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Longitudinal visceral adipose heterogeneity supports digital health stratification of infliximab response loss in Crohn disease

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Abstract

Secondary loss of response to infliximab is common in Crohn disease, but existing monitoring does not capture treatment-related changes in mesenteric adipose tissue. We developed a longitudinal computed tomography enterography-derived visceral adipose tissue heterogeneity score (VHS) and integrated its post-induction percentage change ($\% \Delta \text{VHS}$) with clinical disease behavior to stratify subsequent risk. In 158 patients with paired imaging, low $\% \Delta \text{VHS}$ was independently associated with secondary loss of response (odds ratio 13.4, 95% confidence interval 4.76-43.8). The combined model achieved areas under the receiver operating characteristic curve of 0.825 in the training cohort and 0.818 in the internal testing cohort. Prospective microbiome profiling and descriptive humanized fecal microbiota transplantation experiments provided exploratory evidence consistent with an adipose-gut-microbiome phenotype. These single-center findings support longitudinal imaging heterogeneity as a candidate post-induction digital biomarker but require external validation and prospective evaluation before clinical implementation.

Keywords: crohn disease; infliximab; secondary loss of response; computed tomography enterography; digital health; digital biomarker; longitudinal imaging; microbiome

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Longitudinal CTE-derived VAT heterogeneity stratifies subsequent infliximab loss-of-response risk after induction in Crohn disease. Exploratory microbiome profiling and hFMT observations are consistent with biological plausibility for an adipose-gut-microbiome axis but do not establish causality.

Introduction

Crohn's disease (CD) is a chronic inflammatory bowel disease characterized by recurrent transmural inflammation and a progressive clinical course[1-3]. Infliximab (IFX) has transformed the management of CD and can induce mucosal healing, but therapeutic response remains highly heterogeneous. Up to 40% of patients who initially respond to induction therapy subsequently develop secondary loss of response (SLR) within 1 year, which is associated with clinical relapse and a higher likelihood of surgery[4,5]. Current monitoring strategies rely mainly on systemic inflammatory biomarkers and invasive endoscopic assessment, yet these approaches may not capture localized pathophysiological changes that precede overt treatment failure[6,7]. Spatially resolved mucosal phenotypes have previously stratified anti-TNF induction outcomes, including membrane-TNF imaging, stromal oncostatin M, and multicellular GIMATS programs[47-49]; however, these studies addressed induction response or primary nonresponse rather than SLR. Accurate noninvasive markers for identifying patients at increased risk of subsequent SLR are therefore still needed.

Visceral adipose tissue (VAT) is increasingly recognized as an active component of CD pathophysiology rather than a passive energy reservoir. In CD, hypertrophied mesenteric fat, commonly termed creeping fat, extends around inflamed bowel segments and is a hallmark of transmural disease. Growing evidence indicates that creeping fat is closely linked to bowel wall remodeling, including muscular hypertrophy, fibrosis, and stricture formation, suggesting that it may contribute directly to disease progression rather than merely reflect inflammatory burden[8]. Mechanistically, creeping fat is now regarded as an immunologically active mesenteric compartment enriched with adipocytes, stromal cells, macrophages, and other immune cell populations that produce cytokines, adipokines, and profibrotic mediators[9]. Recent work further suggests that viable intestinal bacteria can translocate across a compromised mucosal barrier into adjacent mesenteric adipose tissue, where they may reshape the local inflammatory milieu and promote adipose expansion, immune activation, and fibrostenotic remodeling[10]. Previous computed tomography (CT)-based studies have mainly used static macroscopic descriptors such as VAT volume, area, and mean attenuation. However, such global measures are unlikely to capture the spatially heterogeneous and dynamically evolving microenvironment of mesenteric adipose tissue during biologic therapy[11-13].

Radiomics enables high-throughput extraction of quantitative imaging features for noninvasive phenotyping. Conventional radiomics usually adopts a whole-region strategy, averaging spatial variation across the region of interest and potentially obscuring localized inflammatory clustering and intrinsic tissue heterogeneity[14,15]. Topology-aware spatial heterogeneity analysis has been proposed to identify and cluster radiomic subregions, allowing more detailed mapping of complex tissue architecture and improved characterization of the local microenvironment[16,17]. Given this framework, together with the focal and structurally disorganized nature of VAT remodeling in CD, we hypothesized that spatially resolved quantification of VAT would provide greater pathophysiological insight than conventional global imaging measures[18-21]. We therefore developed a computed tomography enterography (CTE)-derived VAT heterogeneity score (VHS) to capture subregional

inflammatory variation. By quantifying longitudinal changes between pretreatment and post-induction imaging, we aimed to characterize treatment-related remodeling of VAT during IFX therapy.

Persistent VAT heterogeneity may be related to IFX responsiveness through an adipose-gut-microbiome axis. Creeping fat may arise partly from impaired barrier integrity and bacterial translocation[18,30]. We hypothesized that an unfavorable VHS trajectory marks persistent transmural inflammation and a perturbed mesenteric microenvironment. Dysbiosis with pathobiont expansion and commensal depletion could sustain immune activation and contribute to SLR, but VHS does not directly measure bacterial translocation or microbial function.

In this study, we developed and internally evaluated a longitudinal CTE-based VHS model for post-induction prediction of subsequent SLR in patients with CD receiving IFX. Because both VHS measurements preceded the prediction landmark, this is a post-induction landmark model rather than a pretreatment model. Microbiome and hFMT analyses were exploratory assessments of biological plausibility. We profiled fecal 16S rRNA in a prospective cohort stratified by $\% \Delta \text{VHS}$ ($n = 42$; 21 per group) and used hFMT to examine whether model-defined microbiome patterns accompanied qualitatively different anti-TNF α responses in recipient mice. These analyses were neither independent clinical validation nor proof of causality (Fig. 1).

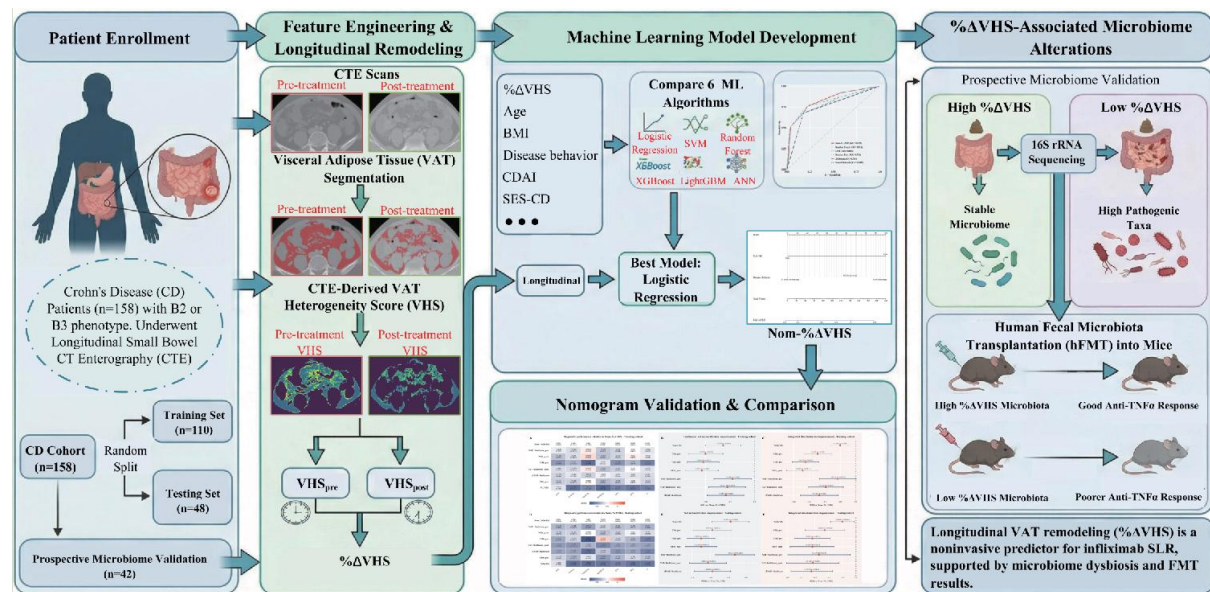


Fig. 1. Overall study design and analytical workflow. The workflow includes patient enrollment, longitudinal CTE-based VAT segmentation and VHS derivation, exploratory comparison of six machine-learning algorithms, development and internal evaluation of the Nom- $\% \Delta \text{VHS}$ model, and prospective microbiome and hFMT-based assessment of biological plausibility.

Materials and Methods

Study population and design

This retrospective study was approved by the Institutional Review Board of the Third Xiangya Hospital, Central South University, which waived the requirement for informed consent because of the retrospective design. We consecutively enrolled patients with CD who received IFX therapy at our center between January 2015 and August 2023. Diagnosis was established according to the European Crohn's and Colitis Organisation guidelines. The IFX regimen comprised an induction phase of 5 to 10 mg/kg administered intravenously at weeks 0, 2, and 6, followed by maintenance infusions every 8 weeks[22].

The inclusion criteria were as follows: (1) first exposure to IFX with completion of the induction regimen; (2) availability of complete CTE examinations within 2 weeks before the first IFX infusion and within 2 weeks after completion of induction; and (3) clinical response assessment at week 14. The exclusion criteria were: (1) prior bowel resection or previous biologic therapy; (2) primary nonresponse to induction; (3) severe CTE image artifacts; (4) incomplete baseline clinical or serologic data; and (5) elective discontinuation of IFX for reasons other than SLR. Eligible patients were allocated to a training cohort and an internal testing cohort in a 7:3 ratio by stratified random sampling.

Outcomes and definitions

Analyses were landmarked at week 14, after completion of IFX induction. Patients categorized as having primary nonresponse during induction were excluded; the at-risk landmark cohort therefore comprised patients judged by a multidisciplinary team to have achieved an initial response by week 14. Subsequent SLR was defined as recurrent clinical disease activity between weeks 14 and 54 after that initial response, with corroboration from radiologic and/or endoscopic findings. Treatment modification, including switching targeted therapy, adding immunosuppressants or corticosteroids, IFX dose intensification or interval shortening, and CD-related surgery, was recorded as a consequence supporting adjudication but was not considered sufficient in isolation.

Objective evidence contributing to adjudication included a Crohn's Disease Activity Index greater than 150 and endoscopic activity with a Simple Endoscopic Score for Crohn's Disease of at least 3[23,24]. A decrease of less than 70 points in the Crohn's Disease Activity Index or less than 50% in the Simple Endoscopic Score for Crohn's Disease from pretreatment was interpreted as inadequate induction response rather than recurrence unless deterioration after the week-14 landmark was documented.

Problem formulation

For each patient $i \in \{1, \dots, N\}$, let $x_i \in \mathbb{R}^p$ denote the observed feature vector constructed from baseline clinical variables and longitudinal VAT-related imaging descriptors derived from paired CTE examinations. Let $y_i \in \{0,1\}$ denote the corresponding clinical endpoint, where $y_i = 1$ indicates SLR and $y_i = 0$ indicates sustained response.

The task was formulated as a patient-level supervised binary classification problem. Specifically, the objective was to estimate the posterior conditional probability

$$P(y_i = 1 | x_i), \quad (1)$$

which represents the patient-specific risk of subsequent SLR given the observed clinical and imaging features. The corresponding prediction rule can be written as

$$\hat{y}_i = \arg \max_{y \in \{0,1\}} P(y | x_i). \quad (2)$$

The proposed framework therefore learns a mapping from baseline clinical information and longitudinal VAT-related imaging descriptors to the posterior probability of future SLR.

Construction of the VAT heterogeneity score

Building on previously reported spatial heterogeneity frameworks[16,17,25], we developed a local feature-driven and topology-aware pipeline to quantify VAT structural heterogeneity from paired pretreatment and post-induction CTE examinations. Briefly, CTE images and VAT masks were resampled to isotropic voxel spacing, and the VAT mask was binarized with retention of the largest connected component to reduce isolated noise. Voxel intensities within VAT were clipped to the 1st and 99th percentiles and normalized within the volume of interest.

For each voxel within the VAT mask, a three-dimensional local window was used to extract 104 radiomic descriptors covering first-order and texture feature families. These local feature vectors were normalized and then clustered using k-means to generate a three-dimensional spatial partition map of VAT habitats. A 26-neighborhood connectivity analysis was performed for each cluster to quantify the degree of spatial fragmentation. Detailed descriptions of the local radiomic feature extraction procedure, feature families, normalization, and computational steps are provided in Supplementary Methods S1 and Supplementary Algorithm S1.

The VAT heterogeneity score (VHS) for patient i at time point $t \in \{\text{pre}, \text{post}\}$ was defined as

$$\text{VHS}_{t,i} = 1 - \frac{1}{v_{\text{total},t,i}} \sum_{k=1}^K \frac{v_{t,i,k,\text{max}}}{n_{t,i,k}}, \quad (3)$$

where $v_{\text{total},t,i}$ denotes the total VAT volume, K is the number of clusters, $n_{t,i,k}$ is the number of connected domains within cluster k , and $v_{t,i,k,\text{max}}$ is the volume of the largest connected domain within cluster k . Higher VHS values indicate greater topological fragmentation and structural disorganization of the VAT microenvironment.

The longitudinal percentage change in VHS was defined as

$$\% \Delta \text{VHS}_i = \frac{\text{VHS}_{\text{pre},i} - \text{VHS}_{\text{post},i}}{\text{VHS}_{\text{pre},i}} \times 100\%. \quad (4)$$

This metric was used as the primary longitudinal VAT descriptor to characterize treatment-related remodeling during IFX induction therapy.

VAT was defined as whole-abdominal visceral adipose tissue from the diaphragmatic dome to the superior margin of the pubic symphysis. As reported in the preceding segmentation protocol[15], masks were generated with semiautomatic threshold-based segmentation (-150 to -50 HU) in 3D Slicer (version 4.11.2) by two abdominal radiologists blinded to clinical outcomes, followed by manual correction and consensus review.

Model development and benchmark models

Model development was performed in the training cohort and evaluated in the internal testing cohort. Candidate predictors comprised baseline clinical variables and the primary longitudinal imaging descriptor, % Δ VHS. Univariable logistic regression was used for exploratory screening, and variables with $P < 0.20$ were entered into multivariable logistic regression. Predictors retained at $P < 0.05$ were incorporated into the final combined clinical-imaging nomogram (Nom-% Δ VHS). The univariable screen was considered exploratory.

To determine the incremental value of longitudinal VAT heterogeneity, Nom-% Δ VHS was compared with a clinical-only model and conventional whole-volume VAT radiomics models. For the conventional radiomics analysis, 1,239 whole-volume VAT radiomic features were extracted from each scan. Feature engineering included normalization, univariable screening using Student's *t* test ($P < 0.05$), redundancy reduction using Pearson correlation analysis ($|r| > 0.90$), and least absolute shrinkage and selection operator regression for radiomics signature construction. Full details of the radiomics feature reduction pipeline and RadScore construction are provided in Supplementary Methods S2.

To explore sensitivity to model class, the same retained predictor set was supplied to five supervised classifiers: random forest, support vector machine, decision tree, XGBoost, and neural network. The classifiers were compared with the logistic regression-based nomogram using the area under the receiver operating characteristic curve and threshold-dependent diagnostic metrics. Because nested repeated cross-validation was not implemented in the available analysis, this comparison was treated as exploratory rather than a tuned head-to-head benchmark. Additional implementation details are provided in Supplementary Methods S3.

Prospective fecal sampling and 16S rRNA sequencing

An exploratory prospective microbiome cohort of 42 patients with CD was enrolled after post-induction imaging and stratified into low and high % Δ VHS groups using the prespecified cutoff derived in the retrospective training cohort ($n = 21$ per group). This cohort was not used for model fitting or performance evaluation. Fresh fecal samples were collected at week 14 before bowel preparation and before treatment modification. Microbial DNA was extracted, the V3-V4 region of the bacterial 16S ribosomal RNA gene was amplified, and libraries were sequenced on an Illumina MiSeq platform. Reads were processed with QIIME 2 and DADA2, and taxonomy was assigned against SILVA. Alpha diversity was assessed using the abundance-based coverage estimator (ACE) at the amplicon sequence variant level. Differential abundance was compared with the Wilcoxon rank-sum test and Benjamini-Hochberg correction. Taxa were classified as persistent, intermittent, or transient according to group prevalence, and a microbial dysbiosis index was calculated from differentially enriched taxa.

Humanized fecal microbiota transplantation and anti-TNF α treatment

Donor fecal samples were collected from patients in the low and high % Δ VHS groups at week 14 and suspended in sterile phosphate-buffered saline. Eight-week-old male C57BL/6J recipient mice received a broad-spectrum antibiotic cocktail in drinking water for 14 days, followed by a 3-day washout and fecal suspension by oral gavage once daily for 3 consecutive days. Twenty-four hours after the final gavage, colitis was induced with 3% dextran sulfate sodium (DSS) in drinking water. Anti-mouse TNF α was administered intraperitoneally at 6 mg/kg on days 1, 4, and 7 after DSS initiation. Experimental groups were healthy control, DSS colitis, DSS colitis plus anti-TNF α , and DSS colitis plus anti-TNF α with hFMT from either the low- or high-% Δ VHS group.

Murine colonoscopy

Colonoscopy was performed on day 10, 72 h after the day-7 anti-TNF α dose and before tissue harvest. Mice were anesthetized with isoflurane, and a miniature endoscope was inserted transanally to examine the distal colon. Images were reviewed in a blinded manner.

Statistical analysis

Clinical and prediction-model analyses were performed in R (version 4.3.1). Continuous variables are presented as mean $\pm$ standard deviation or median (interquartile range), according to their distribution, and were compared with Student's t-test or the Mann-Whitney U test, as appropriate. Categorical variables are presented as number (percentage) and were compared with the chi-squared test or Fisher's exact test. Model analyses used complete cases for the variables required by each model. All tests were two-sided, and $P < 0.05$ was considered statistically significant.

SLR prediction was formulated as a patient-level binary classification problem. Model discrimination was summarized by the area under the receiver operating characteristic curve (AUC) with 95% confidence intervals. Calibration curves and decision-curve analysis were used to examine agreement and potential net benefit. Continuous net reclassification improvement (NRI) and integrated discrimination improvement (IDI) compared Nom-% Δ VHS with the clinical-only model (Clinpre) and the conventional delta-radiomics model (Δ VAT-RadScore). The internal testing cohort was evaluated after model development; threshold-dependent metrics and the fixed-hyperparameter classifier comparison were interpreted descriptively.

For microbiome analyses, the Wilcoxon rank-sum test was used for between-group comparisons and Benjamini-Hochberg adjustment was applied to differential-abundance testing. The hFMT endoscopy panel was treated as descriptive because no donor-level model or quantitative animal endpoint is reported.

Results

Baseline characteristics

A total of 158 patients with CD who received IFX induction therapy were included and randomly assigned to a training cohort (n = 110) and an internal testing cohort (n = 48). Table 1 reports the demographic, disease-related, endoscopic, and serologic distributions in each cohort. No marked between-cohort differences were observed in the measured variables (all $P > 0.05$), although this internal random split does not constitute an external validation sample. Both cohorts were composed mainly of young male patients with a low-normal body mass index. Disease location, behavior, activity indices, and systemic inflammatory markers are summarized descriptively in Table 1.

Table 1. Baseline demographic and clinical characteristics of patients with Crohn disease in the training and internal testing cohorts.

Characteristic	Training cohort (N = 110)	Internal testing cohort (N = 48)	P value
Age (years)	30 (23, 39)	28 (19, 37)	0.13
Sex			0.2
Female	25 (23%)	7 (15%)	
Male	85 (77%)	41 (85%)	
BMI (kg/m ²)	18.7 (16.7, 21.5)	18.5 (16.4, 21.5)	0.5
Disease location			0.077
L1 (ileal disease)	32 (29.1%)	23 (47.9%)	
L2 (colonic disease)	11 (10.0%)	3 (6.3%)	
L3 (ileocolonic disease)	67 (60.9%)	22 (45.8%)	
Disease behavior			0.3
B1 (non stricturing, non penetrating)	50 (45%)	16 (33%)	
B2 (stricturing)	50 (45%)	26 (54%)	
B3 (penetrating)	10 (9.1%)	6 (13%)	
CDAI	158 (101, 230)	170 (120, 248)	0.5
SES-CD	7 (3, 11)	7 (3, 10)	0.7
CRP (mg/L)	21 (5, 47)	18 (5, 77)	0.4
ESR (mm/hour)	43 (24, 70)	34 (21, 74)	0.3
Platelet count ($\times 10^9/L$)	296 (229, 371)	301 (196, 381)	0.9
NLR	4.00 (2.70, 6.00)	4.35 (2.80, 5.65)	>0.9
Albumin (g/L)	35.2 (32.4, 39.6)	35.7 (31.9, 40.2)	>0.9
Creatinine ($\mu\text{mol/L}$)	67 (60, 77)	67 (59, 75)	0.8
SII	1,099 (780, 1,813)	1,361 (754, 1,808)	>0.9

Abbreviations: BMI, body mass index; CDAI, Crohn Disease Activity Index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; NLR, neutrophil-to-lymphocyte ratio; SES-CD, Simple Endoscopic Score for Crohn Disease; SII, systemic immune-inflammation index.

Longitudinal VAT heterogeneity and association with secondary loss of response

To determine whether longitudinal VAT remodeling was associated with treatment outcome, we compared VHS trajectories between patients with sustained clinical response and those who later developed SLR. In the training cohort, paired analysis showed significantly different pretreatment-to-post-induction VHS trajectories between groups (Fig. 2A). Pretreatment VHS (VHSpre) did not differ significantly between sustained responders and patients with SLR (Fig.

2B), indicating limited prognostic value at baseline. By contrast, post-induction VHS (VHSpost) was significantly higher in the SLR group (Fig. 2C), and % Δ VHS showed a substantially less favorable remodeling pattern in patients who subsequently developed SLR (Fig. 2D).

The same overall pattern was observed in the internal testing cohort. Group differences in longitudinal VHS trajectories remained significant (Fig. 2E), whereas VHSpre again showed limited discrimination (Fig. 2F). VHSpost did not differ significantly between groups in the internal testing cohort (Fig. 2G), but % Δ VHS remained significantly different (Fig. 2H). These findings suggest that longitudinal change in VHS carries more information about subsequent SLR than the pretreatment measurement alone; the magnitude and generalizability of this association require external evaluation.

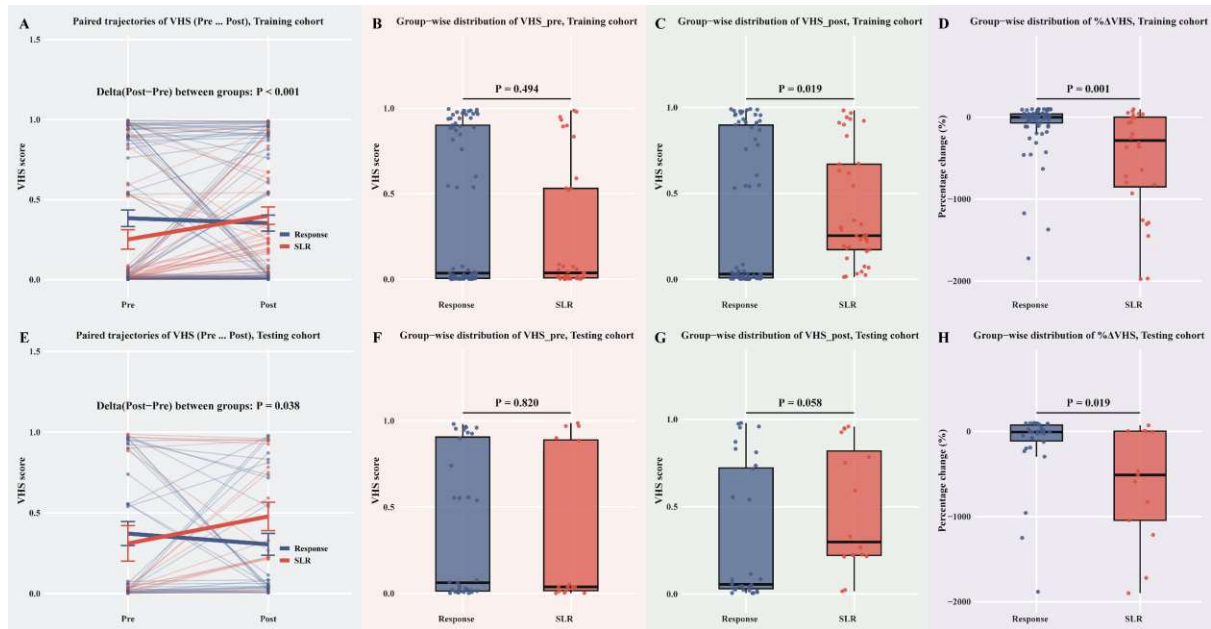


Fig. 2. Dynamic evolution of the visceral adipose tissue heterogeneity score (VHS). The figure shows paired VHS trajectories from pretreatment to post-induction imaging (A, E), together with the group-wise distributions of pretreatment VHSpre (B, F), post-induction VHSpost (C, G), and % Δ VHS (D, H) in patients with sustained clinical response and in those who developed secondary loss of response (SLR). Data are shown for the training cohort (A to D) and the internal testing cohort (E to H).

Comparison with alternative machine learning classifiers

In an exploratory comparison using the same retained predictors, Nom-% Δ VHS yielded the highest nominal AUC in the training cohort (0.825), followed by random forest (0.811), neural network (0.807), support vector machine (0.803), decision tree (0.781), and XGBoost (0.749) (Fig. 3A). In the internal testing cohort, the corresponding AUCs were 0.818, 0.805, 0.725, 0.744, 0.742, and 0.734, respectively (Fig. 3C). Threshold-dependent metric profiles are shown in Fig. 3B and D. Because the alternative classifiers were not tuned with nested repeated cross-validation, these descriptive differences do not establish algorithmic superiority.

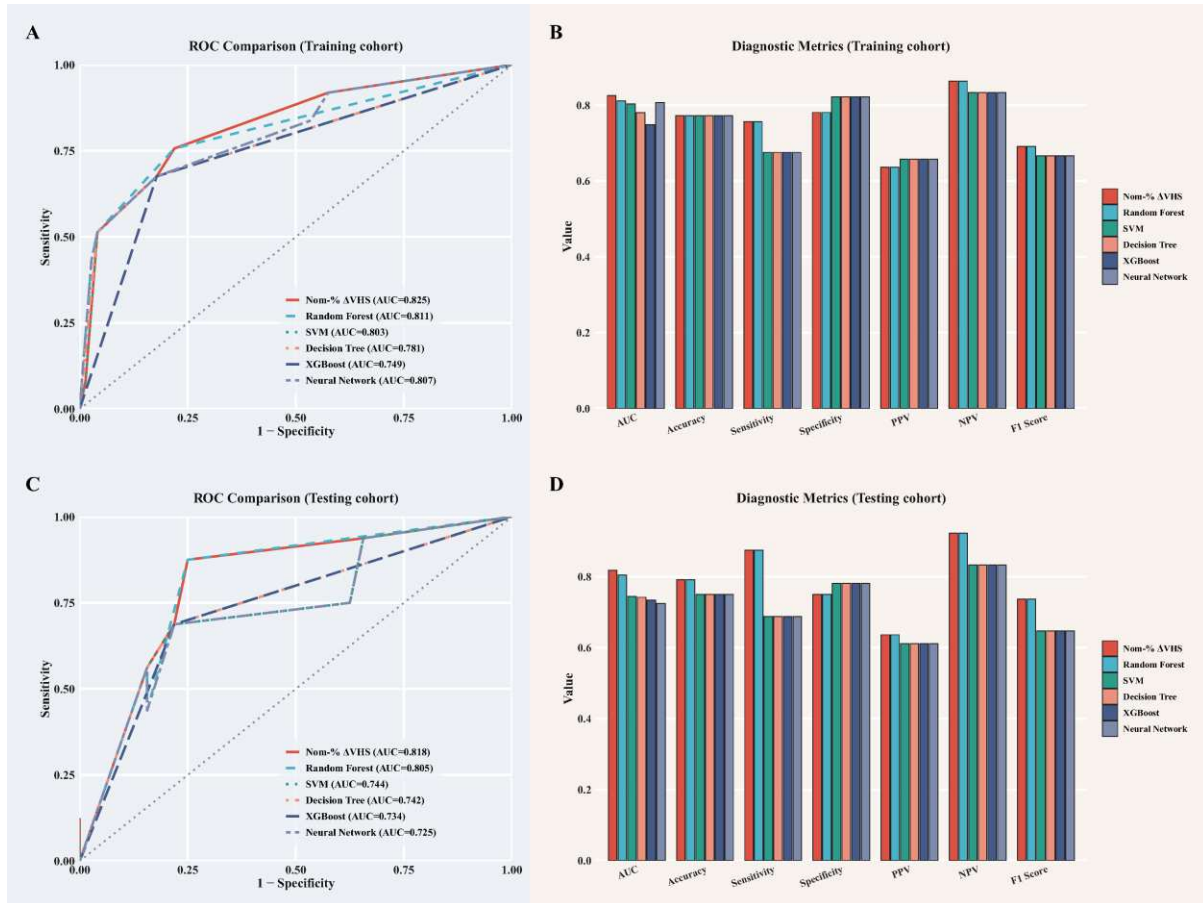


Fig. 3. Exploratory performance comparison between Nom-%ΔVHS and alternative machine-learning classifiers. Receiver operating characteristic curves and threshold-dependent diagnostic metrics are shown for the training cohort (A, B) and internal testing cohort (C, D). The comparison used a common predictor set but was not based on nested repeated cross-validation.

Independent predictors and nomogram development

Univariable and multivariable logistic regression analyses were performed in the training cohort (Table 2). Low %ΔVHS, reflecting absent or limited improvement in VAT heterogeneity, was independently associated with SLR (odds ratio [OR], 13.4; 95% confidence interval [CI], 4.76-43.8; $P < 0.001$). Structuring disease behavior (Montreal B2; OR, 7.05; 95% CI, 2.24-26.2; $P = 0.002$) and penetrating disease behavior (Montreal B3; OR, 13.1; 95% CI, 2.42-83.0; $P = 0.004$) were also independently associated with subsequent SLR.

Table 2. Univariable and multivariable logistic regression analysis for the prediction of secondary loss of response to infliximab in patients with Crohn disease in the training cohort.

Characteristic	Univariable			Multivariable		
	OR	95% CI	P value	OR	95% CI	P value
%ΔVHS						
High	--	--		--	--	
Low	9.62	3.97, 24.9	<0.001	13.4	4.76, 43.8	<0.001
Age (years)	1.01	0.97, 1.05	0.6			
Sex						
Female	--	--		--	--	
Male	0.45	0.18, 1.13	0.088	0.32	0.09, 1.10	0.075
BMI (kg/m ²)	1.02	0.91, 1.13	0.7			
Disease location						
L1 (ileal disease)	--	--				
L2 (colonic disease)	2.13	0.50, 8.95	0.3			
L3 (ileocolonic disease)	1.34	0.54, 3.48	0.5			
Disease behavior						
B1 (non stricturing, non penetrating)	--	--		--	--	
B2 (stricturing)	3.58	1.47, 9.28	0.006	7.05	2.24, 26.2	0.002
B3 (penetrating)	6.83	1.63, 31.9	0.010	13.1	2.42, 83.0	0.004
CDAI	1.00	1.00, 1.00	0.8			
SES-CD	0.99	0.93, 1.04	0.7			
CRP (mg/L)	0.99	0.98, 1.00	0.2			
ESR (mm/hour)	1.00	0.99, 1.01	0.7			
Platelet count (×10 ⁹ /L)	1.00	1.00, 1.01	0.2	1.00	1.00, 1.01	0.052
NLR	0.98	0.85, 1.08	0.7			
Albumin (g/L)	1.00	0.93, 1.07	>0.9			
Creatinine (μmol/L)	0.99	0.96, 1.03	0.7			
SII	1.00	1.00, 1.00	0.8			

Abbreviations: OR, odds ratio; CI, confidence interval; VHS, visceral adipose tissue heterogeneity score; BMI, body mass index; CDAI, Crohn Disease Activity Index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; NLR, neutrophil-to-lymphocyte ratio; SES-CD, Simple Endoscopic Score for Crohn Disease; SII, systemic immune-inflammation index.

These predictors were combined in a clinical-imaging nomogram (Nom-%ΔVHS) for individualized risk estimation (Fig. 4). Calibration curves suggested agreement between predicted and observed risk in the training and internal testing cohorts (Fig. 5A, D), and receiver operating characteristic curves summarized discrimination (Fig. 5B, E). Decision-curve analysis suggested potential net benefit across a range of threshold probabilities (Fig. 5C, F); this clinical utility remains to be tested prospectively.

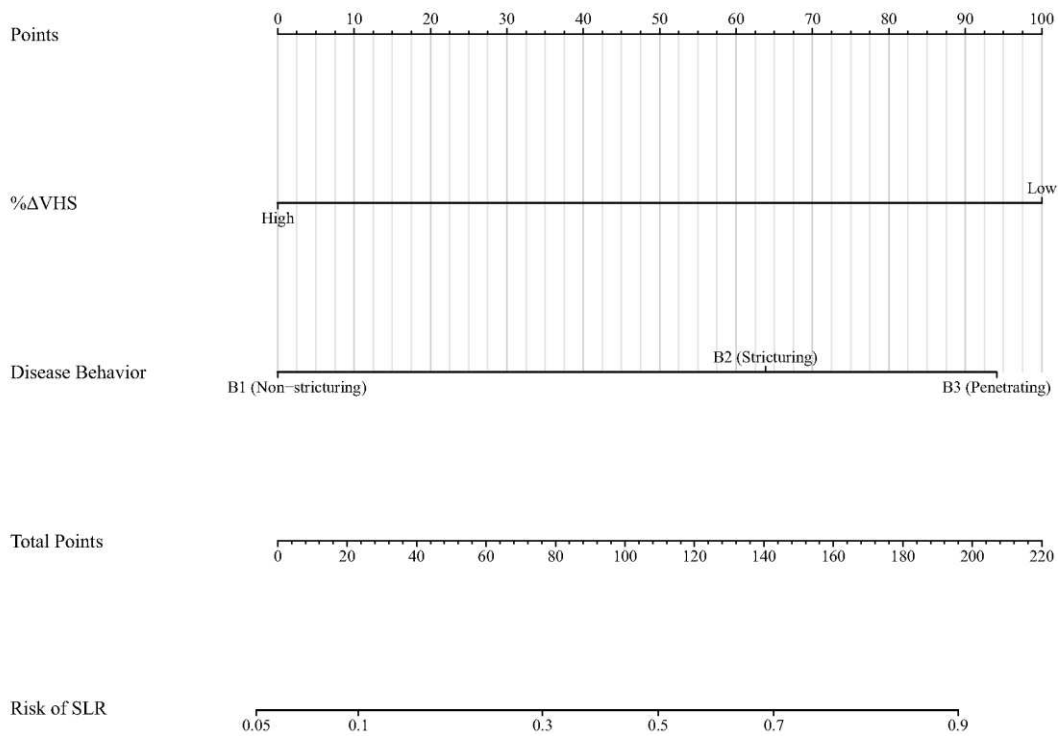


Fig. 4. The combined clinical imaging nomogram (Nom-% Δ VHS), integrating % Δ VHS and baseline disease behavior for individualized estimation of the risk of secondary loss of response to infliximab.

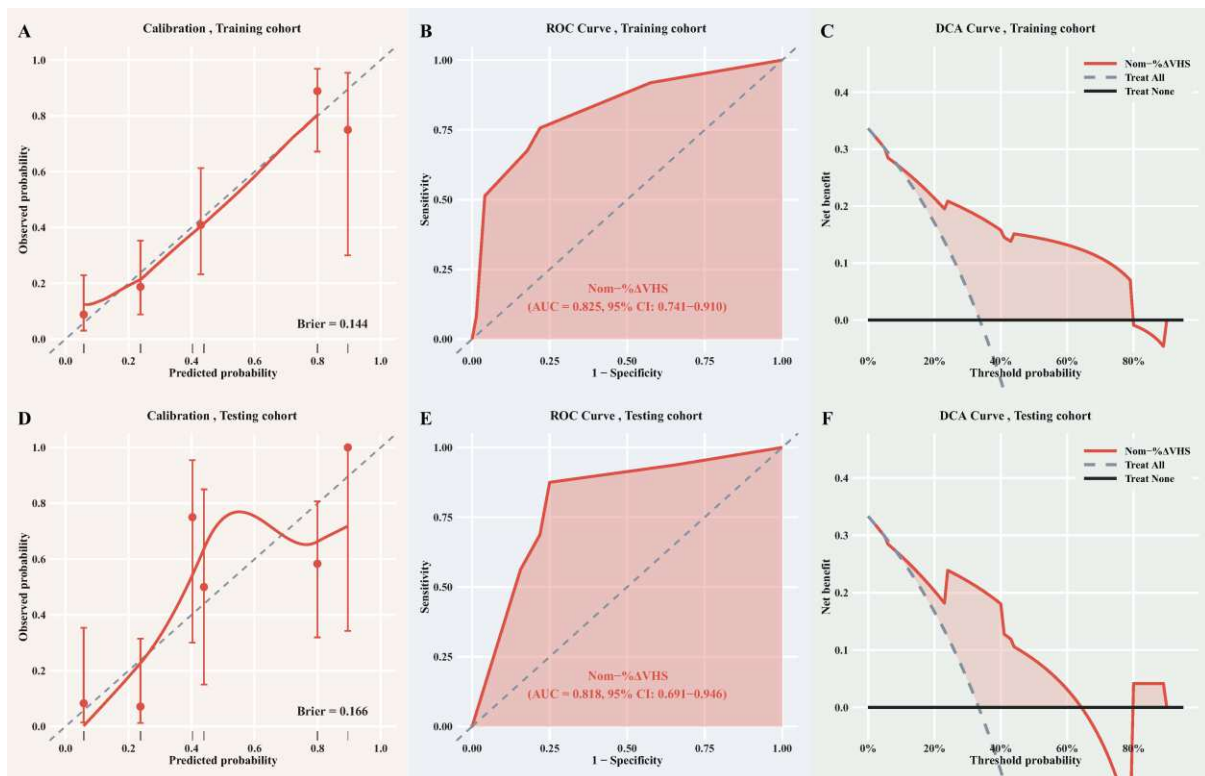


Fig. 5. Internal performance evaluation of the Nom-% Δ VHS model. Calibration curves (A, D), receiver operating characteristic curves (B, E), and decision-curve analyses (C, F) are shown for the training and internal testing cohorts, respectively.

Comparative performance against benchmark models

Nom-% Δ VHS was compared with a clinical-only model and conventional radiomics models (Table 3 and Fig. 6A, D). The model achieved an AUC of 0.825 (95% CI, 0.741-0.910) in the training cohort and 0.818 (95% CI, 0.691-0.946) in the internal testing cohort. These estimates support post-induction risk stratification within the present dataset but do not constitute external validation.

Table 3. Diagnostic performance of VAT-derived heterogeneity and radiomics models.

Metric	Nom-% Δ VHS	% Δ VHS	Clinpre	VHSpre	VHSpst	VAT-RadScorepre	VAT-RadScorepost	Δ VAT-RadScore
Training cohort								
AUC (95% CI)	0.825 (0.741 to 0.910)	0.749 (0.660 to 0.837)	0.674 (0.578 to 0.770)	0.460 (0.350 to 0.570)	0.637 (0.534 to 0.740)	0.777 (0.687 to 0.867)	0.733 (0.636 to 0.830)	0.698 (0.592 to 0.803)
Accuracy	0.773	0.227	0.627	0.536	0.682	0.673	0.664	0.709
Sensitivity	0.757	0.324	0.757	0.189	0.865	0.811	0.811	0.622
Specificity	0.781	0.178	0.562	0.712	0.589	0.603	0.589	0.753
PPV	0.636	0.167	0.467	0.250	0.516	0.508	0.500	0.561
NPV	0.864	0.342	0.820	0.634	0.896	0.863	0.860	0.797
F1 score	0.691	0.220	0.577	0.215	0.646	0.625	0.619	0.590
Internal testing cohort								
AUC (95% CI)	0.818 (0.691 to 0.946)	0.734 (0.596 to 0.872)	0.689 (0.538 to 0.841)	0.521 (0.336 to 0.707)	0.670 (0.508 to 0.832)	0.584 (0.410 to 0.758)	0.469 (0.261 to 0.677)	0.516 (0.333 to 0.698)
Accuracy	0.792	0.250	0.542	0.667	0.667	0.604	0.417	0.479
Sensitivity	0.875	0.312	0.812	0.188	0.875	0.562	0.625	0.312
Specificity	0.750	0.219	0.406	0.906	0.562	0.625	0.312	0.562
PPV	0.636	0.167	0.406	0.500	0.500	0.429	0.312	0.263
NPV	0.923	0.389	0.812	0.690	0.900	0.741	0.625	0.621
F1 score	0.737	0.217	0.542	0.273	0.636	0.486	0.417	0.286

Abbreviations: AUC, area under the receiver operating characteristic curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; Nom-% Δ VHS, nomogram integrating % Δ VHS and pretreatment clinical variables; Clinpre, pretreatment clinical model excluding % Δ VHS; % Δ VHS, percentage change in VHS; VHSpre, pretreatment VHS; VHSpst, post-induction VHS; VAT-RadScorepre, pretreatment VAT radiomics score; VAT-RadScorepost, post-induction VAT radiomics score; Δ VAT-RadScore, delta VAT radiomics score; VHS, visceral adipose tissue heterogeneity score; VAT, visceral adipose tissue.

The Clinpre model and conventional delta-radiomics signature (Δ VAT-RadScore) showed lower discrimination than Nom-% Δ VHS in this internal comparison. NRI and IDI estimates were in the direction favoring Nom-% Δ VHS; in Fig. 6, reference-model-minus-Nom contrasts are displayed as negative values. These reclassification analyses are exploratory and require confirmation in an external cohort.

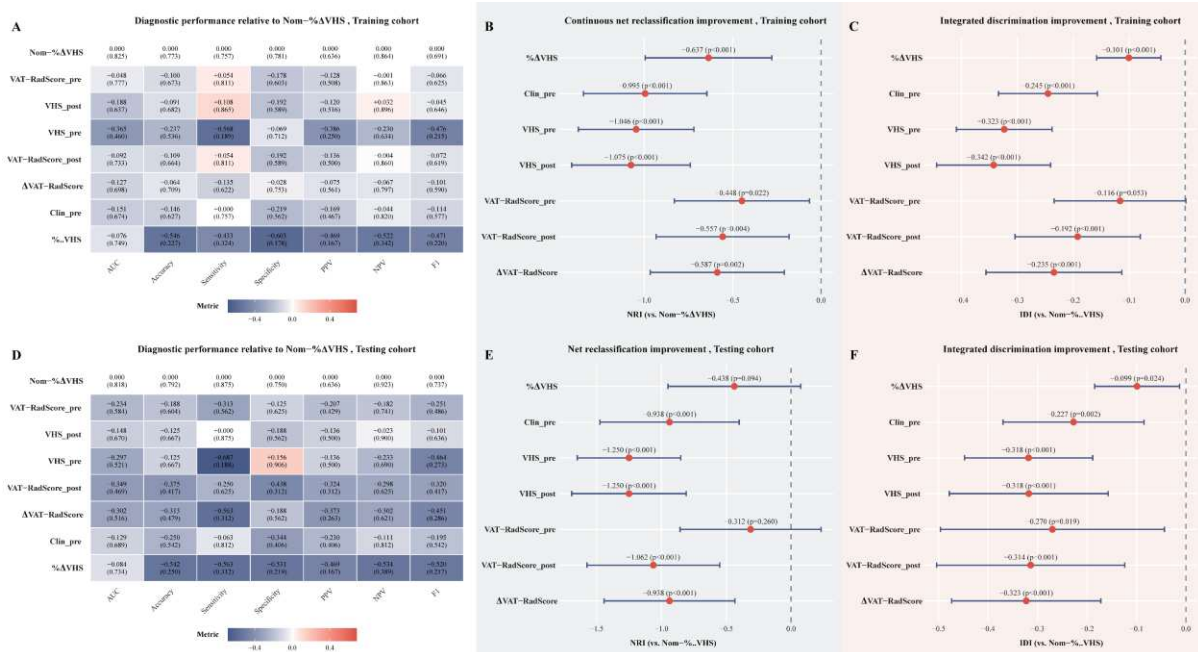


Fig. 6. Internal comparison of Nom-%ΔVHS with benchmark models. Heatmaps summarize diagnostic performance in the training (A) and internal testing (D) cohorts. Forest plots show continuous NRI and IDI for each reference model relative to Nom-%ΔVHS in the training (B, C) and internal testing (E, F) cohorts; negative reference-minus-Nom estimates favor Nom-%ΔVHS.

Microbiome features associated with model-defined %ΔVHS groups and exploratory assessment in hFMT mice

In the prospective microbiome cohort, the low-%ΔVHS group had a lower ACE index at the amplicon sequence variant level than the high-%ΔVHS group ($P < 0.001$; Fig. 7A). Differential-abundance analysis identified *Clostridium innocuum*, *Ruminococcus gnavus*, *Staphylococcus caprae*, and *Fusobacterium nucleatum* as relatively enriched in the low-%ΔVHS group, whereas *Lactobacillus salivarius*, *Rothia mucilaginosa*, and *Dialister pneumosintes* were relatively enriched in the high-%ΔVHS group (Fig. 7B). By relative abundance, persistent taxa accounted for 57.55% of the high-%ΔVHS community and 48.63% of the low-%ΔVHS community, whereas transient taxa accounted for 2.97% and 5.01%, respectively. By taxon count, persistent taxa represented 20.13% and 17.53%, and transient taxa represented 17.64% and 20.22%, in the high- and low-%ΔVHS groups, respectively (Fig. 7C). The microbial dysbiosis index was higher in the low-%ΔVHS group ($P < 0.001$; Fig. 7D). These associations describe model-defined groups and do not demonstrate that dysbiosis caused SLR.

Representative endoscopic images from the hFMT experiment showed greater residual inflammation in mice receiving microbiota from the low-%ΔVHS group than in those receiving microbiota from the high-%ΔVHS group (Fig. 7E). Because animal numbers, quantitative endoscopic or histologic scores, and donor-level analyses are not reported, this observation is descriptive rather than inferential. The hFMT panel is therefore interpreted as exploratory biological support, not functional validation or evidence that the microbiota transferred anti-TNFα resistance.

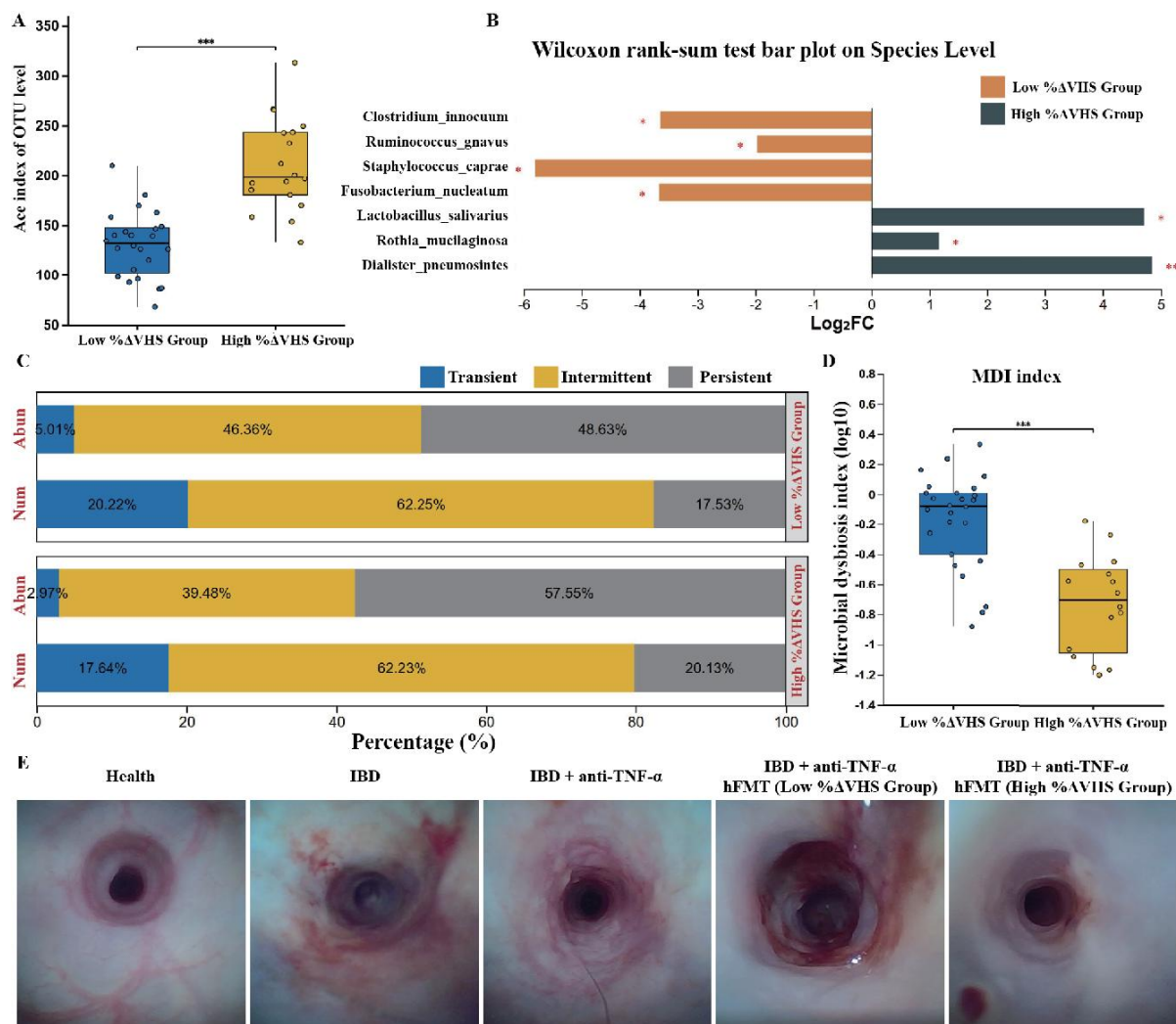


Fig. 7. Microbiome features associated with %ΔVHS stratification and exploratory assessment in hFMT mice. ACE index at the amplicon sequence variant level (A), differential abundance at the lowest reported taxonomic level (B), proportions of persistent, intermittent, and transient taxa by abundance and taxon count (C), and microbial dysbiosis index (D) are shown for the low- and high-%ΔVHS groups. Representative endoscopic images from anti-TNF α -treated hFMT mice are shown in E; no quantitative animal comparison is presented.

Discussion

At a post-induction landmark, longitudinal change in the CTE-derived VAT heterogeneity score (%ΔVHS) was independently associated with subsequent infliximab SLR. A nomogram combining %ΔVHS with baseline disease behavior showed higher internal discrimination than clinical-only and conventional whole-volume radiomics models. These results support the potential value of dynamic mesenteric imaging for risk stratification, while the single-center internal split and exploratory model comparisons preclude claims of external validation or algorithmic superiority.

The longitudinal measurement carried more prognostic information than VHSpre alone. In both cohorts, VHSpre showed limited discrimination, whereas a smaller reduction in heterogeneity during induction was associated with later SLR. This pattern is consistent with

the possibility that the capacity of the VAT microenvironment to remodel during treatment contains information beyond baseline inflammatory burden. An attenuated % Δ VHS reduction may reflect persistent inflammatory cell infiltration, unresolved transmural inflammation, or ongoing fibroinflammatory remodeling within creeping fat after induction[30-32], but these processes were not measured directly.

The topology-aware heterogeneity framework may capture information complementary to conventional whole-volume radiomics, including Δ VAT-RadScore. Related topology-based scores have been used to characterize spatial heterogeneity in solid tumors[17,33-39]. In CD, VAT remodeling is focal and spatially disorganized, so whole-volume summaries may average local variation. Subregional clustering and connectivity metrics retain aspects of spatial organization; however, the apparent advantage of % Δ VHS in this study remains an internal comparison and should be evaluated with prespecified pipelines and external data.

Our findings also strengthen the view that VAT in CD is not merely a passive energy reservoir, but an active inflammatory and fibrogenic compartment. Creeping fat is increasingly recognized as a biologically dynamic mesenteric niche shaped by immune activation, aberrant adipogenesis, extracellular matrix remodeling, and crosstalk with translocated gut bacteria[8-10,30-32]. Within this framework, the present study extends the field from static morphologic assessment to a longitudinal, topology-aware imaging readout that captures how the internal organization of mesenteric fat evolves during therapy. In practical terms, this shifts the focus from how much mesenteric fat is present to whether its internal structure is moving toward resolution or persistence under treatment, which appears to be more relevant to subsequent clinical outcome.

The prospective microbiome analysis provides an orthogonal, exploratory view of the VHS-defined phenotype. Low % Δ VHS was associated with lower richness, a higher microbial dysbiosis index, fewer persistent taxa by relative abundance, and more transient taxa. Longitudinal and treatment-response studies have likewise shown that inflammatory bowel disease activity and therapeutic outcomes are associated with ecosystem instability and therapy-specific microbial profiles[40,41,44,50,51]. Species-level differences in the present cohort should nevertheless be interpreted cautiously because V3-V4 16S sequencing has limited species and functional resolution, and measured covariates that could confound microbiome composition were not fully reported.

The hFMT observations were qualitatively concordant with the patient-level microbiome associations, but they do not establish causality. Representative endoscopic images suggested a less favorable anti-TNF α response after transfer of microbiota from the low-% Δ VHS group. Without quantitative endpoints, donor-level replication, and a model accounting for recipients nested within donors, the experiment cannot show that a microbiome configuration transferred treatment resistance. It is best viewed as hypothesis-generating support for further mechanistic study[44,50,51].

Together, the data motivate a hypothesis in which persistent VAT heterogeneity marks incomplete resolution along an adipose-gut-microbiome axis. Creeping fat has been linked to bacterial translocation and profibrotic stromal programs[30,32,45]. An unfavorable % Δ VHS trajectory could therefore coexist with persistent mesenteric inflammation and ecological

instability during IFX induction. Because bacterial ingress, adipose immune activation, drug clearance, antidrug antibodies, and tissue drug concentrations were not measured here, these mechanisms remain plausible alternatives rather than demonstrated mediators.

Nom-% Δ VHS is intended for risk estimation at the end of induction, not pretreatment prediction. If independently validated, it could help identify patients who warrant closer monitoring or therapeutic drug assessment before overt clinical failure. The present data do not show that management based on Nom-% Δ VHS improves outcomes; dose adjustment, interval shortening, or treatment escalation should therefore continue to follow established inflammatory bowel disease guidance[22,46] until prospective clinical-utility studies are performed.

Several limitations should be acknowledged. First, the imaging cohort was retrospective and single-center, and the random split provided internal evaluation rather than independent validation. The composite SLR adjudication also requires confirmation with a prespecified, reproducible week-14 response definition. Second, contemporaneous IFX trough concentrations and antidrug antibody titers were unavailable, and scanner heterogeneity across the enrollment period may have affected quantitative features despite within-patient normalization. Third, predictor selection was not assessed with the requested 1,000 bootstrap repetitions, and alternative classifiers were not tuned with nested repeated cross-validation; the model-comparison results are therefore exploratory. Fourth, the prospective microbiome cohort was modest in size, potential microbiome confounders were not fully reported, and 16S sequencing has limited taxonomic and functional resolution. Finally, the hFMT report lacks donor-level replication and quantitative endpoints and cannot recapitulate human IFX pharmacology. These biological results should be considered supportive and hypothesis-generating rather than mechanistic validation.

In conclusion, % Δ VHS was associated with subsequent IFX SLR and supported post-induction risk stratification within this single-center internal evaluation. Exploratory microbiome profiling and descriptive hFMT findings were consistent with, but did not prove, biological links among adverse mesenteric remodeling, ecological dysbiosis, and anti-TNF α response. External multicenter validation, prespecified model evaluation, and mechanistic studies incorporating adipose immunobiology, microbial ecology, donor effects, and pharmacologic monitoring are required before clinical implementation.

Ethics Approval and Consent to Participate

This retrospective study was approved by the Institutional Review Board of the Third Xiangya Hospital, Central South University, which waived the requirement for informed consent because of the retrospective design.

Consent for Publication

Not applicable.

Supplementary Materials

Supplementary Materials include Supplementary Methods S1–S3 and Supplementary Algorithm S1 cited in the manuscript.

Data Availability

The deidentified clinical variables, imaging-derived features, and analysis outputs required to interpret the findings of this study are not publicly available because they contain potentially identifiable clinical information. Reasonable requests for the minimum dataset needed to verify and extend the findings will be considered by the corresponding author, subject to institutional approval and applicable data use restrictions.

Code Availability

The code used for image processing, heterogeneity quantification, model development, and statistical analysis is publicly available at <https://github.com/jq-Deng/DeltaVHS-Predictor>.

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Author Contributions

QL, PR, and ZM designed the research. ZM, ZZ, and JD carried out the experiments and performed the data analysis. FS, LY, QW, RG, and GY contributed to the experiments. ZM, ZZ, and JD drafted the manuscript. QL, PR, YC, JL, and HT critically revised the manuscript. All authors read and approved the final manuscript.

Competing Interests

The authors declare no financial or non-financial competing interests.

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Not applicable.

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