FIB-4 score (derived from the combination of AST, ALT, platelets, and age) is recommend using a cut-off > 1.3, as indicative for a secondary risk assessment using ultrasound LS measures as vibration-controlled transient elastography (VCTE). Then patients with VCTE > 8 kPa should be referred to a hepatology specialist for further clinical and diagnostic evaluation [26,27]. However, treatment and prevention of progressive MASLD were previously based on lifestyle modifications (diet and physical activity) only [26]. Recently, new drugs such as glucagon-like peptide-1 agonists (GLP-1, dual agonist for (GLP-1) - glucose-dependent insulinotropic polypeptide (GIP) receptors, and sodium/glucose cotransporter-2 inhibitors (SGLT-2) have shown for the first time an improvement of both cardiovascular and liver steatosis outcomes in T2D patients. [28–32]. Similarly, thyroid hormone (TH) analogues such as Resmetirom, has been approved by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) for the treatment of MASLD patients with F2-F3 fibrosis. [33–37]. In the near future, new molecules namely the analogs of hepatokine, fibroblast growth factor 21 (FGF21), capable of modulating the metabolic profile (glucose and lipid metabolism) in an earlier stage of the disease, prior to overt type 2 diabetes (T2D), will become available. [38–40]. All these evidences emphasize the importance of an early diagnosis and treatment of steatohepatitis. Thus, it is essential to identify patients at risk of disease progression long before the development of liver parenchymal fibrosis associated with advanced liver disease. Cases reported in this narrative study illustrate how transient elastography using appropriate screening cut-offs may provide a very useful tool for the early identification of patients with already ongoing inflammatory liver disease, namely steatohepatitis and referring them for a timely therapy that is essential to prevent the development of irreversible metabolic disease and liver-related complications. The presence of concurrent IBS symptoms in patients with SLD might reflect underlying ileocecal valve incontinence, which can lead to SIBO and increased intestinal permeability. These alterations may consequently trigger intrahepatic inflammation, fueling the progression of liver and systemic diseases associated with MASLD. The significance of this study lies in the identification of a novel diagnostic procedure for the study of the role and importance of ICV incontinence in MASLD and in the mechanisms that trigger hepatic and systemic inflammation as triggers of disease progression.

In addition, the 2 cases in whom the hydro colon ultrasound diagnosis of colon polyps was confirmed subsequently by colonoscopy suggest promising new targets for a possible diagnostic use of such a procedure as complementary tool for the appropriate and timely indication to colonoscopy in subject at risk of colon cancer.

5. Conclusions

We developed and report here the feasibility of a new ultrasound-based technique for diagnosis of ICV incontinence that is worth of being explored as a possible co-factor of small bowel bacterial overgrowth and steatohepatitis triggering in subjects with MASLD. These preliminary results prompt future research aimed to validate in prospective and larger patient cohorts the clinical value of the new x-ray free diagnostic procedure. In addition, the case series described highlight the

importance of using lower TE cut-offs combined with multiple non-invasive tests for the screening of individuals with MASLD for the early identification and treatment of steato-hepatitis since at early stages medical and/or pharmacological intervention have the highest chance of preventing disease progression. Future research should focus as well on exploring the functional roles of SIBO in the progression and response to treatment of subgroups of MASLD patients.

Author Contributions: FB: Methodology, Data curation, Writing – original draft, Writing – review&editing. SA: Methodology, Data curation. Writing–original draft, Writing–review & editing. FF: Methodology, Data curation, Writing – original draft, Writing–review & editing. MRB: Methodology, Writing – original draft, Writing - review&editing. BL: Data curation, Investigation, Writing - review&editing. BL: Writing – original draft, Writing – review & editing. DC: Investigation, Writing – review & editing. GP: Investigation, Writing, Writing – review & editing. SC: Investigation, Writing – original draft, Writing – review & editing.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the University Hospital of Pisa CEAVNO protocol code 22971).

Informed Consent Statement: Written informed consent has been obtained from the patient(s) to publish this paper.:

Data Availability Statement: We encourage all authors of articles published in MDPI journals to share their research data. In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Where no new data were created, or where data is unavailable due to privacy or ethical restrictions, a statement is still required. Suggested Data Availability Statements are available in section “MDPI Research Data Policies” at <https://www.mdpi.com/ethics>.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

TLA	Three letter acronym
LD	Linear dichroism

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