Original Article

Effect of PF-00547659 on Central Nervous System Immune Surveillance and Circulating \( \beta^7 + \) T Cells in Crohn’s Disease: Report of the TOSCA Study

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Abstract

Background and Aims: Progressive multifocal leukoencephalopathy [PML], a brain infection associated with anti-integrin drugs that inhibit lymphocyte translocation from bloodstream to tissue, can be fatal. Decreased central nervous system [CNS] immune surveillance leading to this infection has been reported in patients with multiple sclerosis or Crohn’s disease treated with anti-integrin antibody natalizumab. PF-00547659 is an investigational human monoclonal antibody for inflammatory bowel disease, targeted against \( \alpha_4\beta^7 \)-mucosal addressin cell-adhesion molecule-1 [the integrin ligand selectively expressed in the gut]. We hypothesised that this selective agent would not affect central nervous system immune surveillance.

Methods: Cerebrospinal fluid from five healthy volunteers, and from 10 patients with Crohn’s disease previously treated with immunosuppressants, was evaluated to assess the feasibility of the study. Subsequently, 39 patients with active Crohn’s disease and previous immunosuppression were evaluated over 12 weeks of PF-00547659-induction therapy. We measured total lymphocytes, T cell subsets in cerebrospinal fluid, and circulating \( \beta^7 + \) memory cells. Disease activity was assessed using the Harvey–Bradshaw Index.

Results: Patients treated with PF-00547659 had no reduction of cerebrospinal fluid lymphocytes, T-lymphocyte subsets, or CD4:CD8 ratio, whereas circulating \( \beta^7 + \) memory cells increased.
1. Introduction

Inhibition of lymphocyte trafficking from blood vessels into tissue offers the promise of treatment for a variety of inflammatory diseases. Lymphocyte trafficking is a complex process that is mediated in part by binding of integrins, such as LFA-1 and α4β7, to cell adhesion molecules, such as ICAM-1 and VCAM-1. However, this category of drugs has significantly lost momentum due to the high incidence of progressive multifocal leukoencephalopathy [PML]. The first two anti-integrin agents reported to inhibit lymphocyte trafficking were: 1) efalizumab [indicated for the treatment of psoriasis] and 2) natalizumab (indicated for the treatment of multiple sclerosis [MS] and Crohn’s disease [CD]).

In patients treated with natalizumab, three clinical risk factors for PML have been identified: 1) treatment duration [ie, 25–48 months]; 2) previous exposure to JCV, measured by the presence of anti-JCV antibodies; and 3) history of previous immunosuppressant therapy. More than 1% of patients who had all three risk factors developed PML.

As a result, therapies with greater selectivity have been investigated. The purpose of our study was to investigate the effects of 8 weeks of induction therapy with high-dose PF-00547659 on the cellular elements of CNS immune surveillance in patients with active CD and a history of immunosuppressive therapy.

2. Methods

2.1. Study design

The study was executed in three parts under two protocols [Figure 1]:

1. CSF from an initial group of six healthy volunteers [designated Cohort 0, in the first protocol] was studied to determine whether TransFix® could stabilise CSF lymphocytes and their surface markers in order to permit use of a central flow cytometry laboratory.
2. Two cohorts were evaluated in the second protocol. Cohort 1 included 10 volunteers with CD who underwent two lumbar punctures [LP] with a 14-day interval, and then received treatment with PF-00547659. In this cohort we addressed two questions: i) do patients with CD who received previous immunosuppressant therapy have a sufficient number of CSF lymphocytes to provide reliable flow cytometry data; and ii) is the intra–individual variability low enough to permit conclusions regarding treatment effect?
3. After review of the results of Cohort 1, Cohort 2 included 39 additional patients with CD. CSF was collected via LP before and after three 225-mg doses of PF-00547659. The drug was administered subcutaneously at least 24 h after the first LP, and then every 28 days. The study was conducted in compliance with the Declaration of Helsinki, good clinical practice guidelines, and national regulations for protection of human subjects. The research protocol and subject compensation were approved by the relevant institutional review board or ethics committee at each centre, and all human participants gave written informed consent. Patients were compensated for their participation. Data were analysed by the sponsor according to a statistical analysis plan approved by the authors.

2.2. Patient populations

Subjects in Cohort 0 were healthy volunteers aged 18–55 years, with BMI 18–30 kg/m² and body mass > 50 kg. Patients in Cohorts 1 and
2.3. Lumbar puncture and CSF
Patients who met inclusion criteria underwent LP using a 22G 90-mmatraumatic spinal anaesthesia needle with introducer [http://www.imd-inc.com/spinalanesthesia.php]. In healthy volunteers, 10 mL of CSF were withdrawn and preserved in TransFix®. In patients with CD, 14 mL of CSF were withdrawn; 10 mL preserved with TransFix® were transferred to a central laboratory for flow cytometry; 3 mL were analysed at the local laboratory for culture, protein, glucose, and cell count; and 1 mL was evaluated for JCV DNA and anti-JCV antibody.

2.4. Study drug
PF-00547659 was administered by subcutaneous injection every 4 weeks. The 225-mg dose was selected as the highest repeated dose given in the Phase I trial.21

2.5. Study visits
Healthy volunteers had a single visit. Patients with CD returned every 4 weeks for treatment, assessment of disease activity with HBI,22 evaluation of adverse events, physical examination, electrocardiogram [ECG], and laboratory tests. All patients who underwent the first LP were permitted to receive the study drug. At each LP visit, venous blood was also withdrawn for analysis by flow cytometry. In Cohort 1, the second LP was performed before the administration of the study drug and 14 ± 4 days after the first LP. In Cohort 2, the second LP was performed 2 ± 1 weeks after the latest dose of study drug [i.e. at Week 9–11 after baseline]. The final study visit was performed 4 weeks after the third dose of study drug, at which time patients who had a clinical response to the study drug were allowed to enrol in an open-label extension study.

2.6. Study endpoints
The primary endpoint was the percentage change in absolute lymphocyte count in the CSF of patients with CD after receiving three monthly doses of PF-00547659. Secondary endpoints included clinical response (defined as a reduction in disease activity [HBI score] by ≥ 3 points) and remission (defined as an HBI score < 5 points). Additional analyses included characterisation of lymphocyte subpopulations in both CSF and blood before and after treatment with PF-00547659.

2.7. Pharmacodynamic, efficacy, and safety evaluations
2.7.1. Flow cytometry
Flow cytometry data were analysed by WinList® 7.0 [Verity Software, ME]. All CSF and blood samples were analysed with a BD FACSCanto-II® [Becton Dickinson, San Jose, CA] by LabCorp® [Mechelen, Belgium] in a regulated environment. Assays were validated for analysis of CSF and blood samples.

2.7.2. Cerebrospinal fluid
CSF samples were collected in TransFix®-containing tubes. In Cohort 0, they were analysed within 2 h, then stored at room temperature for 24 and 48 h for repeat studies. In Cohorts 1 and 2, they were transported to a central laboratory within 24 h of LP. Details are presented in the Supplementary material, available at ECCO-JCC online.
2.8. Study oversight
The protocol for this study was developed by the authors and approved by ethics committees and, where appropriate, national or regional authorities. A data-monitoring committee adjudicated any patients with unexplained neurological findings or suspected potential PML. All authors had full access to all data. All the authors reviewed and approved the final manuscript, and made the decision to submit the manuscript for publication and all vouch for the veracity and completeness of the data, the analyses and the fidelity of the study to the protocol.

2.9. Statistical analysis
The analysis was designed to show that the percentage decrease from baseline in absolute lymphocyte count in CSF is < 50% if the true percentage change is approximately 10%. Assuming a standard deviation of the paired differences [post-dose vs baseline] on the log scale of 0.99, approximately 15 patients were required to yield approximately 83% power with α = 0.10 [one-sided]. Assuming a dropout rate of 25%, enrolment of 20 patients was planned to yield approximately 15 paired samples for analysis. The primary end point—change from baseline in absolute lymphocyte count—was tested using a paired t-test on the log-transformed data. The estimates and the confidence intervals [CI] were constructed for the geometric mean ratio [GMR]. For results to be deemed statistically significant, the 10% lower confidence limit of the percentage decrease from baseline of the GMR had to be > 0.5. The data reported were obtained from a cleaned locked database for inclusion in this manuscript.

3. Results
As shown in Table 1, healthy volunteers in Cohort 0 were similar in age to patients with CD. One volunteer was replaced due to a traumatic LP. CD patients had long-term, active disease, and a significant subset had stomas. All 49 CD patients from both Cohorts 1 and 2 had previous treatment with both immunosuppressants and anti-TNF agents. In Cohort 2, 17/23 [74%] patients tested had positive anti-JCV serology [Table 1].

3.1. CSF/TransFix® stability—cohort 0
The absolute counts of lymphocytes and subpopulations were unaffected by storage for up to 48 h after collection in four of the five donors; in one donor [Volunteer #3] absolute counts decreased by 26.0% to 51.9% at 24 h and 72.6% to 81.8% at 24 h after collection. Post-collection storage of CSF samples did not influence the frequency of lymphocyte subpopulations in CSF in any of the five volunteers [Supplementary Table S1 and Supplementary Figure S1, available at ECCO-JCC online].

3.2. Pre-interventional CSF characteristics—cohort 1
In patients with CD, the numbers of lymphocytes and lymphocyte subpopulations were within published reference ranges.21,24 Pre-treatment CSF lymphocyte and lymphocyte subset counts were similar between LP 1 and 2 for most patients evaluated in Cohort 1 [Supplementary Table S2 and Supplementary Figure S2, available as Supplementary data at ECCO-JCC online]. The mean fold change in lymphocytes using a mixed model from LP1 to LP2 [90% CI] was 0.94 [0.71, 1.25].

3.3. Effects of PF-00547659—cohort 2
3.3.1. Immunological effects
After treatment, there was a small increase in the numbers of overall CSF lymphocytes across several subsets [CD3+; CD4+; CD8+]; the CD4:CD8 ratio was similar before and after treatment [Figure 2; Supplementary Table S3, available at ECCO-JCC online]. Among the nine patients who continued to receive immunosuppressant therapy during the study, baseline numbers of T-lymphocytes and subsets were about 20% lower than in the other 28 patients, but the differences were neither significant nor below the range seen in healthy volunteers [Table 1]. Concurrent use of systemic glucocorticoids was associated with lower pre-treatment CSF CD4+, but not CD8+ T-lymphocytes, in a dose-related manner [data not shown]. CSF CD4:CD8 ratios were similar both before and after treatment, and in patients receiving or not receiving concomitant immunosuppressants. As in healthy subjects, very few NK and B cells were seen. Post-treatment changes were similar in magnitude regardless of ongoing immunosuppressant treatment [Table 2]. No JCV DNA was detected in 20 pre-treatment or 19 post-treatment samples of CSF.

In peripheral blood, the only change associated with PF-00547659 treatment was a 1.45-fold mean increase [90% CI: 1.31, 1.61] in β7+ central memory T-lymphocytes between baseline and 3.3.2. Anti-JCV antibody seropositive patients
In the 19 anti-JCV antibody seropositive patients, baseline peripheral blood and CSF CD3 cells were increased [90% CI: 1.24, 1.56]. The mean percentage increase of T-lymphocytes in peripheral blood and CSF samples from LP1 and LP2 was similar [90% CI: 1.07, 1.12]. The change in CSF lymphocytes across several subsets [CD3+; CD4+; CD8+; CD4:CD8 ratio was similar before and after treatment for most patients evaluated in Cohort 1 [Supplementary Table S2 and Supplementary Figure S2, available as Supplementary data at ECCO-JCC online].

Table 1. Demographic and baseline characteristics.

| Characteristic                  | Cohort 0 [n = 6] | Cohort 1 [n = 10] | Cohort 2 [n = 39] |
|--------------------------------|------------------|-------------------|-------------------|
| Age [years]                    | 37.7 [12.1]      | 40.9 [15.9]       | 37.4 [10.6]       |
| Gender [male/female]           | 5/1              | 8/2               | 13/26             |
| Weight [kg]                    | 81.2 [9.1]       | 67.8 [12.6]       | 69.6 [17.0]       |
| Duration of Crohn’s disease [years] | N/A              | 13.3 [6.3]       | 12.6 [6.3]       |
| Anti-JCV antibody seropositive [n] | N/A              | 5                 | 17                |
| Intestinal stoma present [n]  | N/A              | 3                 | 11                |
| Harvey–Bradshaw Index total score, median [min, max] | N/A              | 10.0 [8, 12]     | 9.0 [5, 10]      |
| High sensitivity C-reactive protein, mg/L, median [min, max] | N/A              | 16.4 [0.7, 67.2] | 5.0 [0.5, 79.5]  |
| Duration anti-TNF use [years]  | N/A              | 3.2 [1.6]         | 3.6 [2.9]         |
| Ongoing immunosuppressant [n]  | N/A              | 1                 | 9*                |
| Ongoing glucocorticoids [n]    | N/A              | 7                 | 12                |

All values are mean [standard deviation] values, unless otherwise noted. CV, coefficient of variation; JCV, John Cunningham virus; N/A, not applicable; TNF, tumour necrosis factor.

* Only 23 patients tested [in Cohort 2].

† Three patients each continued to receive azathioprine, 6-mercaptopurine, and methotrexate during the study.
3.4. Clinical effects

3.4.1. Clinical response

All treated patients \([n = 49]\) were evaluated for clinical effects. Of the 35 patients in Cohorts 1 and 2 with an evaluable HBI [ie, patients without stoma], 28 [80\%] achieved a clinical response and 27 [77\%] achieved clinical remission at Week 12. Enrolment with hsCRP \(\leq 5.0\) mg/L vs > 5.0 mg/L had no impact on rates of remission or response. The mean HBI decreased significantly from 8.7 [1.7] to 3.6 [2.7] points \([p < 0.001]\). The reduction in HBI was \(-6.6 \pm 1.72\) for patients who continued to receive immunosuppressants, and \(-4.8 \pm 3.02\) for those who did not. The HBI without the points assessed for stool count was evaluated in patients with stomas [Supplementary Table S5, available as Supplementary data at ECCO-JCC online]. The improvement in this partial HBI was similar in patients with intact GI tracts and those with stomas. The only disease biomarker assessed was hs-CRP, the geometric mean value for which decreased 29.8\% in all patients.

3.4.2. Safety

Treatment-emergent adverse events were reported by 46 [94\%] patients. No patient withdrew from the study due to an adverse event. The most common adverse events are listed in Table 4; 23 patients had adverse events considered related to study treatment, including four injection-site reactions. The most common events considered related to study treatment were arthralgia and nasopharyngitis, reported in four patients each. One patient who did not experience clinical response discontinued from the study; two other patients withdrew consent; and one was lost to follow-up.
Table 2. Changes in cerebrospinal fluid T cell subsets by concurrent immunosuppressant use—Cohort 2 [geometric mean, 95% confidence interval].

| Population                  | Concurrent immunosuppressant use |
|-----------------------------|----------------------------------|
|                             | Pre-treatment | Post-treatment | Pre-treatment | Post-treatment |
|                             | N             |               |               |               |
| CD3+                        | 28            | 22            | 9             | 8             |
| CD3+/CD4+                   | 387 [262, 570]| 512 [313, 837]| 299 [118, 759]| 397 [202, 783]|
| CD3+/CD4+CD8+               | 260 [173, 393]| 348 [211, 576]| 207 [79, 542] | 293 [143, 602]|
| CD3+/CD4+CD8+               | 107 [75, 153] | 134 [84, 213] | 82 [34, 199]  | 96 [52, 175]  |
| CD3–/CD8                    | 2.43 [1.96, 3.01] | 2.58 [2.03, 3.28] | 2.53 [1.77, 3.62] | 3.03 [2.11, 4.36] |
| CD3–/CD4+CD45RO+CD27+/β7+   | 7 [4, 11]     | 7 [4, 11]     | 6 [2, 20]     | 7 [2, 21]     |
| CD3–/CD19+                  | 4 [2, 6]      | 3 [2, 5]      | 7 [2, 24]     | 7 [3, 18]     |

Values are cells/mL [apart from CD4:CD8].

Table 3. Blood lymphocytes by flow cytometry before and after treatment with 225 mg PF-00547659—Cohort 2 [geometric mean, 95% CI].

| Population                  | Pre-treatment [n = 39] | Post-treatment [n = 32] | Mean fold change |
|-----------------------------|------------------------|-------------------------|------------------|
| Lymphocytes                 | 1482 [1273, 1724]      | 1670 [1423, 1961]       | 1.10 [0.99, 1.23]|
| CD3+/CD4+                   | 715 [591, 865]         | 806 [653, 995]          | 1.11 [0.97, 1.27]|
| CD3+/CD8+                   | 305 [246, 377]         | 326 [260, 410]          | 1.05 [0.92, 1.20]|
| CD4:CD8                     | 2.01 [1.91, 2.89]      | 2.47 [1.97, 3.10]       | 1.05 [0.95, 1.17]|
| CD3+/CD8+CD45RO+CD27+/β7+  | 61 [51, 73]            | 91 [71, 116]            | 1.44 [1.28, 1.64]|

Values are cells/mL [apart from CD4:CD8].

Table 4. Treatment-emergent adverse events reported for > 4 patients—Cohorts 1 and 2.

| Event                        | Number of patients [%] |
|------------------------------|------------------------|
| Any adverse event            | 46 [93.9]              |
| Gastrointestinal             | 17 [34.7]              |
| Abdominal pain               | 5 [10.2]               |
| General/site                 | 15 [30.6]              |
| Fatigue                      | 6 [12.2]               |
| Infections and infestations  | 21 [42.9]              |
| Nasopharyngitis              | 8 [16.3]               |
| Musculoskeletal and connective tissue | 16 [32.7] |
| Arthralgia                   | 9 [18.4]               |
| Nervous system               | 9 [18.4]               |
| Headache                     | 6 [12.2]               |

Five serious adverse events [SAEs] were reported in four patients. One had enterocutaneous fistula and fatigue, one had nephrolithiasis and two patients reported exacerbations of CD. All events resolved and none was considered to be related to study treatment.

3.4.3. Clinical laboratory parameters

None of the laboratory abnormalities was reported as an AE by the investigators. No significant elevations in liver tests were observed [ie. bilirubin was always < 2 × ULN and transaminases were always < 3 × ULN], and no cases met the criteria of Hy’s Law.

4. Discussion

The selective anti-MAdCAM monoclonal antibody PF-00547659 was developed to mitigate the risk of PML seen with natalizumab. We hypothesised that its lack of binding to VCAM-1 would result in preserved lymphocyte translocation into the CSF, thereby minimising the risk of PML. Of the known risk factors for PML in natalizumab-treated patients, only the mechanism by which previous immunosuppressant exposure increases risk is not understood.

We were concerned that previous immunosuppression might lower total lymphocyte count or the number of CD4+ T-lymphocytes in CSF, which would make our results uninterpretable. Because of inter-laboratory variability in flow cytometry data, we used a central laboratory, with up to 24-h transport time for this critical test. For this study to be valid, we needed to ensure that CSF samples would be stable and results would be interpretable in our patient population. The first part of the study demonstrated that: 1) CSF T-lymphocyte surface markers were stabilised with TransFix®, a stabilizer for blood lymphocytes, for ≥ 24 h; 2) patients with CD and previous immunosuppressant exposure had CSF lymphocyte counts and subpopulations similar to healthy subjects; and 3) within-subject variability in CSF lymphocyte counts was low enough to evaluate the primary objective.

We then demonstrated that a 12-week induction course of anti-MAdCAM-1 monoclonal antibody PF-00547659 did not affect the number or nature of lymphocytes in the CSF. The increase in circulating β7+ central memory T-lymphocytes in PF-00547659-treated patients likely reflects pharmacodynamic activity in drug-mediated reduction of integrin-mediated lymphocyte extravasation into the gut, as previously reported in animal models. A recent publication suggests that vedolizumab treatment may result in accumulation of T-reg cells in the circulation of ulcerative colitis patients, which may limit expansion of T-effector cells in the peripheral blood and favour suppression of systemic inflammation.

Natalizumab was the first anti-adhesion molecule to be approved for treatment of patients with CD. As mentioned earlier, its acceptance has been limited by the increased risk of PML, a life-threatening opportunistic infection caused by the John Cunningham virus that attacks the CNS. Natalizumab blocks the α4-integrins on leukocytes, which target both MAdCAM-1 and VCAM-1 the gut, and VCAM-1 in the CNS, and therefore can result in profound depression of CSF lymphocytes, especially CD4+ T-lymphocytes. This
effect can persist for 6 months after treatment with natalizumab.\textsuperscript{24} Interestingly, no case of PML has been reported more than 6 months after cessation of natalizumab therapy unless another immunosuppressive agent was given.\textsuperscript{29}

The emergence of PML has underscored the importance of CNS immune surveillance of latent JCV reactivation. Previous evidence suggests that VCAM-1 plays a central role in the CNS immune response to viruses.\textsuperscript{26} In contrast, our results suggest that MAdCAM-1 does not mediate leukocyte migration into the CNS compartment in patients with CD.

Although MAdCAM-1 is considered mostly gut-selective, it was found to be upregulated in choroid plexus epithelium during experimental autoimmune encephalomyelitis [EAE], an autoimmune animal model of MS.\textsuperscript{27} Monoclonal antibodies against MAdCAM-1 have been shown to effectively prevent the development of actively induced EAE and decrease the number of CNS leukocytes in this model,\textsuperscript{30} although conflicting evidence has been reported.\textsuperscript{31} It was therefore important to confirm that MAdCAM inhibition would have no impact on CNS lymphocytes.

Vedolizumab, an anti-integrin monoclonal antibody targeted against the \(\alpha_4\beta_7\) integrin, is effective in the treatment of IBD.\textsuperscript{32,33} It was the first of the next generation of integrin antibodies with a more selective mode of action—it specifically targets the \(\alpha_4\beta_7\) integrin on circulating lymphocytes and blocks its interaction with MAdCAM-1, but does not inhibit \(\alpha_4\beta_7\) binding to VCAM-1. Thus, vedolizumab prevents leukocyte migration to the intestinal mucosa. Vedolizumab did not affect T-lymphocyte populations in the CSF of healthy volunteers 5 weeks after a single administration,\textsuperscript{34} nor did it inhibit immune surveillance of the CNS in primates.\textsuperscript{35} However, the effect of vedolizumab in IBD patients’ CNS immune surveillance has not yet been studied.

In conclusion, as measured by the numbers of overall CSF lymphocytes, lymphocyte subsets [CD3\(^+\), CD4\(^+\), CD8\(^+\)] and the CD4:CD8 ratio, selective binding of MAdCAM-1 with PF-00547659 does not impair normal CNS immune surveillance in patients with CD and previous immunosuppression, regardless of anti-JC virus antibody seropositivity. Whether the long-term risk of CNS opportunistic infections with high cumulative doses of PF-00547659 is increased cannot be categorically ruled out with the data generated in this study. However, our data provide a good biological rationale to suggest that PF-00547659 treatment of IBD should have a lower risk of PML than do non-selective anti-integrin therapies.

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**Conflict of Interest**

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Author Contributions

The following chart identifies the roles of each of the co-authors.

| Author                        | Author roles* | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|------------------------------|---------------|---|---|---|---|---|---|---|---|---|
| Geert D’Haens                 |               | X | X | X | X |
| Severine Vermeire            |               | X | X | X | X |
| Harald Vogelsang             |               | X | X | X | X |
| Matthieu Allez               |               | X | X | X | X |
| Pierre Desreumaux            |               | X | X | X | X |
| Andre Van Gossum             |               | X | X | X | X |
| William J. Sandborn          |               | X | X | X | X |
| Daniel C. Baumgart           |               | X | X | X | X |
| Richard M. Ransohoff         |               | X | X | X | X |
| Gail M. Comer                |               | X | X | X | X | X |
| Alaa Ahmad                   |               | X | X | X | X | X | X |
| Fabio Cataldi                |               | X | X | X | X | X |
| John Cheng                   |               | X | X | X | X |
| Robert Clare                 |               | X | X | X |
| Kenneth J. Gorelick          |               | X | X | X | X |
| Annamarie Kaminski           |               | X | X | X | X |
| Vivek Pradhan                |               | X | X | X | X | X |
| Sunday Rivers                |               | X | X | X | X |
| Matthew O. Sikpi             |               | X | X | X | X | X |
| Yinhua Zhang                 |               | X | X | X | X |
| Minu Hassan-Zahraee          |               | X | X | X | X |
| Walter Remisch               |               | X | X | X | X |
| Olaf Stuve                   |               | X | X | X | X |

*1: study concept and design; 2: acquisition of data; 3: analysis and interpretation of data; 4: drafting of the manuscript; 5: critical revision of the manuscript for important intellectual content; 6: statistical analysis; 7: obtained funding; 8: technical or material support; 9: study supervision.

Supplementary Data

Supplementary data are available at ECCO-JCC online.

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