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# PICaSSO Histologic Remission Index (PHRI) in Ulcerative Colitis – Development of a Novel Simplified Histological Score for Monitoring Mucosal Healing and Predicting Clinical Outcomes and its Applicability in an Artificial Intelligence System

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APB: Data acquisition, Analysis and interpretation of data, Critical revision of manuscript for important intellectual content
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The study was approved by West Midlands Research Ethics Committee (17/WM/0223). All patients gave informed consent to participate in the study.

## ABSTRACT

Histological remission is evolving as an important treatment target in ulcerative colitis (UC). We aimed to develop a simple histologic index, aligned to endoscopy, correlated with clinical outcomes, and suited to apply to an artificial intelligence (AI) system to evaluate inflammatory activity.

### Methods

Utilizing a set of 614 biopsies from 307 UC patients enrolled into a prospective multicentre study, we developed the PICaSSO histologic remission index (PHRI). Agreement with multiple other histologic indices and validation for inter-reader reproducibility were assessed. Finally, to implement PHRI into a computer-aided-diagnosis (CAD) system, we trained and tested a novel deep learning strategy based on a convolutional neural network architecture to detect neutrophils, calculate PHRI and identify active from quiescent UC using a subset of 138 biopsies.

### Results

PHRI is strongly correlated with endoscopic scores (MES, UCEIS, and PICaSSO) and with clinical outcomes (hospitalisation, colectomy, and initiation or changes in medical therapy due to UC flare-up). A PHRI score of 1 could accurately stratify patients' risk of adverse outcomes (hospitalisation, colectomy, and treatment optimisation due to flare-up) within 12 months. Our inter-reader agreement was high. (ICC 0.84). Our preliminary AI algorithm differentiated active from quiescent UC with 78% sensitivity, 91.7% specificity and 86% accuracy.

### Conclusions

PHRI is a simple histological index in UC, and it exhibits the highest correlation with endoscopic activity and clinical outcomes. A PHRI-based AI system was accurate in predicting histologic remission.

**Key words**:

Inflammatory bowel disease (IBD); ulcerative colitis (UC); endoscopic remission (ER); mucosal healing (MH); histologic remission (HR); histologic healing (HH); histological index; artificial intelligence (AI); machine learning; computer aided diagnosis

**Significance of this study (TBD)**

We developed a new simple histologic index for UC, PICaSSO Histogic Remission Index (PHRI), which could be successfully implemented into an artificial intelligence model to detect histological remission.

**What is already known on this topic?**

Histological activity in UC is associated with poor outcomes and histological remission has been proposed as a treatment target in UC. Multiple histological indices have been developed to define disease activity, however they have not been widely adopted in clinical practice due to their complexity. Machine learning models are powerful tools that can complement and support pathologists in their histopathological evaluation.

**What are the new findings?**

PHRI is a new score based simply on the presence or absence of neutrophils (Yes/No) and it provides excellent diagnostic accuracy, the strongest correlation to endoscopic activity among several histologic scores, minimal inter-rater variability, and excellent prediction of long-term clinical outcome. An AI algorithm based on PHRI was able to accurately determine histologic remission.

**How might it impact on clinical practice in the foreseeable future?**

PHRI can help standardise histological assessment of UC in a most practical and easy way. A machine learning model based on PHRI can further facilitate the histologic reading and improve diagnostic performance.

## INTRODUCTION

Histological assessment plays a critical role in determining inflammatory activity and monitoring treatment response in ulcerative colitis (UC). Histologic remission (HR) (also referred as histologic healing, HH) is an emerging treatment target and is an important outcome in UC clinical trials due to its association with favourable outcomes [1–10]. However, challenges remain on how to incorporate histology into clinical practice mainly due to: (1) the lack of a universal definition of HR to guide pathologists, and (2) the lack of a sensitive, easily applicable histologic score/index. Ideally, this index would be: (a) informative of and correlated with endoscopic assessment of disease activity, (b) representative of recovery/healing status of damaged mucosa, and (c) predictive of disease outcomes.

The histopathologic characteristics of UC are those of a chronic active colitis with a relapsing and remitting course, and consist of three fundamental components: (1) active inflammation ('*activity*'), which is the neutrophil infiltration in cryptal epithelium as well as in the lamina propria; (2) chronic inflammation, characterized by expansion of mononuclear cell (lymphocyte and plasma cell) infiltrates in the lamina propria, often accompanied by basal plasmacytosis and eosinophilia; and (3) cryptal architecture/structure distortion ('*chronicity*'), characterized by irregularity and variation of crypts in size, shape, orientation, and inter-cryptal distance; which is the result of mixed repetitive injury and regeneration of crypts.

Over the past decades more than 30 histological scores have been developed, although their adoption in clinical practice remains modest [11,12]. Similarly, different definitions and criteria of HR have been proposed, ranging from 'elimination of mucosal ulceration/erosion' to 'complete histological normalization' [1,3,13–18]. Almost all investigators now agree that the absence of neutrophilic

infiltration ('neutrophil-free' mucosa) is the key to a HR definition due to its association with favourable clinical outcomes [2,4,5,19–22]. Indeed, two independent international expert panels, recently recommended to define HR as the absence of neutrophil infiltration (*i.e*, elimination of histological activity) [22,23].

With the advent of digital pathology, artificial intelligence (AI) algorithms are increasingly employed into histopathologic evaluation and diagnosis, as seen in many imaging-focused fields in medicine. For example, it is being widely introduced in oncopathology using convolutional neural network (CNN) based learning [24]. But, thus far, to the best of our knowledge, no computer aided diagnosis (CAD) system has been developed to perform histologic scoring and assess HR in UC. Part of the reason is that the complexity and mixed subjectivity of the existing histologic scores makes it difficult to build and train deep learning algorithm, supervised and unsupervised.

Recently, we conducted a prospective international multicentre study to develop the PICaSSO (**P**addington **I**nternational virtual **C**hromoendo**S**copy **S**cOre) endoscopic score [25–27], a new tool for assessing endoscopic activity and remission in patients with UC by using high-definition virtual electronic chromoendoscopy (HD-VCE). The PICaSSO endoscopic score had better correlation than Mayo Endoscopic Score (MES) and UC Endoscopic Index of Severity (UCEIS) with multiple histological scores [27]. The current study is distinct, and a step further, from all our previous published studies on PICaSSO endoscopic score, as it focuses on creating a new *UC histological score* that can be used quickly and easily by histopathologists in clinical practice, as well as in trials, and can be incorporated into an artificial intelligence (AI) algorithm. Using the PICaSSO project as a platform, in the present study we meticulously analysed the mucosal biopsies taken from the same

colonic areas assessed endoscopically, with a focus on identifying the specific histopathologic component(s) associated with histologic-endoscopic correlation and with the risk of adverse clinical outcomes. Ultimately, we aimed to develop a simplified and novel histological score that could accurately reflect microscopic mucosal inflammation and healing, predict clinical outcome, respond to therapy, and be readily implementable into a machine learning algorithm and thus easily adopted into clinical practice and trials. Creating a simplified histologic score, PHRI, that is an objective histologic instrument was the main aim, as current use of histologic scores in clinical practice is limited. The primary aim of PHRI was to create a simple "neutrophil-only" histologic evaluation that predicted specified clinical outcomes. An additional purpose was that an ideal histologic index should go beyond the limit of endoscopic evaluation.

## PATIENTS AND METHODS

### Study Population, Endoscopic Evaluation and Clinical Follow-Up

A total of 307 UC patients were prospectively enrolled from 11 centres in Europe and North America into the international multicentre PICaSSO study. Ethics approval was obtained from West Midlands Research Ethics Committee (17/WM/0223) and institutional ethics committees of all centres. All patients gave informed consent to participate in the study. The protocol for endoscopic and histological evaluation have been described in details in a previous publication [27]. Briefly, each patient underwent white light HD colonoscopy to determine MES and UCEIS [28,29], followed by VCE (iSCAN, Pentax, Japan) to determine PICaSSO score, which is comprised of mucosal and vascular subscores (PMS and PVS) [25,27]. In the same areas of rectum and sigmoid assessed and video recorded on endoscopy, at least 2 targeted mucosal biopsies were taken resulting in a total of

614 biopsies for histopathological analysis. Targeted biopsies were taken from the most inflamed area or showing the most representative features of endoscopic remission determined by PICaSSO [26]. All patients were then followed up for at least 12 months with regular clinic visits to document the following prespecified adverse clinical outcomes: (1) hospitalization as a result of UC relapse, (2) colectomy, and (3) initiation or changes in medical therapy for UC flare-up including steroids, immunosuppressants and biologics (after excluding adverse effects, immunogenicity or low drug levels).

**Phase I – Deep Histological Analyses and Histologic-Endoscopic-Clinical Outcome Correlations**

The hematoxylin-eosin (H&E) stained glass slides of colorectal biopsies were scanned at 40× (0.25 microns per pixel) using Aperio Digital Pathology Scanning system (Leica Biosystem, IL, USA). The high-definition digitised slides were centrally hosted and read by a group of 6 gastrointestinal pathologists (XG, MV, VV, DZ, GDH, ESR) experienced in IBD who were blinded to the endoscopic data. For each biopsy from each segment, the worst features were scored applying five different histological scoring schemes - Geboes Score (GS) [30], Robarts Histological Index (RHI) [31], Nancy Histological Index (NHI)[32], ECAP (Extent, Chronicity, Activity, and Plus) Score [33,34], and Villanacci Simplified Score (VSS)[35]. The average values of each score and subscore for both rectum and sigmoid were also separately analysed.

The endoscopic-histologic correlation was analysed in multiple steps in order to identify the histopathologic features/components that specifically corresponded to the different endoscopic features/patterns of disease activity and remission, as well as predicted the risk of specified clinical outcomes at follow-up.

**Phase II - Development and Assessment of PICaSSO Histologic Remission Index (PHRI)**

Based on a solid conclusion from our multistep and comprehensive histologic-endoscopic-clinical outcome correlation analyses in the phase I study and following a modified Delphi roundtable discussion between the expert pathologists, the presence of *neutrophil infiltration* was identified as the key element in UC histopathology that determines the disease activity, mucosal healing and clinical outcome. Subsequently a novel simplified histological scoring scheme, PICaSSO Histologic Remission Index (PHRI), was proposed, as detailed in Table 1. This index takes into account solely the neutrophil infiltration in both epithelium and lamina propria, as illustrated in Supplementary Figure 1. Ulcer and erosion were not included because the histologic features of ulcers and/or erosions may not always been apparent in the biopsies due to sampling variation. We standardized particularly the criteria of '*cryptitis*' (any number of neutrophils infiltrating the epithelium of any number of crypt/gland) and '*crypt abscess*' (cryptitis with any number of neutrophils overflowing into cryptal lumina and any degree of cryptal epithelial cell injury), given that a clear and standardized histological criteria of cryptitis and crypt abscess are still lacking. The pathologists also completed a standardized training module representative of several histological pictures displaying all the histological features before scoring the slides.

The new PHRI was then used to re-analyse the aforementioned histologic-endoscopic correlations, to compare it with the other five histological indices, and its prediction of clinical outcome at 12 months follow-up. We also explored the additional prognostic benefit of PHRI in further stratifying the risk of disease relapse in patients who were already in endoscopic remission (ER) defined by MES of 0. The PHRI scores of rectum and sigmoid were considered individually as well as combined in the total

score (*PHRI_total, i.e.*, the sum of PHRI scores of both rectum and sigmoid) or the maximum score (*PHRI_max, i.e.,* the higher score between rectum and sigmoid). The latter, PHRI_max, was chosen. If not otherwise specified, the term PHRI refers to the highest score in the examined areas, PHRI_max.

**Phase III - Validation of PHRI**

To validate PHRI, the same pathologists assessed 50 digital slides (about half quiescent and half active UC) and scored PHRI and the other five selected indexes. The validation cases were randomly selected and relabelled by a non-pathologist investigator from the same study group. The pathologists were blinded to clinical and endoscopic information and performed the histological scoring independently.

**Phase IV- Development of AI Algorithm**

In this exploratory study we included 138 biopsies, randomly selected from the study collection, that were representative of different grades of inflammation from the 614 collected in the whole study. We developed a Convolutional Neural Network (CNN) classifier to detect the neutrophils in whole slide images (WSIs) and classify them into either histological remission or non-remission based on the presence of neutrophils. The detailed design of the convolutional neural network is reported in the AI supplementary appendix. Briefly, a first model identified patches (areas of the WSI) containing neutrophils, while a second model, using a multiple instance learning approach, combined the features of each patch in the slide into a final dichotomous result (presence or absence of active disease) following the PHRI. (Figure 1)

**Statistics**

Statistical Software R (R Core Team, https://www.R-project.org/) was used. The strength of the correlation of continuous and categorical variables was measured with Spearman's (ρ) correlation coefficient. Coefficients of 0.8-1.0 were considered as 'very strong', 0.6-0.79 as 'strong', 0.4-0.59 as 'moderate', and 0.2-0.39 as 'weak'. Spearman correlations were compared by drawing 100 bootstrap samples for each pair of variables and computing the corresponding quantiles. Wilcoxon and Fishers exact tests were used to determine the differences between continuous and binary distributions respectively. For AUROC analysis we used R-package pROC (https://CRAN.R-project.org/package=pROC). Predictive modelling was performed by R-package CARRoT (https://CRAN.R-project.org/package=CARRoT). Details are reported in the statistical supplementary appendix.

The Cox proportional hazard model was used to calculate the probability of survival without specified clinical outcomes for different cut-offs of PHRI. The difference between groups of patients was assessed by hazard ratio test and survival analysis implemented via R-package survival (https://CRAN.R-project.org/package=survival).

To assess the inter-rater agreement of the histological scorings, we used one-way intra-class correlation (ICC) coefficient by means of R package irr (http://cran.r-project.org/package=irr). In order to test the hypothesis of the ICC being larger than 0.5 against the alternative we needed a minimum of 40 histology images to reach the power of 0.8 with a type I error of 0.05 [36]. According to Landis and Koch benchmarks [37], ICC of <0.2, 0.2 to 0.4, >0.4 to ≤0.6, >0.6 to 0.8, and >0.8 was considered 'poor', 'fair', 'moderate', 'good', 'substantial', and 'almost perfect', respectively. Results

of all statistical tests were considered significant at $p < 0.05$. Statistical power was computed in the PICaSSO endoscopic and histologic study recently published [27] based on correlation of PICaSSO endoscopic score and histologic scores compared with standard MES and also on specified clinical outcome rates and a sample size of 302 was determined.

The diagnostic performance of the artificial intelligence CAD for the detection of active UC was reported as sensitivity (SN), specificity (SP), positive predictive value (PPV), negative predictive value (NPV) and accuracy (ACC).

## RESULTS

614 biopsies from 307 UC patients were analysed. 168 (54.7%) patients were in ER as defined by MES 0, while the others had endoscopically active disease at the time of study. None of the patients were on topical therapy or had Montreal E1 disease. 270 (88%) patients completed follow-up for 12 months. The detailed demographic data of the study subjects are shown in Table 2.

### Phase I. Neutrophils as the key determinant in histologic-endoscopic-clinical correlation

All five histological indices (VSS, RHI, NHI, GS and ECAP) correlated strongly with all of the endoscopic scores in the same regions of bowel (rectum and sigmoid colon) (Spearman's $\rho = 0.55$ to 0.78), as illustrated by the heatmaps (Figure 2). All histological indices also showed a weak to moderate correlation with the pre-specified adverse clinical outcomes at 12 months ($\rho = 0.34$ to 0.42) (Figure 2).

Looking further into the correlations between the various histopathologic components (Supplementary Table 1) and endoscopic scores (represented by the mucosal and vascular subscores

of PICaSSO score), the neutrophil infiltration in the lamina propria and in epithelium, especially that in lamina propria and the combination of both, generally showed the strongest correlation ($\rho = 0.60$ to 0.76), as compared to the other histologic features that also showed correlation to some degree (moderate to strong, $\rho = 0.43$ to 0.64) ($p < 0.05$) (Figure 3). Similarly, neutrophil infiltration also showed a stronger correlation, although overall weak/moderate ($\rho = 0.40$ to 0.45), with clinical outcomes at 12 months compared to other histologic features ($\rho = 0.24$ to 0.37) ($p < 0.05$) (Figure 3).

**Phase II. PHRI for Histologic-Endoscopic and Clinical Outcome Correlations**

***PHRI correlated best with endoscopic disease activity***

PHRI correlated strongly with the endoscopic scores, and the strength of its correlation was the best among all the histologic indices ($p < 0.05$) (Figure 2).

***Correlation of PHRI with specified clinical outcomes and relapse risk***

For the entire cohort, the PHRI all showed a similar moderate correlation with the specified adverse clinical outcomes at 12 months ($\rho$ values around 0.4). Additionally, the average PHRI scores were significantly higher in those who had specified adverse clinical outcomes at 12 months than in those with no events (Supplementary Table 2, Supplementary Figure 4).

Furthermore, we performed a multivariable logistic regressions to explore whether other histologic features (chronic inflammation, basal plasmacytosis, and eosinophilia) could improve PHRI prediction of specified clinical outcomes. We found that the addition of none of these histological features further improved PHRI prognostic outcome ability (Figure 4).

Patients with PHRI > 0 compared with those with PHRI = 0 had significantly more negative clinical events (outcomes) at 12 months (48.65% (54/111) vs 13.91% (21/151), $p < 0.00001$), as shown in Supplementary Figure 4-C. In addition, analysis by Receiver Operating Characteristic (ROC) curve, as shown in Supplementary Table 3-A, the best cut-off values of PHRI for predicting the specified clinical outcomes at 12 months in the entire cohort was 1 (≤1 versus >1).

**Cox proportional hazards curves of PHRI in predicting specified clinical outcome**

We then further analysed with the Cox proportional hazards curves by using value 0 or 1 as the cut-off score of PHRI (or individual PHRI of rectum or sigmoid), the patients' event rates of specified clinical outcomes during 12 months follow-up were significantly stratified, as shown in Figure 5. The predicative power of PHRI in any form were almost the same.

**Subgroup analysis of patients with only endoscopic remission**

When we singled out the patients who were in endoscopic remission (ER) as defined by MES 0, the histologic-endoscopic-clinical outcome correlations became weak in all aspects. In the Phase I of the study, for this particular subpopulation of patients, of whom only a few had residual mild neutrophil infiltration in colorectal biopsies (5.7% with neutrophils in lamina propria, and 5.4% in epithelium), the correlations between histological and endoscopic scores (represented by PICaSSO mucosal score and PICaSSO vascular score) ($\rho < 0.30$) and between histological scores and specified clinical outcome (indicative of relapse in this particular patient population) both became weak or near zero ($\rho = 0$ to 0.12) (Supplementary Figure 2). Nevertheless, neutrophil infiltration was the single histologic feature that remained correlated, though weakly (slightly over 0.1) (Supplementary Figure 3).

In the phase II of the study, in patients in ER (MES 0), of which only 10.9% had PHRI > 0 (presence of neutrophilic infiltration) and 89.1% had PHRI of 0 (no neutrophilic infiltration), the correlation between PHRI and endoscopic scores also turned to be much weaker ($\rho$ = 0.24 to 0.36) (Supplementary Table 4). However, PHRI still appeared generally superior to most of the other histologic indices ($p < 0.05$), as represented by their correlation with PICaSSO score and its mucosal and vascular subscores (Supplementary Figure 2). Moreover, the correlation between PHRI scores and prespecified clinical outcomes was also very weak, but still performed better than the other histological scores ($p < 0.05$), (Supplementary Figure 2 and Supplementary Table 4). Consistent with this, patients with PHRI > 0 seemed to have a higher disease relapse rate at 12 months, as compared to those with PHRI 0 (11.76% (2/17) vs 9.3% (12/129)), although the differences did not reach statistical significance ($p > 0.05$) (Supplementary Figure 4-D). Lastly, the best cut-off value of PHRI for predicting the relapse at 12-months in patients in ER seemed to be 1 (≤1 versus >1), although further analysis with Cox proportional hazards curves failed to satisfactorily stratify the patients' relapse risk (Figure 5C).

**Phase III. Validity and reliability of PHRI**

The inter-rater agreement among pathologists on all of the histological scores was excellent, as reflected by ICCs: RHI 0.77 (95% CI 0.69-0.85), NHI 0.85 (95% CI 0.79-0.90), GS 0.82 (95% CI 0.75-0.88), ECAP 0.87 (95% CI 0.82-0.92), VSS 0.77 (95% CI 0.71-0.86), and PHRI 0.84 (95% CI 0.78-0.90). The differences between the ICCs of each index were not statistically significant. Overall, inter-observer agreement for PHRI was almost perfect, although not necessarily significantly superior to the other histological indices. The breakdown of ICC on each of the histologic components of

different histological indices were also analysed. For any given histological score, we had the best agreement on assessment for the neutrophil-related parameters, as shown in Supplementary Table 5.

**Phase IV. Convolutional Neural Network (CNN) classifier able to detect neutrophils**

We divided our cohort in two sets, training and testing, with similar patient characteristics to avoid overfitting our system and ensuring its generalisability. 70% of the biopsies were used to train the model and 30% to test it. To train the proposed models and optimize the hyperparameters involved, 15% of the training set was used as validation. In the testing set, our CAD to detect neutrophils had SE 0.71, SP 0.95, PPV 0.85, NPV 0.89, and accuracy 0.88, these results were in line with those of the validation cohort, see Table 3. Figure 6 shows the class activation maps (CAMs) to highlight the regions of interest at patch-level in which the proposed model paid attention to predict the samples. The highlighted regions match with the areas containing neutrophils. For the histological remission prediction, the diagnostic performance, expressed as the same characteristics, was 0.78, 0.92, 0.88, 0.85, and 0.86, respectively. See Table 3.

## DISCUSSION

We developed a novel and simpler histologic remission index for UC, the PHRI, that correlates well with endoscopic disease activity and with clinical outcomes and it can be easily implemented into a CNN model. The development process of this histological index differs from that of existing scores. PHRI was the result of a joint collaboration between pathologists and endoscopists aiming to develop a histologic score aligned to the endoscopic score and goes beyond endoscopic evaluation [27]. Our work has several strengths. Firstly, the histological study was part of a large international multicentre prospective study with the precise focus on endoscopic-histological-clinical correlation. We included

a large number of matching biopsies taken immediately after and exactly from the same areas where endoscopic assessment was performed, rather than limiting the comparison to a patient-level. Second, instead of including multiple diagnostic features as in other histological indices, we limited the PHRI to one parameter only, *neutrophil infiltration* (active inflammation), the single factor identified by multiple comparative analyses, to be most relevant to both endoscopic features and clinical outcomes. Our independent finding echoes Pai's study [21] and is consistent with a gathering consensus on the importance of neutrophils in the definition of disease activity and histologic remission.

The most notable advantage of PHRI is its simplicity. PHRI requires only identifying the presence or absence of infiltrating neutrophils within the lamina propria and glandular epithelium, in a straightforward dichotomous way of 'Yes or No' (present or absent). It also avoids the usual activity grading (*e.g*., mild, moderate, and severe) by arbitrary visual scale or estimate of percentage values, which is somewhat subjective. As found by other investigators and shown by our own inter-rater agreement data, the assessment for neutrophils has always been the most reproducible characteristic [38,39] Presence of ulceration/erosion, often included in other indexes, was eliminated from PHRI as we considered it a potential source of variability with little contribution to the score's accuracy. Indeed erosions/ulcers might not be visible on biopsy histology [22], the distinction between the two is not always possible and, more importantly, patients with erosions/ulcers inevitably have more extensive neutrophilic infiltration anyway. Adopting this simplified '*neutrophil-only*' approach, we expect that the histological readings would be maximally objective and reproducible. The addition of other histologic components that also had some degree of impact on endoscopic features and/or clinical outcomes did not add significant benefit from a practical point of view and would have instead complicated the development of the artificial intelligence algorithm. We feel that compared to the

other currently available histological scores, PHRI is the easiest to apply in daily practice as a universal histological indicator and quantitative measurement (grading tool) of disease activity in UC. To promote its adoption in clinical practice and AI development we are also proposing a histopathological reporting template (Table 1).

Another advantage is that of PHRI makes it easier to perform histological scoring on multiple biopsies from different segments of colon in patients with extensive colitis, to achieve an entire assessment and generate a global (total, maximum, or average) score per colon. This approach would appreciate the globality and increase the overall accuracy of the histological assessment.

In our analysis, we found that the PHRI scores of rectum and sigmoid were similar in terms of their correlation with endoscopy and prediction of clinical outcome. In addition, the highest score and the total score of PHRI (PHRI_max and PHRI_total) had the same value of application and significance. Therefore, it is our preference to set the global score as the highest/worst score among all biopsies (PHRI_max) or simply the score of the histologically worst biopsy only, considering that the total number of biopsies being taken and the extent of disease vary in different patients and different clinicians. Finally, the successful development of a computer aided UC histologic diagnosis and scoring system based on PHRI, to the best of our knowledge the first in the field of IBD, supports the notion that a simplified score is readily implementable into an AI model. This may complement rapidly advancing development of AI systems for endoscopic scoring of UC, including prediction of histology from endoscopic scores by a number of authors including us [40–48]. Although preliminary, these findings are particularly promising in light of the rapid integration of CAD systems into clinical

practice. The potential benefits of this change are extraordinary, but their discussion exceeds the objectives of our study.

Admittedly, our work has a few limitations. First, our patients' follow-up protocol did not include the endoscopic and histological reassessments at 12 months (not standard of care in all centres), and second it only lasted 12 months, whereas some clinical outcomes might be observed even after 36 months.[49] Third, we did not follow-up patients using patient-reported outcomes similar to other studies [1, 15] as symptoms do not relate well to histology or endoscopy. Fourth, we have not yet tested sufficiently the global score of PHRI throughout different regions of the entire colon in patients with pan-colitis, although we did include two sites (rectum and sigmoid) in the present study which recruited a diverse cohort of patients. We recruited patients as part of standard of care and this included flexible sigmoidoscopy or colonoscopy. External validation in large cohort in the context of specific clinical trials is necessary and will be conducted as the next step. Lastly, some histological interpretations were challenging and required further discussion (details in histological appendix). An unsolved issue remains the suboptimal performance of all the current histological indices for patients who have reached ER where residual neutrophils are lacking or scarce. In this particular patient population histological indices, including our new PHRI, lose correlation with either endoscopic scores or with relapse rate. The predictive power of PHRI in assessing the relapse risk in these patients was also limited, although a biopsy finding of PHRI >0 in patients who are otherwise in ER would still be of interest. By using a different histological scoring, Narula et al also failed to show the significance of the impact of histologic activity on the relapse in this subpopulation of patients [50]. The reasons for this shortfall may be several. First, the small number of cases with histologic but not endoscopic activity (only 10% had PHRI >0 in our patients) underpowered the analysis. Second, the

heterogeneous distribution of residual inflammation in treated UC might have generated an underestimation of disease activity. Third, the recurrence of UC is not simply arising from the minimal residual inflammation but is the result of the reactivation of dysregulated mucosal immunologic mechanisms.

Nonetheless, in our opinion, as compared to any of the other histological indices, PHRI is the simplest one, while it is also most objective and sensitive. Since a pathologist needs only to identify neutrophils, which is a part of routine in reading biopsy slides as clinical histopathological evaluation, one can have the PHRI score immediately without making additional effort and spending extra time. Thus, the PHRI score can also be easily included into the pathology reports, which would be something that infrequently happen at present. Therefore, we believe that PHRI can be applied efficiently in clinical practice.

In conclusion, PHRI is a simple and reproducible histological index that correlates strongly with endoscopic activity and predicts clinical outcomes in UC. It is therefore ideally suited for adoption in clinical practice as well as for consideration in clinical trials and central readouts if further validated to fulfil requirements of US Food and Drugs Administration (FDA) or European Medicines Agency (EMA) requirements. We suggest using a PHRI cutoff of 0 to define HR, and a cutoff of 1 to stratify low vs high risk of adverse outcomes. The dichotomous nature of PHRI (*i.e.*, presence or absence of neutrophils) allowed the development of machine learning algorithm with high diagnostic accuracy for detection of the disease activity and HR in UC. Further studies are ongoing to validate the deep learning based computer-aided classifier before it can be adopted in clinical practice.

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**Table 1. PICaSSO Histologic Remission Index (PHRI)**

| Histologic Finding | Score |
|---|---|
| **Neutrophil infiltration in lamina propria** | |
| Absent (No) | 0 |
| Present (Yes) | 1 |
| **Neutrophil infiltration in epithelium** | |
| Absent (No) | 0 |
| Present (Yes) | |
| - Surface epithelium[#] | 1 |
| - Cryptal epithelium (cryptitis) | 1 |
| - Crypt abscess | 1 |
| **Total Score** = sum of all above (maximum 4)* | |

**Criteria** for the scoring histologic components:

1) "*Neutrophil infiltration"* (in either lamina propria or epithelium): Any number (even only one) of neutrophil(s) is acceptable. (Evaluation under high power view at 40x is required if "absence of neutrophil" is determined. Neutrophil in the lamina propria must be outside of capillary lumina.)
2) *"Crypt abscess"*: cryptitis with any number of neutrophils or any amount of neutrophilic exudate overflowing into cryptal lumen AND any degree of cryptal epithelial cell injury.

[#] If a biopsy has no intact surface epithelium but shows features of erosion/ulceration ( *e.g.*, granulation tissue, or/and inflammatory exudates), also score 1.

*When there are multiple biopsies from different segments of bowel, the maximum/highest/worst score (PHRI_max) among all biopsy sites will be the preferred '*global score'*.

**Table 2. Demographic Data of Study Subjects**

| Characteristics | Number of Patients, mean ± sd, or n (%) |
|---|---|
| Total patients | 307 |
| Age (y) | 48.4 ± 14.8 |
| Gender | |
| Male | 182 (59.3%) |
| Female | 125 (40.7%) |
| Extent of disease | |
| Proctitis | |
| Left-sided | 130 (42.3%) |
| Subtotal or total | 172 (56.0%) |
| Unknown | 5 (1.6%) |
| Disease duration (y) | 15 ± 10.8 |
| Endoscopic activity | |
| Mayo endoscopic score (MES) | |
| MES 0 (Remission) | 168 (54.7%) |
| MES 1 | 47 (15.3%) |
| MES 2 | 56 (18.2%) |
| MES 3 | 31 (10.1%) |
| Missing data* | 5 (1.6%) |
| UCEIS - Rectum | |
| Remission (1) | 209 (68.1%) |
| Mild (2-4) | 62 (20.2%) |
| Moderate (5-7) | 33 (10.7%) |
| Severe (>7) | 1 (0.3%) |
| Missing data* | 2 (0.7%) |
| PICaSSO score - Rectum | |
| Remission (≤3)[#] | 220 (71.7%) |
| Active (>3)[#] | 85 (27.7%) |
| Missing data* | 2 (0.6%) |
| Medical treatments in last 12 months | |
| No treatment | 14 (4.6%) |
| 5-ASA | 234 (76.2%) |
| Corticosteroids | 74 (24.1%) |
| Immunomodulators | 68 (22.1%) |
| Biologics | 118 (38.4%) |

* missing data due to solid stool present precluding the endoscopic scoring of these segments. These patients were not included in the overall analysis; [#] please refer to our other publication (*Reference 26*)

**Table 3. Classification results reached during the validation and the test stage with the neutrophil identification model and the activity of ulcerative colitis prediction.**

| | Neutrophil infiltration | | UC activity | |
|---|---|---|---|---|
| | **Validation set** | **Test set** | **Validation set** | **Test set** |
| **Sensitivity (SE)** | 0.8136 | 0.8043 | 0.6700 | 0.7800 |
| **Specificity (SP)** | 0.9253 | 0.9429 | 0.9000 | 0.9167 |
| **PPV** | 0.8683 | 0.8810 | 0.8000 | 0.8750 |
| **NPV** | 0.9076 | 0.9017 | 0.8182 | 0.8462 |
| **F1-Score** | 0.8108 | 0.8409 | 0.7400 | 0.8235 |
| **Accuracy** | 0.8783 | 0.8952 | 0.8371 | 0.8600 |