

Defining faecal calprotectin thresholds as a surrogate for endoscopic and histological disease activity in ulcerative colitis – a prospective analysis

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Abstract

Background

Faecal calprotectin (FCal) levels are used as a surrogate marker for mucosal inflammation, but thresholds for defining endoscopic or histological disease activity in ulcerative colitis (UC) remain unclear.

Methods

Using validated indices, prospective measurements of FCal, symptoms (Simple Colitis Clinical Activity Index, SCCAI), endoscopic (Ulcerative Colitis Endoscopic Index of Severity, UCEIS) and histological activity (Nancy index) were made over 6 months in patients enrolled into the TrueColours UC web-based monitoring programme. Repeated measurements correlation was performed between FCal and SCCAI, UCEIS and Nancy indices using definitions for remission and active disease (UCEIS: remission ≤ 1 , active ≥ 4 ; Nancy: remission ≤ 1 , active ≥ 2 ; Combined criteria: remission UCEIS ≤ 1 and Nancy ≤ 1 , active UCEIS ≥ 4 and Nancy ≥ 2). Receiver operating characteristic curves investigated FCal thresholds after maximising sensitivity for active disease.

Results

In 39 patients followed prospectively for 6 months, correlation coefficients between FCal and SCCAI, UCEIS and Nancy indices were 0.271 (95% CI 0.114-0.415), 0.741 (95% CI 0.289-0.922) and 0.876 (95% CI 0.605-0.965) respectively. Median FCal thresholds for remission using endoscopic, histologic, or combined criteria were 71 $\mu\text{g/g}$ (range 8-624), 91 $\mu\text{g/g}$ (range 8-858) and 67 $\mu\text{g/g}$ (range 8-479), respectively. The FCal threshold above which active

disease was confirmed was $187\mu\text{g/g}$ for UCEIS (AUC 0.915), $72\mu\text{g/g}$ for Nancy (AUC 0.824) and $187\mu\text{g/g}$ for combined endoscopic and histologic criteria (AUC 0.936).

Conclusions

Correlation between FCal and symptoms in UC is weak. In contrast, the correlation between FCal and endoscopic or histological activity is strong. An $\text{FCal} \geq 72\mu\text{g/g}$ indicates histological inflammation (Nancy ≥ 2) and $\geq 187\mu\text{g/g}$ indicates endoscopically active disease ($\text{UCEIS} \geq 4$), whether combined with histopathology or not.

Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease. When active, symptoms can include bloody diarrhoea and urgency. In recent years there has been a paradigm shift in the management, now aiming for combined clinical and endoscopic remission. For symptoms, the treatment target is resolution of rectal bleeding and normalisation of bowel habit (1).

Although there are multiple symptom-based indices for UC (2), the Simple Clinical Colitis Activity Index (SCCAI) is increasingly used in clinical practice (3). Symptoms alone however, are not an accurate marker of the degree of inflammation.

For endoscopy, mucosal healing is the treatment target (1, 4). Mucosal healing is associated with sustained remission (5), decreased need for hospitalisation (6-8), decreased corticosteroid use (9), decreased colectomy (5, 6, 8) and decreased risk of colorectal cancer (10). Endoscopy is therefore increasingly used to guide decision making. The UCEIS is the only validated endoscopic index for UC (11).

Histological inflammation can persist despite mucosal healing, representing residual disease activity (12, 13). Persistent histological inflammation in UC is associated with an increased risk of clinical relapse, hospitalisation, colectomy and colorectal cancer (13-15). The absence of any acute inflammatory infiltrate of neutrophils represents complete histological remission for UC (16), but this is not yet recommended as a target of treatment in either clinical trials or practice (1, 17). There are no fewer than 26 histological activity indices for UC, but only two are validated, the Nancy Index (18) and the Robarts' Histopathology Index (19).

Endoscopy is invasive, uncomfortable, time-consuming and expensive. Surrogate markers that reflect the severity of mucosal inflammation have therefore been investigated. Faecal

calprotectin (FCal) has been demonstrated to be useful, correlating closely with endoscopic activity (20-23). The level of FCal is proportional to neutrophil migration in the gastrointestinal tract, but thresholds for FCal that indicate endoscopic mucosal inflammation remain debated, ranging from 60-250 µg/g (20, 23-25). For histological activity, FCal values from 40-170 µg/g have been associated with histologic remission (23, 26-28).

There is further need to correlate FCal with validated endoscopic and histological disease activity indices. This was the aim of our prospective study, which also aimed to explore FCal thresholds for defining endoscopic or histologically active disease and to determine whether these thresholds are altered by combining endoscopic and histological criteria.

Methods and Data

Patients and data collection

Patients: Patients were recruited as part of the TrueColours Ulcerative Colitis (TCUC) programme (29) to a prospective study. TCUC is a real-time web-based platform that is able to collate longitudinal data entered by patients or healthcare professionals. The programme functions through sending email prompts that directly link the patient to relevant questionnaires regarding demographics, symptoms, quality of life and outcomes (such as corticosteroid use). It is also able to collate pathology (blood tests, endoscopy, histopathology) and FCal results. TCUC was piloted at the John Radcliffe Hospital in Oxford in 66 patients for 6 months in 2016-17. Patients were recruited from the Inflammatory Bowel Disease clinic and all patients had an established diagnosis of UC according to established criteria (30). Data were collected on symptoms (daily), quality of life (fortnightly), outcomes (every 3 months), endoscopy and biopsy (0 & 6 months).

Symptoms: Patients were prompted to complete the Simple Clinical Colitis Activity Index (SCCAI) daily. The SCCAI ranges from 0-19 (0=best, 19=worst). The median SCCAI for the 5 days prior to each FCal measurement was used as the symptom score.

Endoscopy: The Ulcerative Colitis Endoscopic Index of Severity (UCEIS) is routinely used by endoscopists in Oxford to assess endoscopic activity. All endoscopists have received appropriate training (<https://e-learning.ecco-ibd.eu/course/view.php?id=40>). The worst affected area at flexible sigmoidoscopy was scored. Endoscopy was performed at the start and end of the 6 month study, with an additional procedure in the event of a relapse.

Histopathology: At the time of endoscopy, mucosal biopsies were taken from the worst affected area. Histological activity was assessed by a specialist GI histopathologist using the Nancy Index (18). The Nancy Index is reproducible, responsive and simple, ranging from 0 to 4 (normal and worst respectively), and is routinely used in clinical practice in Oxford. It correlates closely with the Robarts Histological index (31). Each endoscopic procedure and the resulting histopathology result was classified as an “event”. Neither the endoscopist nor histopathologist were blinded to other clinical information, except for the FCal level. All results were date-stamped to allow longitudinal analysis. Results were entered into TCUC.

Faecal calprotectin: The *IBDoc*[®] FCal test (Buhlmann Laboratories), is a pre-packaged kit that provides the equipment and instructions to allow patients to test their own stool at home. Accompanying software (*CalApp*[®]) converts their smartphone into a test cassette reader. To achieve graphic representation of FCal within the TCUC programme, communication between *IBDoc*[®] and TCUC was developed. Endoscopic and histological results were considered within the time interval of 14 days before (25 results) or after (27 results) the measurement of FCal at home.

Although 66 patients were monitored using TCUC, only those who performed FCal were included in the analysis. All patients gave their written consent to participate in the study (Hampshire B Research Ethics Committee C Ref: 16/SC/0103).

Statistical analysis

To determine strength of correlation, repeated measurements between FCal and SCCAI, UCEIS and Nancy indices were examined. For correlation of repeated measurements, the assumption of independence is violated, as many individual patients provide more than one

data point. To allow for this, an approach designed for repeated measurements correlation was used (32). This novel approach yields greater power than other techniques (33).

There is no consensus on criteria for endoscopic, histological, or combined endoscopic and histologic remission or active disease (34-36). Definitions used for remission and active disease groups in this study are shown in Table 1. For UCEIS (range 0-8), patients with a UCEIS of 2 or 3 were classified as an intermediate group (n=10/39) to enable separation between the groups. This is because it does not necessarily follow that a UCEIS of 2 or 3 represents active disease, even if remission is defined as ≤ 1 , since patients do not suddenly move from one disease activity state to another. While the definitions of remission and active disease groups (as presented in Table 1) were used in the analysis, two other threshold values of UCEIS (2 and 3) were additionally examined. Exact numbers of events analysed are presented in the Results section.

Table 1: Definition of remission and active disease for UCEIS, Nancy and their combined criteria

TYPE OF INDEX	DISEASE ACTIVITY GROUP	
	Remission	Active
ENDOSCOPIC	UCEIS ≤ 1	UCEIS ≥ 4
HISTOLOGIC	Nancy ≤ 1	Nancy ≥ 2
COMBINED CRITERIA	UCEIS ≤ 1 and Nancy ≤ 1	UCEIS ≥ 4 and Nancy ≥ 2

UCEIS = Ulcerative Colitis Endoscopic index of Severity, Nancy = Nancy histopathology index

For statistical analysis and data processing, several software programmes and programming languages were used. The R free software for statistical computing version 3.4.4 (37) and Python 2.7 with Matplotlib 2.2.2 (38) or graphics. The repeated measurements correlation coefficients r were computed using the `rmcorr` package in R (33). For strength of association, an r of 0-0.19 is regarded as very weak, 0.2-0.39 as weak, 0.40-0.59 as moderate,

0.6-0.79 as strong and 0.8-1.0 as very strong correlation (39). The continuous variables of FCal between remission and active categories were compared using the Mann-Whitney *U* test.

Thresholds were estimated using the R packages ‘pROC’ (40) and ‘OptimalCutpoints’ (41) for the receiver operating characteristic (ROC) curve analysis with weighted Youden’s index (42). The weighted Youden’s index is a function of sensitivity and specificity, which seeks a ROC operating point that maximises the vertical distance between the ROC curve and the diagonal or chance line, while keeping unequal weighted contributions from the sensitivity and specificity. Due to the limited sample size and skewed values of the predictor FCal measurement, the midpoint method was used to estimate thresholds. The midpoint method corresponds to computing the average of the optimal threshold and the next highest predictor value. Optimal FCal thresholds were found by maximising sensitivity for active disease, while penalising false negative errors (43). This approach was taken to ensure that no patient with active disease was incorrectly labelled as being in remission. Other diagnostic metrics such as positive and negative predictive values (PPV and NPV respectively) with the positive and negative diagnostic likelihood ratios (LR+ and LR- respectively) were estimated at the optimal cut-off. Statistical significance was accepted at $p < 0.05$.

Results

In total, 66 patients were recruited to the TrueColours study. 48/66 (73%) performed at least one FCal measurement throughout the 6 month study, with a median of 4 (range 1-7) measurements per patient. 39 patients had SCCAI, UCEIS and Nancy data associated with FCal levels. This resulted in 198 paired SCCAI and FCal events, 52 paired UCEIS and FCal events and 52 paired Nancy and FCal events. Repeated measurements correlation coefficients (r) between FCal and SCCAI, UCEIS and Nancy are presented in Table 2. There was weak correlation between FCal and symptoms (SCCAI), but strong correlation between FCal and endoscopy (UCEIS) and very strong correlation with histopathology (Nancy index).

Table 2: Correlation coefficients between the levels of FCal and indices

	<i>r</i>	95%CI	<i>p-value</i>
SCCAI	0.271	(0.114, 0.415)	<0.001
UCEIS	0.741	(0.289, 0.922)	<0.003
Nancy	0.876	(0.605, 0.965)	<0.0001

r = repeated measurements correlation coefficients. For absolute values of r , 0-0.19 is regarded as very weak, 0.2-0.39 as weak, 0.40-0.59 as moderate, 0.6-0.79 as strong and 0.8-1.0 as very strong correlation (39)

95% CI = 95% confidence intervals

SCCAI = Simple Clinical Colitis Activity Index, UCEIS = Ulcerative Colitis Endoscopic Activity Index, Nancy = Nancy Histopathological Index

Using the criteria for the indices specified in Table 1, patient events were stratified into remission and active groups. Those events that were ‘in between’ and did not meet criteria for either remission or active disease (ie UCEIS score of 2 or 3) were excluded. The number of FCal measurements and number of active disease events are summarised in Table 3.

Table 3: Number of events included per index and combined criteria

Disease activity assessment	Endoscopic UCEIS ≤ 1 or ≥ 4	Histologic Nancy \leq or ≥ 2	Combined Criteria UCEIS <u>AND</u> Nancy
Number of patients	n=29	n=39	n=26
Excluded patients *	n=10	n= 0	n=13
FCal measurements:			
total	39	52	31
per patient	1 (1-3)	1 (1-3)	1 (1-3)
Active disease events			
total:	18	27	18
per patient	1 (1-3)	1 (1-3)	1 (1-3)

All values are presented as counts or median (range).

* Excluded patients: did not meet criteria for either remission or active disease: UCEIS: remission ≤ 1 , active ≥ 4 ; Nancy: remission ≤ 1 , active ≥ 2 ; Combined criteria: remission UCEIS ≤ 1 and Nancy ≤ 1 , active UCEIS ≥ 4 and Nancy ≥ 2 .

The distributions of FCal levels in remission and active disease groups are summarised in Table 4. This shows lower median values of FCal levels in remission and active disease group ($p < 0.001$), regardless of whether endoscopic, histologic or combined criteria are used.

Table 4: Distribution of faecal calprotectin in remission and active disease groups

Indices used for classification	Calprotectin ($\mu\text{g/g}$)		Mann-Whitney <i>U</i> test <i>p-value</i>
	Remission*	Active*	
UCEIS	71 (8-624)	672 (214-1313)	< 0.0001
Nancy	91 (8-858)	585 (73-1313)	< 0.0001
Combined criteria	67 (8-479)	593 (214-1089)	< 0.0001

*Values presented as median (range), UCEIS: remission ≤ 1 , active ≥ 4 ; Nancy: remission ≤ 1 , active ≥ 2 ; Combined criteria: remission UCEIS ≤ 1 and Nancy ≤ 1 , active UCEIS ≥ 4 and Nancy ≥ 2 .

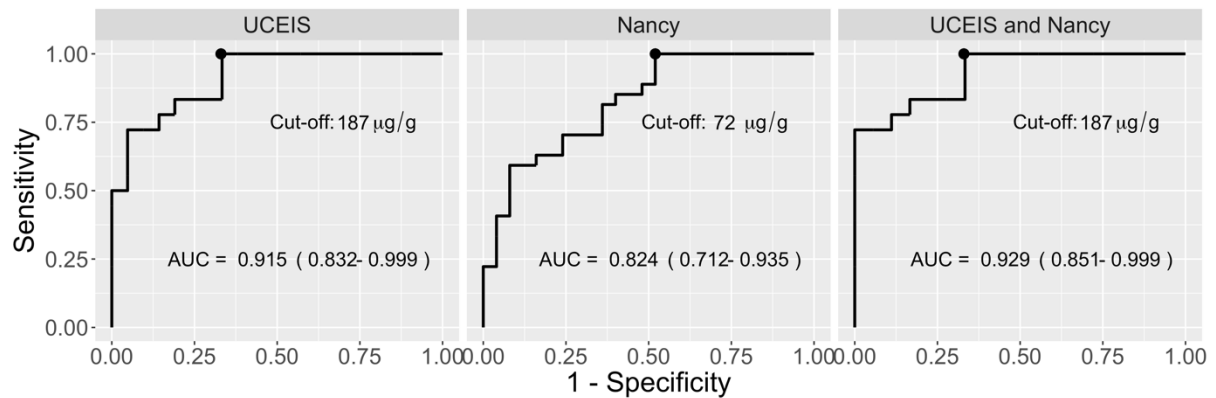
ROC analysis with weighted Youden's index criterion was performed for the UCEIS, Nancy and their combined criteria. Using the midpoint method, the optimal thresholds for FCal that indicated disease activity were estimated as 187 µg/g (midpoint between 160 µg/g and 214 µg/g), 72µg/g (midpoint between 71 µg/g and 73 µg/g) and 187µg/g (midpoint between 160 µg/g and 214 µg/g) respectively (Table 5 and Figure 1).

Table 5: The optimal faecal calprotectin thresholds for detecting endoscopic (UCEIS), histologic (Nancy) or their combined criteria for disease activity, based on weighted Youden's index

	UCEIS	Nancy	UCEIS and Nancy
Cut-off (FCal µg/g)	187	72	187
Sensitivity	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)
Specificity	0.67 (0.48-0.86)	0.48 (0.28-0.68)	0.67 (0.44-0.88)
PPV	0.72 (0.62-0.86)	0.67 (0.6-0.77)	0.68 (0.56-0.87)
NPV	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)
LR+	3.0 (1.63-5.49)	1.92 (1.32- 2.8)	3.0 (1.56-5.76)
LR-	0 (0-0)	0 (0-0)	0 (0-0)
AUC	0.915 (0.832-0.999)	0.824 (0.712-0.935)	0.936 (0.853-1.0)

Values are presented as median (95% CI). FCal = IBDoc[®] faecal calprotectin (measured in µg/g of faeces) PPV = positive predictive value, NPV = negative predictive value, LR+ = positive diagnostic likelihood ratio, LR- = negative diagnostic likelihood ratio, AUC = area under curve

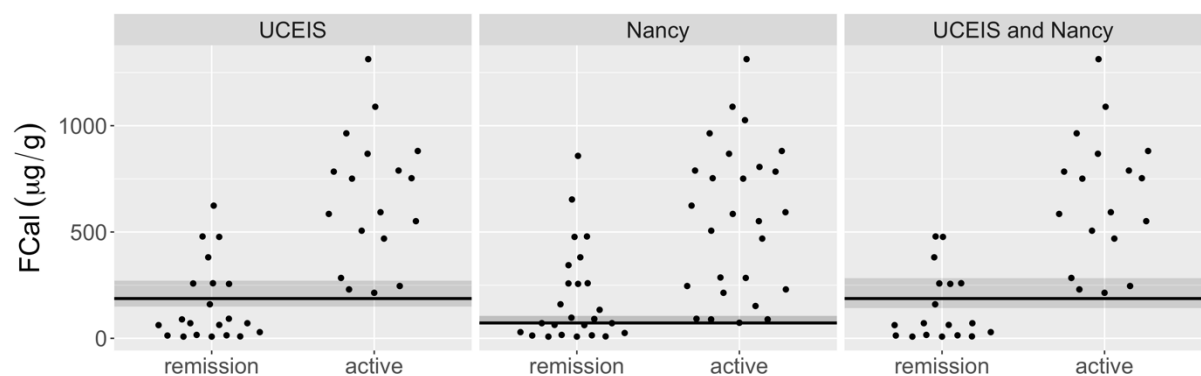
Figure 1. Receiver operator characteristic curves of the indices and the FCal thresholds for detecting active disease



UCEIS: active disease ≥ 4 ; Nancy: active disease ≥ 2 ; Combined criteria: active disease UCEIS ≥ 4 and Nancy ≥ 2 .

Distributions of FCal levels within remission and active disease groups for the UCEIS and Nancy indices are shown in Figure 2, with the threshold denoted by the solid line with corresponding 95% confidence intervals. The thresholds were optimised to avoid misclassifying a patient with active disease into the remission group, by minimising both the number of false negatives and false positives, while keeping the relative cost of false negatives higher.

Figure 2. Distribution of FCal levels within remission and active disease groups with corresponding thresholds and 95% confidence intervals.



UCEIS: remission ≤ 1 , active ≥ 4 ; Nancy: remission ≤ 1 , active ≥ 2 ; Combined criteria: remission UCEIS ≤ 1 and Nancy ≤ 1 , active UCEIS ≥ 4 and Nancy ≥ 2 .

While the remission and active disease phase were defined in Table 1 and used throughout the current analysis, two endoscopic thresholds (UCEIS of 2 and 3), separating the remission and the active disease states, were additionally explored. Setting the endoscopic threshold for the active disease phase as UCEIS ≥ 2 or ≥ 3 and keeping other variables exactly as presented in Table 1, the FCal threshold was 21 $\mu\text{g/g}$. Conversely, an increase of the UCEIS score above 4 (that is, UCEIS ≥ 5 , ≥ 6) for the lowest endoscopic threshold for active disease, did not affect the FCal threshold which remained at 187 $\mu\text{g/g}$, however PPV decreased to 0.61 and 0.46 for UCEIS ≥ 5 and ≥ 6 respectively. Once the threshold was placed at UCEIS ≥ 7 , the FCal threshold increased to 532 $\mu\text{g/g}$ (PPV 0.67, NPV 1.0).

Discussion

Surrogate markers of remission that help avoid follow-up endoscopies will facilitate the management of ulcerative colitis to benefit individual patients and healthcare utilisation. This is the first prospective study to use validated endoscopic and histologic indices to determine the thresholds of FCal that have the greatest sensitivity for detecting active disease.

There was weak correlation between FCal and symptoms ($r=0.27$) whereas the correlation between FCal and endoscopic activity ($r=0.741$) or histopathology ($r=0.876$) was much stronger. This is biologically plausible given that FCal is a largely neutrophil and macrophage-derived protein and microscopic disease activity in UC is characterised by the presence of neutrophils, including crypt abscesses (18). This is also consistent with a previous retrospective analysis reported a similarly strong correlation between the UCEIS and FCal (r values between 0.60 and 0.70) (22).

For our study, sensitivity was maximised to 1.0, to avoid mislabelling patients with active disease as being in remission. This was considered clinically appropriate, since the consequence of decisions based on the detection of active disease were considered greater than those of disease in remission. The FCal threshold for detecting endoscopic activity (defined as UCEIS ≥ 4) was 187 $\mu\text{g/g}$ which is lower than the 250 $\mu\text{g/g}$ threshold suggested in 2012 (20), but consistent with thresholds reported from Denmark and Asia (22, 23). A lower threshold is less likely to misclassify patients with active disease as being in remission, as illustrated in Figure 2.

The initial reason that a UCEIS ≥ 4 was chosen to define active endoscopic disease was that this was considered a point at which therapy is often escalated, although this threshold has

not been validated. When UCEIS ≥ 2 and ≥ 3 were included as active disease, this resulted in thresholds as low as 21 $\mu\text{g/g}$ which would have no utility in clinical practice. Interestingly when UCEIS ≥ 4 , ≥ 5 and ≥ 6 were examined, all resulted in the same FCal threshold of 187 $\mu\text{g/g}$, justifying UCEIS ≥ 4 as an appropriate cut-off.

FCal thresholds for detection of histological disease activity can be expected to be more stringent than those for endoscopic activity and indeed the Fcal threshold cut-off for histologic activity was $>72 \mu\text{g/g}$. Combining endoscopic and histological indices did not provide any advantage to considering endoscopic criteria alone, because in all events where the UCEIS was ≥ 4 , the Nancy index was also ≥ 2 . We have previously shown that the correlation between the Nancy index and the Robarts Histological Index is very strong ($r=0.86$) [Correlation between endoscopic and histological activity in ulcerative colitis using validated indices. Irani N, Wang LM, Collins GS, Keshav S, Travis SPL. *J Crohns Colitis* 2018 [ePub Jun 11](#)] and others have shown in a retrospective study that the Geboes histological index performs in a similar fashion [Magro et al Gut 2018].

There is currently no international agreement on the definitions of remission or active disease in ulcerative colitis. The definition of remission in this study was a score of ≤ 1 for both the UCEIS and Nancy indices. These thresholds were selected because it was considered unlikely that medical management would be escalated if either the UCEIS or Nancy index were ≤ 1 , assuming that these objective criteria are used for making treatment decisions, rather than symptoms alone.

Limitations of this study include that the endoscopist was not blinded to clinical information, since current symptoms were likely discussed at the time of endoscopy. However, it is known that scoring the UCEIS is unaffected by clinical information (44). No central reading was

performed, but the UCEIS accounts for 88% of interobserver variation in endoscopy (11) and the Nancy has good inter-reader reliability with an intraclass correlation coefficient of 0.86 (18). While numbers in this study are small and too few to compare UCEIS = 0 with UCEIS = 1, the prospective design and use of validated indices are respective strengths.

FCal is a useful surrogate marker for detecting endoscopic or histological remission or disease activity in ulcerative colitis. The threshold based on ROC analysis suggests that a FCal level $<187 \mu\text{g/g}$ is not associated with active endoscopic disease (UCEIS ≥ 4) and that an FCal $<72 \mu\text{g/g}$ is not associated with histological inflammation (Nancy ≥ 2). Importantly for clinical practice, neither level would misclassify patients with active disease as being in remission. Using these thresholds in clinical practice may help to avoid endoscopic procedures for those patients with FCal levels $<187 \mu\text{g/g}$.

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