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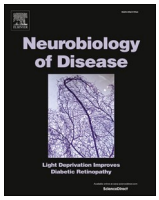

The gut-brain axis: Correlation of choroid plexus volume and permeability with inflammatory biomarkers in Crohn's disease

Cristiana Bonifacio [a,1], Giovanni Savini [b,c,1], Christian Reca [a,b], Federico Garoli [a,b], Riccardo Levi [b,c], Giulia Vatteroni [a,b], Luca Balzarini [a], Mariangela Allocca [d], Federica Furfaro [d], Arianna Dal Buono [e], Alessandro Armuzzi [b,e], Silvio Danese [d], Michela Matteoli [f,g], Maria Rescigno [b,h], Gionata Fiorino [d,i], Letterio S. Politi [b,c,*]

[a] Radiology Department, IRCCS Humanitas Research Hospital, Via Manzoni 56, 20089, Rozzano, Milan, Italy
[b] Department of Biomedical Sciences, Humanitas University, Via R. Levi Montalcini 4, 20072, Pieve Emanuele, Milan, Italy
[c] Neuroradiology Unit, IRCCS Humanitas Research Hospital, Via Manzoni 56, 20089, Rozzano, Milan, Italy
[d] Department of Gastroenterology and Endoscopy, IRCCS San Raffaele Hospital and Vita-Salute San Raffaele University, Milan, Italy
[e] IBD Center, Department of Gastroenterology, IRCCS Humanitas Research Hospital, Via Manzoni 56, 20089, Rozzano, Milan, Italy
[f] Laboratory of Pharmacology and Brain Pathology, Neuro Center, IRCCS Humanitas Research Hospital, Via Manzoni 56, 20089, Rozzano, Milan, Italy
[g] Institute of Neuroscience, National Research Council of Italy (CNR) c/o Humanitas Mirasole S.p.A, Via Manzoni 56, 20089, Rozzano, Milan, Italy
[h] IRCCS Humanitas Research Hospital, Via Manzoni 56, Rozzano, 20089 Milan, Italy
[i] Gastroenterology and Digestive Endoscopy, San Camillo-Forlanini Hospital, Rome, Italy

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ABSTRACT

Background: The dysregulation of the gut-brain axis in chronic inflammatory bowel diseases can cause neuropsychological disturbances, but the underlying mechanisms are still not fully understood. The choroid plexus (CP) maintains brain homeostasis and nourishment through the secretion and clearance of cerebrospinal fluid. Recent research has demonstrated the existence of a CP vascular barrier in mice which is modulated during intestinal inflammation.

This study investigates possible correlations between CP modifications and inflammatory activity in patients with Crohn's disease (CD).

Methods: In this prospective study, 17 patients with CD underwent concomitant abdominal and brain 3 T MRI. The volume and permeability of CP were compared with levels of C-reactive protein (CRP), fecal calprotectin (FC), sMARIA and SES-CD scores.

Results: The CP volume was negatively correlated with CRP levels (R = -0.643, p-value = 0.024) and FC (R = -0.571, p-value = 0.050). DCE metrics normalized by CP volume were positively correlated with CRP (K-trans: R = 0.587, p-value = 0.045; Vp: R = 0.706, p-value = 0.010; T1: R = 0.699, p-value = 0.011), and FC (Vp: R = 0.606, p-value = 0.037).

Conclusions: Inflammatory activity in patients with CD is associated with changes in CP volume and permeability, thus supporting the hypothesis that intestinal inflammation could affect the brain through the modulation of CP vascular barrier also in humans.

1. Introduction

Crohn's disease (CD) is a chronic inflammatory bowel disease (IBD) of unknown etiology characterized by a remitting and relapsing course (Torres et al., 2017). The dysregulation of the intestinal mucosal immune system seems to be of paramount importance, leading to chronic

Abbreviations: (CD), Crohn's disease; (CP), choroid plexus; (DCE) MRI, dynamic contrast-enhanced; (CRP), C-reactive protein; (FC), fecal calprotectin; (IBD), inflammatory bowel disease; (PVB), vascular barrier in the CP; (GVB), gut vascular barrier; (sMARIA), Simplified Magnetic Resonance Index of Activity; (ICV), intracranial volume; (K-trans), transfer constant; (Ve), fractional volume of extravascular extracellular space; (Vp), fractional plasma volume.

* Corresponding author at: Department of Biomedical Sciences, Humanitas University, Via R. Levi Montalcini 4, 20072, Pieve Emanuele, Milan, Italy.
E-mail address: letterio.politi@hunimed.eu (L.S. Politi).

[1] These authors contributed equally.

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transmural inflammation in genetically predisposed individuals (Günther et al., 2021).

During the course of the disease, patients affected by CD may experience both intestinal and extra-intestinal manifestations: among the latter, neuro-psychological disturbances, such as anxiety and depression, are frequent (Bartocci et al., 2023; Gajendran et al., 2018; Leone et al., 2019), with higher rates in CD patients compared to healthy individuals especially when the disease is active (Mikocka-Walus et al., 2015). Furthermore, neuro-psychological symptoms are associated with reduced adherence to medications and poor response to CD treatment (Bannaga and Selinger, 2015; Mittermaier et al., 2004).

The relationship between intestinal inflammation and brain changes is still not fully understood, but several studies have pointed to the existence of a "gut-brain axis" (Mayer et al., 2022). In particular, the choroid plexus (CP) has been proposed to facilitate communication between the gastrointestinal tract and the brain (Carloni et al., 2021). The CP performs various functions such as synthesizing, secreting, and clearing the cerebrospinal fluid, as well as modulating the blood-cerebrospinal fluid barrier during inflammatory and infectious processes within the central nervous system (Engelhardt et al., 2001; Kaur et al., 2016; Thompson et al., 2022). It has been hypothesized that CP modifications are involved in the pathophysiology of certain neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease (Choi et al., 2022; Tadayon et al., 2020). Additionally, changes in CP volume have been observed in patients with neurological and psychiatric disorders including multiple sclerosis, Alzheimer's disease and depression (Althubaity et al., 2022; Choi et al., 2022; Müller et al., 2022; Ricigliano et al., 2021). Finally, changes in CP volume, perfusion and microstructure were shown to occur also in healthy aging (Alisch et al., 2021; Eisma et al., 2023).

In a murine model of IBD, Carloni et al. recently described the existence of a vascular barrier in the CP (PVB) that responds to the inflammatory state of the intestine (Carloni et al., 2021). During intestinal inflammation, the PVB undergoes first an increase and later a shut off of its permeability (Carloni et al., 2021). This later closure of the PVB upon the opening of the gut vascular barrier (GVB) (Spadoni et al., 2015) might prevent large bacterial molecules traveling through the damaged GVB to penetrate into the brain. The study demonstrated a correlation between modifications of PVB in CP and behavioral disturbances in mice. These disturbances, such as anxiety-like behavior and deficit in short-term memory, are similar to the neuro-psychological symptoms experienced by nearly 40% of CD patients during active disease (Bartocci et al., 2023). Although it was hypothesized that chronic inflammation in IBD could determine a dysregulation of the gut-brain axis also in humans (Carloni et al., 2021), there is still lack of data regarding CP alterations promoted by intestinal inflammation in CD patients, which could potentially lead to changes in brain function (Solár et al., 2020; Strominger et al., 2018). Therefore, we aimed to investigate the effects of intestinal and systemic inflammation on the CP within a population of patients with CD with different inflammatory activity of the disease. Specifically, we evaluated the possible association between C-reactive protein (CRP), fecal calprotectin (FC) inflammatory biomarkers and the volume and permeability of CP as measured in vivo with brain MRI.

2. Materials and methods

2.1. Study cohort

In this prospective, single-center study we enrolled 17 subjects aged between 18 and 70 with established diagnosis of CD, confirmed by clinical, endoscopic, and histological investigations and disease activity defined when at least one of the following criteria was matched: SES-CD $\geq$ 3, CRP $\geq$ 0.5 mg/dl, FC $\geq$ 50 μg/g.

Given the preliminary nature of this study and the lack of previously published results regarding the measurement of choroid plexus volume and permeability in the context of systemic and/or gastrointestinal inflammation, a statistical power analysis to find the optimal sample size was not performed.

The study was approved by the local Ethical Committee and written informed consent was obtained from each participant.

Each patient underwent a 3 T MRI session including both abdominal imaging for CD evaluation and brain imaging for the study of the effects of CD activity on CP.

Additionally, all subjects underwent endoscopic evaluation. Blood samples were collected for inflammatory biomarkers including CRP and FC.

Upon diagnostic evaluation, all subjects were treated with monoclonal antibody or anti-inflammatory medications according to good clinical practice.

2.2. Image acquisition

Each patient underwent a 3 T MRI session including both abdominal imaging for CD staging and brain imaging for the study of the effects of CD activity on CP. The study was performed using a Siemens SkyraFit 3 T MRI scanner, an 18-channel body coil and a 64-channel head/neck coil.

The protocol for abdominal imaging included axial and coronal T1w images (VIBE, TE1/TE2/TR = 1.29–1.34/2.52–2.57/3.97–4.2 ms, FA = 9°, reconstructed voxel size = 0.6 × 0.6 × 3.0–1.4 × 1.4 × 1.5 mm3), axial, coronal and sagittal T2w images (HASTE, TE/TR = 91–113/1100-1200 ms, FA = 121–138°, reconstructed voxel size = 0.5 × 0.5 × 4.0–0.8 × 0.8 × 4.0 mm3), axial T2w images with fat saturation (HASTE, TE/TR = 96/1200 ms, FA = 160°, reconstructed voxel size = 0.5 × 0.5 × 4.0 mm3, SPAIR), coronal T2w TrueFISP images (TE/TR = 1.72/489.13 ms, FA = 48°, reconstructed voxel size = 0.7 × 0.7 × 4.0 mm3), DWI (SS-SE-EPI, TE/TR = 58/6700 ms, reconstructed voxel size = 1.6 × 1.6 × 4.0 mm3, b = 0–50-650-1000s/mm2, SPAIR) and post-contrast axial and coronal T1w images (same parameters as pre-contrast T1w images).

The brain MRI protocol included the following sequences: 3D T1w (MP2RAGE, TE/TR/TI1/TI2 = 2.98/5000/700/2500 ms, FA1/FA2 = 4/5°, 1 mm isotropic resolution), 3D T2w FLAIR (SPACE, TE/TR/TI = 368/7000/2100 ms, 1 mm isotropic resolution), 2D DWI (SS-SE-EPI, TE/TR = 69/4000 ms, 2.3 mm isotropic resolution, 30 diffusion directions, b = 1000s/mm2), and 3D dynamic contrast enhanced (DCE) images (VIBE, TE/TR = 1.6/4.62 ms, FA = 15°, reconstructed voxel size = 1.1 × 1.1 × 1.6 mm3, temporal resolution = 6 s, 55 measurements) with injection of gadobutrol (0.1 mmol/kg body weight injected at 2 ml/s after 5 measurements).

2.3. Radiological evaluation

A senior radiologist with 20 years of experience in IBD evaluated the abdominal images and computed the Simplified Magnetic Resonance Index of Activity (sMARIA) for each patient (Ordás et al., 2019).

A senior neuroradiologist with 21 years of experience evaluated the brain images.

2.4. Processing of brain images

MP2RAGE images were processed to perform brain tissue segmentation (SPM12 ("https://www.fil.ion.ucl.ac.uk/spm/software/spm12/," n.d.)), to create a binary mask of the lateral ventricles, and to manually segment the CP of the lateral ventricles (Fig. 1). The total intracranial volume (ICV), lateral ventricle CP volume and total lateral ventricles volume were computed.

DCE images were processed using FSL (Jenkinson et al., 2012) and Quantiphyse (Chappell et al., 2009). MP2RAGE and DCE images were rigidly co-registered using ANTs ("http://stnava.github.io/ANTs/," n.d.) and T1 maps obtained from MP2RAGE images (Marques et al., 2010) (qMRLab (Karakuzu et al., 2020)) were used to fit the extended Tofts

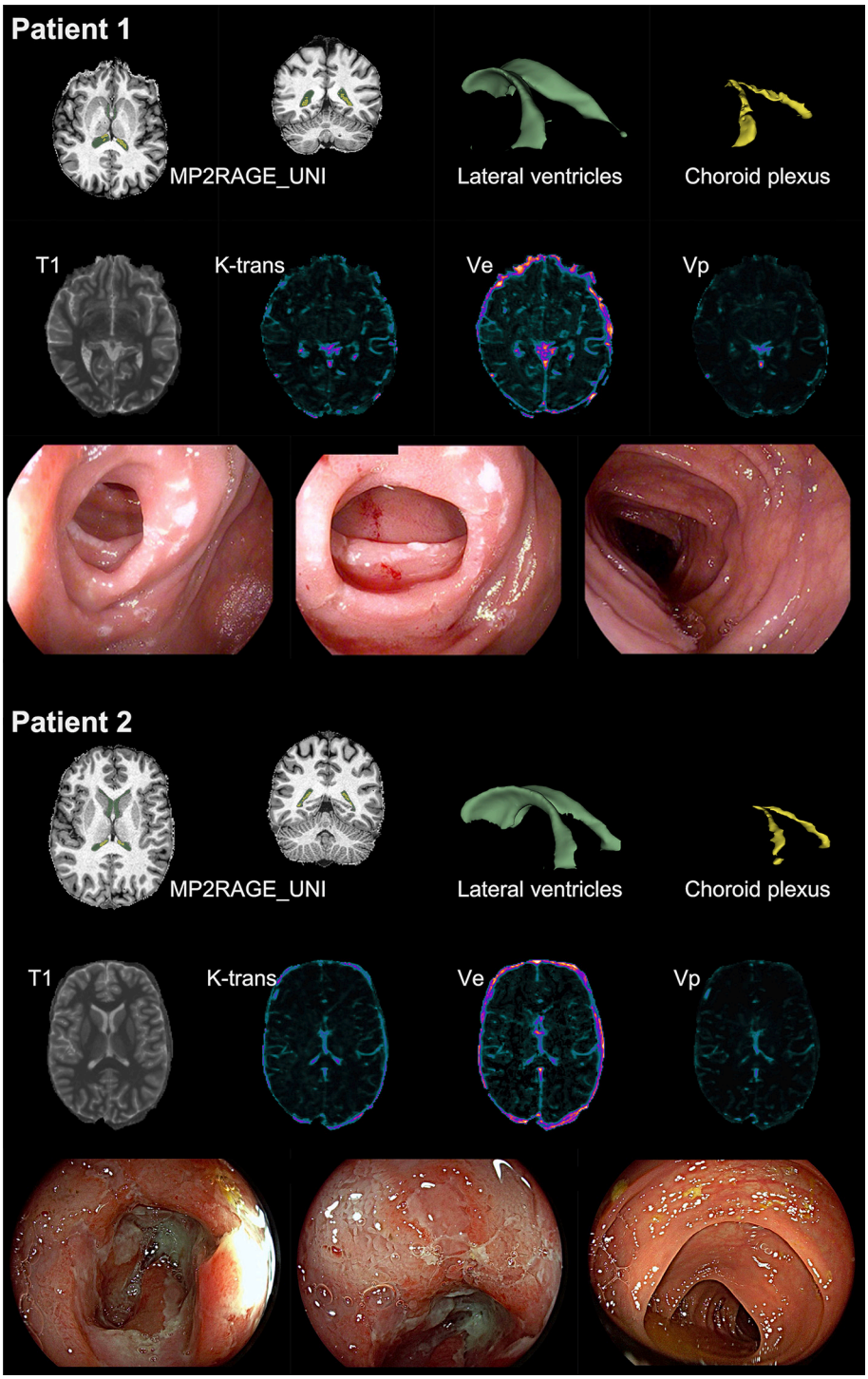

Fig. 1. Brain MR images and features of two representative subjects with respective endoscopic images. Patient 1 presented a large volume of choroid plexus and low levels of C-reactive protein and fecal calprotectin (SES-CD 4), while patient 2 presented small choroid plexus and high levels of C-reactive protein and fecal calprotectin (SES-CD 12).

model (Bergen et al., 2020; Tofts et al., 1999). The CP binary mask was used to compute the corresponding mean value of T1, transfer constant (K-trans), extracellular volume (Ve) and plasma volume (Vp), which were also normalized by CP volume (Fig. 1).

2.5. Statistical analysis

Each continuous feature was assessed for normality distribution using Shapiro-Wilk test. The association between variables was assessed by Pearson Correlation Coefficient (parametric test) or Spearman Correlation Coefficient (non-parameter test). In particular, we assessed the association between the age and CP volume of patients, between the volume and T1 and DCE values of the CP, and between inflammatory biomarkers (CRP and FC) and sMARIA and SES-CD scores in respect to MRI parameters (volume, T1 and DCE parameters of the CP). Statistical significance level was set for p-value < 0.05. All statistical analyses were performed in Python (version 3.9) and Scipy library (version 1.9.2).

3. Results

Of the 17 patients enrolled, 2 were excluded from analyses due to motion-induced brain imaging artifacts.

Demographic, clinical and imaging data of the 15 analyzed subjects are summarized in Table 1.

The CP volume was negatively associated with CRP levels ($R = -0.643$, p-value = 0.024) and FC ($R = -0.571$, p-value = 0.050) (Fig. 2). CP volume normalized with total intracranial volume (ICV) was negatively associated with CRP levels ($R = -0.615$, p-value = 0.033). No correlation was found between patient's age and CP volume ($R = 0.20$, p-value = 0.47).

A positive association was found between CP volume and T1 ($R = 0.488$, p-value = 0.05).

There was no association among inflammatory biomarkers and DCE parameters or T1 measurements. Upon normalization of the above-mentioned DCE metrics by CP volume, we observed a positive association between CRP and K-trans ($R = 0.587$, p-value = 0.045), Vp ($R = 0.706$, p-value = 0.010) and T1 ($R = 0.699$, p-value = 0.011), as well as between FC and Vp ($R = 0.606$, p-value = 0.037) (Table 2). No correlations were found among CP metrics and sMARIA or SES-CD scores.

Results of correlation analyses between MRI variables and markers of inflammation and disease activity are reported in Table 2.

Fig. 1 shows brain MRI and endoscopic images of two representative subjects.

Table 1
Demographic, clinical, endoscopic, abdominal and brain MRI data of the analyzed patients with CD ($n = 15$).

Demographic data			
Age (yrs, median [IQR])	33 [27–51]		
Sex (M [%] / F [%])	9 [60%] / 6 [40%]		
Clinical data			
Smoker (y [%] / n [%])	4 [27%] / 11 [73%]		
HBI (median [range])	4 [2−20]		
Montreal classification	Age at diagnosis A1: 1 (7%) A2: 10 (67%) A3: 4 (27%)	Location L1: 10 L2: 4 L3: 1 L4: 1	Behavior B1: 4 (27%) B2: 9 (60%) B3: 2 (13%) P: 0
Therapy at MRI examination	Anti-inflammatory drugs Corticosteroids: 1 (7%) Mesalazine: 4 (27%)	Monoclonal antibodies Adalimumab: 4 (27%) Ustekinumab: 1 (7%)	No treatment 5 (33%)
Surgery before MRI (y [%] / n [%])	4 [27%] / 11 [73%]		
Endoscopic data			
SES-CD (median [range])	7 [0–25]		
Rutgeerts score (if applicable)	i2: 3 i4: 1		
Inflammatory biomarkers			
C-Reactive Protein (mg/dL, median [IQR])	0.45 [0.23–2.26]		
Fecal Calprotectin (μg/g, median [IQR])	522 [386–2000]		
Abdominal imaging			
sMARIA score (AU, median [IQR])	5 [3.5–12.5]		
Disease location at MRI	Ileal: 8 Colonic: 3 Ileocolonic: 2 Ileocecal: 5		
CP Volumetry			
CP volume (mm^3, median [IQR])	1128 [1012−1333]		
Lateral ventricles volume (mm^3, median [IQR])	10,593 [8255–14,074]		
Intracranial volume (lt, median [IQR])	1.42 [1.34–1.51]		
CP Permeability			
K-trans (min^{-1}, median [IQR])	0.65 [0.46–0.73]		
Ve (%, median [IQR])	39.64 [31.78–54.90]		
Vp (%, median [IQR])	3.33 [2.90–4.75]		
CP Relaxometry			
T1 (s, median [IQR])	1.88 [1.81–1.93]		

4. Discussion

In this prospective, single-center study, we observed that systemic and intestinal inflammation markers in patients with CD are correlated with morphological and functional changes of the CP.

CD is associated with extra-intestinal manifestations and, among others, with cognitive and psychiatric disorders, including anxiety and depression (Bartocci et al., 2023; Gajendran et al., 2018; Mikocka-Walus et al., 2015). Several MRI studies reported the existence of structural and functional alterations in the brain of patients with CD that could underlie such disorders (Agostini et al., 2013, 2023; Bao et al., 2015; Kornelsen et al., 2020), but the link between the intestinal inflammation and its effects in the brain has never been explained. Inflammatory processes of the intestine affect the permeability of the gut-vascular barrier and it has been recently proposed that brain alterations might analogously originate from the modulation of the PVB in response to intestinal inflammation. The activation of the inflammatory response prompts the closure of the PVB subsequent to the opening of the gut vascular barrier. This closure is facilitated through the up-regulation of the wingless-type, catenin-beta 1 (Wnt/b-catenin) signaling pathway, thereby preventing the passage of large molecules (Carloni et al., 2021).

To date, no studies have investigated the modifications of the CP in patients with IBD in vivo. In this preliminary study we used MRI to measure the CP volume and permeability in patients with CD and assessed possible associations with systemic and intestinal markers of inflammation. We found that CP volume decreases with increasing values of both CRP and FC, thus supporting recent findings of a direct effect of intestinal inflammatory processes on CP (Carloni et al., 2021). Normalization of CP volume by ICV did not alter the strength of this correlation. Previous studies reported an increase of CP volume with advancing disease stage in neurodegenerative and neuroinflammatory diseases like Alzheimer's disease and multiple sclerosis respectively. However, to the best of our knowledge, there is currently no clinical study investigating the choroid plexus in the context of systemic and/or gastrointestinal inflammation, thus originating outside the central nervous system and not directly involving it. Therefore, we cannot automatically assume that CP show analogous response to neurodegenerative/neuroinflammatory diseases and inflammation arising outside the central nervous system, as inflammatory cells and molecules mediator of inflammation traveling in opposite directions might trigger different mechanisms and behaviors in the CP.

Even though previous studies reported the existence of an association between the age and the CP volume of healthy individuals (Alisch et al., 2021; Eisma et al., 2023), we observed no significant correlation between these two variables, possibly suggesting that the number of subjects included in this study is too low to detect this association and/or that the effects of disease activity and inflammation might overcome the changes arising with advancing age.

Previous studies suggested that brain changes could be driven by the alteration of the PVB permeability induced by intestinal inflammation that varies in different disease phases (Carloni et al., 2021). Based on this hypothesis, we measured the permeability of the CP through DCE-MRI to assess a possible association between inflammation and CP behavior in a population of patients with CD. Future studies investigating link between CD inflammatory phase and neuro-psychological disturbances are warranted. Although we did not find significant direct correlations among DCE metrics and inflammation markers, we found that normalization of these parameters by CP volume led to strong

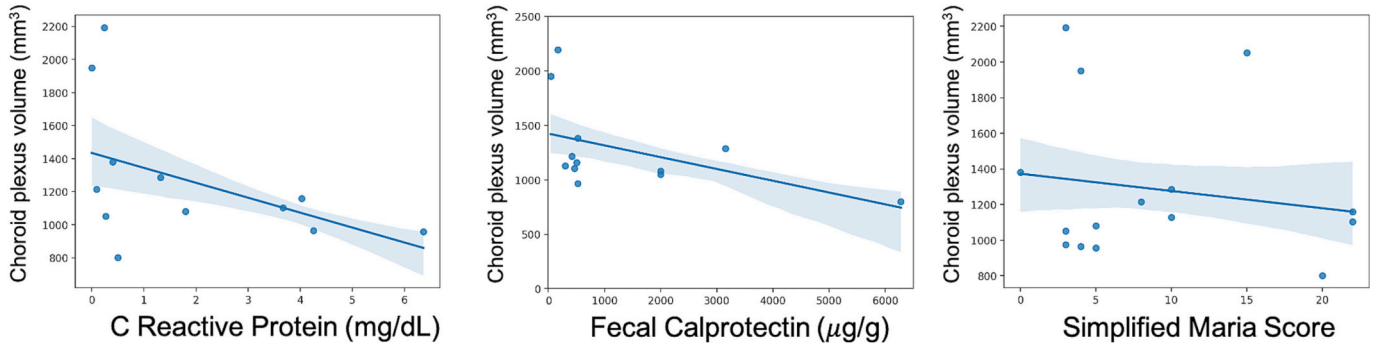

Fig. 2. Association between CP volume and markers of inflammation and disease. Scatter-plots show the association between CP volume and CRP (left), and between CP volume and FC (middle). No significant association was identified between the CP volume and the radiological sMARIA score (right). Linear regression lines and 95% confidence intervals are displayed.

Table 2
Univariate statistical analysis correlation between C-Reactive Protein, Fecal Calprotectin and sMARIA and SES-CD scores in respect to quantitative volumetric MRI and DCE variables. [a]Pearson's Correlation test, [b] Spearman's Correlation test.

	CRP	Fecal Calprotectin	sMARIA score	SES-CD
Volumetric Measurements				
CP volume	**R = −0.643[b] p = 0.024**	**R = −0.571[b] p = 0.050**	$R = -0.092^{b}$ $p = 0.745$	$R = -0.357^{b}$ $p = 0.160$
CP volume / IC volume	**R = −0.615[b] p = 0.033**	$R = -0.550^{b}$ $p = 0.064$	$R = -0.099^{b}$ $p = 0.729$	$R = -0.069^{b}$ $p = 0.791$
DCE parameters				
K-trans	$R = 0.259^{a}$ $p = 0.417$	$R = 0.140^{a}$ $p = 0.664$	$R = -0.261^{b}$ $p = 0.348$	$R = -0.145^{a}$ $p = 0.578$
Ve	$R = 0.126^{a}$ $p = 0.697$	$R = 0.252^{a}$ $p = 0.429$	$R = -0.272^{b}$ $p = 0.327$	$R = 0.092^{a}$ $p = 0.726$
Vp	$R = 0.238^{b}$ $p = 0.457$	$R = 0.091^{b}$ $p = 0.778$	$R = -0.063^{b}$ $p = 0.824$	$R = 0.370^{b}$ $p = 0.143$
T1	$R = -0.357^{a}$ $p = 0.255$	$R = -0.487^{a}$ $p = 0.108$	$R = 0.131^{b}$ $p = 0.641$	$R = 0.044^{a}$ $p = 0.868$
Normalized DCE parameters				
K-trans / CP volume	**R = 0.587[a] p = 0.045**	$R = 0.368^{a}$ $p = 0.240$	$R = -0.156^{b}$ $p = 0.578$	$R = 0.133^{b}$ $p = 0.612$
Ve / CP volume	$R = 0.441^{a}$ $p = 0.152$	$R = 0.462^{a}$ $p = 0.130$	$R = -0.191^{b}$ $p = 0.496$	$R = 0.169^{a}$ $p = 0.518$
Vp / CP volume	**R = 0.706[b] p = 0.010**	**R = 0.606[b] p = 0.037**	$R = 0.088^{b}$ $p = 0.755$	$R = 0.397^{a}$ $p = 0.114$
T1 / CP volume	**R = 0.699[a] p = 0.011**	$R = 0.550^{a}$ $p = 0.064$	$R = 0.200^{b}$ $p = 0.476$	$R = 0.259^{a}$ $p = 0.316$

correlation with CRP. The normalization was applied to account for possible partial-volume effects arising because of the small size and high surface-to-volume ratio of the CP, and for the dependance between CP volume and T1 that could affect both T1 values and DCE parameters, which computation depends on T1. Therefore, our results suggest the existence of a link between PVB permeability and systemic and intestinal inflammation in patients with CD, in agreement with the recent findings that intestinal inflammation causes modulation of the PVB permeability in mice (Carloni et al., 2021). It must be acknowledged, however, that the molecules used to investigate PVB permeability are very different in size (molecular weight of 70 kDa for Cy7/Cy3-conjugated dextran (Carloni et al., 2021) vs 604 Da for gadobutrol contrast agent ("https://www.bayer.com/sites/default/files/2020-11/gadovist-pm-en_0.pdf," n.d.)), and it cannot be excluded that different mechanisms of transmission are being probed in the two experiments. While clinical imaging studies are bound to the use of gadolinium-based contrast agents, preclinical studies are might be fundamental to compare these tracers and reproduce in preclinical models the results obtained in vivo in humans in the present study. Moreover, it is not currently known whether and how CP volume and permeability are related. Therefore, here we underline only the strength of the correlation, the meaning of which is still to be investigated.

It is known that CD manifestations, both neurological and abdominal, do not strictly correlate with disease activity (Gracie et al., 2016; Peyrin-Biroulet et al., 2016; Smith and Gaya, 2012; Targownik et al., 2015) and that, as in other pathologies, the systemic inflammation correlates with manifestations in other organs independently from the severity of the disease. This is the case, for example, of psoriasis where high CRP levels are associated with an increased risk of cardiovascular comorbidity and mortality (González-Cantero et al., 2021). Such a mechanism might explain the lack of an association between brain MRI measurements and sMARIA and SES-CD scores (that capture intestinal pathology).

We acknowledge that the impact of this study is limited by the small sample size that prevents further and more robust statistical analyses, like for example a multivariate linear regression analysis that would allow a more thorough investigation into the contribution of each variable, including mixed terms like T1 divided by volume, to the statistical model of CRP. Another drawback of a small sample size is the limited generalizability of results. However, the strength of the correlations observed with such a low number of cases is very promising for future studies aiming to extend the cohort of patients. Another limiting factor is represented by the absence of a neuropsychological assessment of patients to be compared against CP volume and permeability. Moreover, a longitudinal study is required to assess possible modifications of CP volume and permeability within the same subject in the different inflammatory phases of the disease.

5. Conclusions

This is the first study that prospectively investigates the modification of CP in CD patients in vivo. We found that CD activity is associated with changes in CP volume and permeability. This result further supports the existence of the gut-brain vascular axis and a possible involvement of the CP in CD and is a starting point for future studies investigating the mechanisms of communication between the gastrointestinal tract and the brain in CD.

Ethics approval and consent to participate

The study was approved by the local Ethical Committee *"Comitato Etico Indipendente IRCCS Istituto Clinico Humanitas"* and written informed consent was obtained from each participant.

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Data statement

The data underlying this article cannot be shared publicly for the privacy of individuals that participated in the study. The data will be shared on reasonable request to the corresponding author.

CRediT authorship contribution statement

Cristiana Bonifacio: Writing – review & editing, Investigation, Data curation, Conceptualization. **Giovanni Savini:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Christian Reca:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Federico Garoli:** Formal analysis, Data curation. **Riccardo Levi:** Writing – original draft, Methodology, Formal analysis, Data curation. **Giulia Vatteroni:** Project administration, Conceptualization. **Luca Balzarini:** Writing – review & editing, Supervision. **Mariangela Allocca:** Investigation, Data curation. **Federica Furfaro:** Investigation, Data curation. **Arianna Dal Buono:** Investigation, Data curation. **Alessandro Armuzzi:** Writing – review & editing, Validation. **Silvio Danese:** Writing – review & editing, Methodology, Conceptualization. **Michela Matteoli:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Maria Rescigno:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Gionata Fiorino:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Letterio S. Politi:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The following authors disclose conflicts of interest:

Federica Furfaro received consulting fees from Amgen, AbbVie and lecture fees from Janssen and Pfizer; Mariangela Allocca received consulting fees from Nikkiso Europe, Mundipharma, Janssen, AbbVie and Pfizer; Gionata Fiorino received consultancy fees from Ferring, MSD, AbbVie, Takeda, Janssen, Amgen, Sandoz, Samsung Bioepis, Celltrion; Silvio Danese served as a speaker, consultant, and advisory board member for Schering-Plow, Abbott (AbbVie) Laboratories, Merck, UCB Pharma, Ferring, Cellerix, Millenium Takeda, Nycomed, Pharmacosmos, Actelion, Alfa Wasserman, Genentech, Grunenthal, Pfizer, AstraZeneca, Novo Nordisk, Cosmo Pharmaceuticals, Vifor, and Johnson and Johnson; Alessandro Armuzzi has received consulting and/or advisory board fees from AbbVie, Allergan, Amgen, Arena, Biogen, Boehringer Ingelheim, Bristol-Myers Squibb, Celltrion, Eli-Lilly, Ferring, Galapagos, Gilead, Janssen, MSD, Mylan, Pfizer, Protagonist Therapeutics, Roche, Samsung Bioepis, Sandoz, and Takeda; lecture and/or speaker bureau fees from AbbVie, Amgen, Arena, Biogen, Bristol-Myers Squibb, Celltrion, Eli-Lilly, Ferring, Galapagos, Gilead, Janssen, MSD, Mitsubishi Tanabe, Novartis, Pfizer, Roche, Samsung Bioepis, Sandoz, Takeda, and Tigenix; and research grants from MSD, Pfizer, Takeda and Biogen; Maria Rescigno reports payment or honoraria for speaking engagements from Fondazione Internazionale Menarini; Riccardo Levi has received funds from Esaote.

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All remaining authors declare no financial or non-financial competing interests. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

Data availability

The data underlying this article cannot be shared publicly for the privacy of individuals that participated in the study. The data will be shared on reasonable request to the corresponding author.

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