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ORIGINAL ARTICLE

AI-based digital pathology provides newer insights into lifestyle intervention-induced fibrosis regression in MASLD: An exploratory study

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Abstract

Background and Aims: Lifestyle intervention is the mainstay of therapy for metabolic dysfunction-associated steatohepatitis (MASH), and liver fibrosis is a key consequence of MASH that predicts adverse clinical outcomes. The placebo response plays a pivotal role in the outcome of MASH clinical trials. Second harmonic generation/two-photon excitation fluorescence (SHG/TPEF) microscopy with artificial intelligence analyses can provide an automated quantitative assessment of fibrosis features on a continuous scale called qFibrosis. In this exploratory study, we used this approach to gain insight into the effect of lifestyle intervention-induced fibrosis changes in MASH.

Abbreviations: AI, artificial intelligence; BL, baseline; BMI, body mass index; CPA, collagen proportionate area; EOI, end of intervention; FIB-4, fibrosis-4 index; HOMA-IR, homeostatic model assessment of insulin resistance; MASH, metabolic dysfunction-associated steatohepatitis; MFS, MASLD fibrosis score; NASH CRN, Nonalcoholic Steatohepatitis Clinical Research Network; RLI, routine lifestyle intervention; SHG/TPEF, second harmonic generation/two-photon excitation fluorescence; SLI, strengthened lifestyle intervention.

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Methods: We examined unstained sections from paired liver biopsies (baseline and end-of-intervention) from MASH individuals who had received either routine lifestyle intervention (RLI) ($n=35$) or strengthened lifestyle intervention (SLI) ($n=17$). We quantified liver fibrosis with qFibrosis in the portal tract, periportal, transitional, pericentral, and central vein regions.

Results: About 20% (7/35) and 65% (11/17) of patients had fibrosis regression in the RLI and SLI groups, respectively. Liver fibrosis tended towards no change or regression after each lifestyle intervention, and this phenomenon was more prominent in the SLI group. SLI-induced liver fibrosis regression was concentrated in the periportal region.

Conclusion: Using digital pathology, we could detect a more pronounced fibrosis regression with SLI, mainly in the periportal region. With changes in fibrosis area in the periportal region, we could differentiate RLI and SLI patients in the placebo group in the MASH clinical trial. Digital pathology provides new insight into lifestyle-induced fibrosis regression and placebo responses, which is not captured by conventional histological staging.

KEYWORDS
fibrosis regression, lifestyle intervention, metabolic dysfunction-associated steatohepatitis, qFibrosis

1 | INTRODUCTION

With changes in lifestyle in the 21st century, metabolic dysfunction-associated steatotic liver disease (MASLD) has become a major public health problem, affecting up to a third of the global adult population.[1–3] Metabolic dysfunction-associated steatohepatitis (MASH) represents the progressive manifestation of MASLD, characterized by more severe liver damage than isolated steatosis, and can progress to advanced fibrosis, cirrhosis, and hepatocellular carcinoma.[4–6] Notably, the stage of liver fibrosis is the strongest histological predictor of liver-related morbidity and mortality in MASH. As a metabolic liver disease, MASH is strongly associated with obesity, type 2 diabetes, cardiovascular disease, and other metabolic conditions.[7–11] The proposed theory of 'multiple hits', which is a widely accepted theory of the pathophysiology of MASH, refers to a series of environmental regulators (e.g. diet, lifestyle, and gut microbiome) acting on susceptibility genes or epigenetic background to alter the response to dietary calorie excess.[5,12]

Recently, the U.S. FDA granted conditional approval to the thyroid hormone receptor-beta agonist resmetirom, the first licensed drug treatment for adults with noncirrhotic MASH and moderate-to-severe fibrosis.[13] However, the complex pathophysiology and high phenotypic heterogeneity of MASH hinder drug development efforts.[1,14–17] Lifestyle intervention, such as moderate physical exercise and a hypocaloric healthy diet, may lead to sustained weight loss and is currently the proven effective treatment for MASH.[18,19] Substantial evidence has confirmed that lifestyle intervention significantly reduces serum liver enzyme levels and improves liver fat content and fibrosis in patients with MASH.[20–22] Most intervention studies have been undertaken in small patient cohorts, and liver disease has been investigated either via imaging methods (liver ultrasonography, magnetic resonance imaging, or transient elastography) or several non-invasive diagnostic tests.[20–22] Consequently, the specific effects of lifestyle intervention intensity on liver fibrosis regression remain unclear and poorly quantified.

Key points

The impact of lifestyle intervention on the reversal of liver fibrosis at the microscopic level remains uncertain. Using AI-based digital pathology called qFibrosis for quantitative assessment of liver fibrosis, we observed that SLI-induced fibrosis regression was mainly in the periportal region.

Furthermore, unexpectedly high and variable placebo responses have posed a challenging issue in MASH clinical trials. A recent meta-analysis showed that approximately 20% of MASH patients in the placebo group experienced fibrosis regression or two-point MASLD activity score improvement without worsening liver fibrosis.[23] In MASH clinical trials with less than 20% placebo response rates, a drug is likely to be successful.[24–27] Conversely, when placebo response rates exceed 20%, drugs are more likely to fail.[28–31] The significant impact of lifestyle factors on the outcomes of MASLD

clinical trials complicates determining whether the results of a tested drug can be attributed to the medication or may arise due to lifestyle modifications.

The application of second harmonic generation/two-photon excitation fluorescence (SHG/TPEF) microscopy, coupled with artificial intelligence (AI) analyses, can provide an accurate and repeatable automated quantitative assessment of liver fibrosis features on a continuous scale, called qFibrosis.[32–34] By integrating pathological structural features of collagen with automated computer-aided image analysis tools, qFibrosis enables the identification of individual collagen fibres, fibrosis localization within liver samples, and accurate quantification of physical characteristics.[32,34] Hence, in this exploratory study, we employed this automated digital pathology approach to investigate the effect of lifestyle intervention intensity on liver fibrosis in individuals with biopsy-confirmed MASH to determine whether we could detect more subtle changes in liver pathology than can be detected by conventional histological examination.

2 | METHODS

2.1 | Study population

We conducted a retrospective study involving adult patients with histologically confirmed MASH from two medical centres in China (i.e. the First Affiliated Hospital of Wenzhou Medical University and the Beijing Friendship Hospital) who had undergone two liver biopsies at different periods, before and after lifestyle intervention. Patients were eligible for inclusion in the study if they: (1) had age between 18 and 75 years; (2) had undergone two liver biopsies; (3) had a confirmed MASH diagnosis based on the first liver biopsy; (4) received lifestyle intervention advice between the two liver biopsies; and (5) provided written informed consent. Patients were excluded if they: (1) had a history of excessive alcohol consumption (>10 g per day for women and >20 g per day for men); (2) were diagnosed with other chronic liver diseases, such as viral hepatitis, autoimmune hepatitis, primary biliary cholangitis, or Wilson's disease; (3) developed drug-induced or secondary fatty liver diseases; (4) used potentially hepatoprotective agents, such as pioglitazone, gliflozins, or glucagon-like peptide-1 receptor agonists during the lifestyle intervention; (5) were pregnant or lactating; (6) developed hepatocellular carcinoma or other benign or malignant tumours; and (7) had missing records of important parameters. Ethical approval for the study was obtained from the ethics committees of each participating centre, and written informed consent was obtained from each patient.

2.2 | Study design

All patients received generic advice regarding diet and physical activity during their initial consultation, and based on the intensity of the lifestyle intervention, the patients were categorized into a strengthened lifestyle intervention (SLI) group and a routine lifestyle intervention (RLI) group.

In addition to receiving a generic advice regarding diet and physical activity, patients in the SLI group also received detailed guidance from a physician regarding a comprehensive lifestyle intervention regimen aimed at intensifying physical activity levels, implementing dietary changes, and developing healthy lifestyle habits. This included advice on: (1) the energy balance, nutrients and weight monitoring; (2) the dietary pyramid and portion size; and (3) physical activity, when and how much. Specifically, each patient in the SLI group followed a personalized diet plan developed by a dietitian, restricting their daily calorie intake. The dietary approach primarily consisted of a low-calorie, low-fat and high-protein diet, along with a balanced distribution of essential nutritional elements. Furthermore, it incorporated appropriate supplementation of vitamins, minerals, and dietary fibre. Dietitians closely monitored patients in the SLI group throughout the intervention period. The dietitians supervised their daily meals and dietary adherence via acceptance photos through WeChat (a popular messaging app in China). Additionally, weekly phone consultations were conducted to discuss progress, address concerns, and provide ongoing support. Patients in the SLI group also adhered to an aerobic and resistance exercise program tailored to their individual needs and capabilities. Exercise selection considered medical, social, and patient-specific factors, ensuring safety and effectiveness. Activities such as running, badminton, jumping over a rope, resistance training, walking, dancing, tai chi, or swimming were recommended based on patients' abilities.

In contrast, the RLI group involved patients who self-regulated their diet and physical exercise without the same level of structured intervention provided to the SLI group. All patients were scheduled for hospital outpatient visits every 3–6 months.

2.3 | Clinical and laboratory data

Clinical variables of patients with MASH within 1 day of each liver biopsy, including biochemical and body composition parameters, were collected and measured. Body mass index (BMI), fibrosis-4 (FIB-4) index, MASLD fibrosis score (MFS), and homeostatic model assessment of insulin resistance (HOMA-IR) score were calculated.

Paired percutaneous liver biopsies were performed for histological examination using a 16-gauge needle under ultrasound guidance. Two liver biopsy specimens were obtained from each patient: one at baseline (BL) and the other at the end of intervention (EOI). Pathological examinations were performed by two independent board-certified pathologists from the respective centres who were unaware of the patient's clinical and biochemical data. Discrepancies were resolved through discussion, reaching a consensus. Inter-observer variability was assessed to evaluate the agreement between the pathologists (Method S1).

2.4 | SHG/TPEF microscopy and qFibrosis

Unstained sections from patients' paired liver biopsies (BL and EOI) were examined using SHG/TPEF microscopy with AI analyses.[34] Genesis®200, a fully automated, stain-free multi-photon fluorescence imaging microscope, was applied to scan the liver sections. Then, the resulting images were analysed using AI-based algorithms (HistoIndex Pte. Ltd).[34] The same image acquisition parameters were consistently applied to all samples across the two participating centres. The samples were laser excited at 780 nm, SHG signals were recorded at 390 nm, and TPEF signals were recorded at 550 nm. Image tiles were obtained at 20× magnification, featuring a resolution of 512 × 512 pixels and a dimension of 200 × 200 μm^2. Multiple adjacent image tiles were captured to encompass the entire organizational area on each slide.

qFibrosis is the overall output parameter derived from AI analyses, quantifying the severity of liver fibrosis in liver biopsy specimens. Unlike the semiquantitative histological measurement, qFibrosis is a continuous linear measurement based on the SHG index to take quantitative readings of liver fibrosis features, including fibre deposition, length, width, and area of collagen fibres. The liver lobules are classified into five regions: portal tract, periportal, transitional, pericentral, and central vein. The periportal and pericentral regions are defined as areas located approximately 100 μm away from the portal tract and central vein regions, respectively. This measurement of 100 μm is an approximation based on one-tenth of the average distance between the portal tract and central vein in a normal liver. Each liver region has 28 fibrosis parameters, resulting in 140 liver fibrosis parameters. The periportal, transitional, and pericentral regions represent the perisinusoidal region. Additionally, the collagen proportionate area (CPA), that is, another indicator of the severity of liver fibrosis and a significant predictor of long-term adverse clinical outcomes in patients with MASH, was defined as the proportion of collagen area to the total liver tissue area.[35]

2.5 | Statistical analysis

Based on their distribution, continuous variables were reported as means ± SD or medians (first quartile, third quartile). Categorical variables were expressed as percentages. The Wilcoxon signed-rank test was applied to compare the changes in liver fibrosis parameters from BL to EOI between the RLI and SLI groups. Progressive/No change/Regressive (P/N/R) analysis was used to compare liver fibrosis dynamics (i.e. progression, stabilization, or regression). The number of cases in each subgroup was divided by the total number of patients in each subgroup to calculate the corresponding percentages. The Pearson's chi-squared test was employed for the P/N/R analysis. p-values < .05 were statistically significant, and all statistical tests were two-tailed.

3 | RESULTS

3.1 | Lifestyle intervention-induced liver fibrosis regression

The clinical and biochemical characteristics of patients with histologically proven MASH in pre- and post-intervention from the SLI group ($n = 17$) and the RLI group ($n = 35$) are shown in Table 1. A reduction in BMI and waist circumference was observed following lifestyle intervention, particularly in the SLI group. The clinical and biochemical characteristics pre- and post-intervention suggest that lifestyle intervention could somewhat reduce the progression of MASH, with prominent improvement in liver function tests and liver histology features. Furthermore, compared to the RLI group, patients in the SLI group showed significant liver fibrosis regression ($p < .001$). These results suggest that the lifestyle intervention was working as intended and that increasing the intensity of lifestyle intervention yielded better liver histological outcomes.

Based on the Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) score, the P/N/R analyses of pathological fibrosis stages in both lifestyle intervention groups showed that liver fibrosis progression mostly tended to be reversed or stalled after lifestyle intervention (Figure 1A). The liver fibrosis regression rates in the RLI and SLI groups were 20% (7/35) and 65% (11/17), respectively. With the increase in the intensity of lifestyle intervention, the SLI group showed a more pronounced fibrosis regression ($p = .002$).

The paired liver specimens from MASH patients were scanned with Genesis®200 and quantified with qFibrosis. The AI computer-assisted measurements of the 104 biopsies revealed a median length of 12.80 mm (8.25–28.47 mm) and a median tissue width of .64 mm (ranging from .25 to 1.71 mm). The median number of portal tracts observed was 8 (ranging from 3 to 62). In both lifestyle intervention groups, the absolute changes in qFibrosis measurement from BL to EOI revealed a more prominent liver fibrosis decrease in the SLI group (−.4516 ± .3647) than in the RLI group (.0397 ± .2467, $p = .01$, Figure 1B). In addition, CPA was reduced post-intervention in the SLI group (−1.2887 ± 1.4418, Figure 1C).

The P/N/R analyses of the fibrosis changes from BL to EOI based on qFibrosis stage (20% and 41% in the RLI and SLI groups, respectively) and qFibrosis continuous value (43% and 53% in the RLI and SLI groups, respectively) showed the efficacy of the intervention in both lifestyle intervention groups and distinguished fibrosis progression in subjects in the SLI group that was not recognized by the current histological fibrosis staging system (Figure 1D). The qFibrosis stage was determined by evaluating the qFibrosis continuous value to assess the severity of liver fibrosis.

3.2 | Periportal fibrosis regression

AI-based digital pathology analyses were performed to investigate the changes in liver fibrosis areas in five regions. The zonal analysis

TABLE 1 Clinical and biochemical characteristics of patients with biopsy-proven MASH and paired liver biopsies at baseline (BL) and end of intervention (EOI) in both the routine lifestyle intervention (RLI) group and the strengthened lifestyle intervention (SLI) group.

	RLI group ($n=35$)			SLI group ($n=17$)			*p*-value			
	BL (1)	EOI (2)	Δ (3)	BL (4)	EOI (5)	Δ (6)	1 vs. 4	1 vs. 2	4 vs. 5	3 vs.6
Demographics										
Age (years)	44.0±13.5	-	-	39.5±11.6	-	-	.239	-	-	-
Male sex, *n* (%)	22 (62.9%)	-	-	11 (64.7%)	-	-	.897	-	-	-
Body weight (kg)	72.2±11.6	71.1±11.4	−1.1±4.5	74.0±10.4	66.3±10.6	−7.6±4.3	.597	.152	<.001	<.001
Body mass index (kg/m^2)	26.2±2.8	25.8±2.7	−.4±1.7	26.3±2.8	23.6±2.5	−2.7±1.6	.901	.204	<.001	<.001
Waist circumference (cm)	90.9±8.6	91.4±9.5	−.1±4.6	90.9±6.5	85.3±8.1	−5.9±9.0	.982	.880	.020	.006
Previous T2DM, *n* (%)	8 (22.9%)	-	-	0 (.0%)	-	-	.032	-	-	-
Newly diagnosed T2DM, *n* (%)	-	-	4 (14.8%)	-	-	2 (11.8%)	-	-	-	.774
Laboratory parameters										
WBC ($\times10^{12}$/L)	6.1±1.4	6.2±1.6	.1±1.2	5.9±2.3	6.0±1.8	.1±1.4	.708	.557	.713	.998
RBC ($\times10^{12}$/L)	4.9±.7	5.0±.7	.0±.3	4.9±.7	4.9±.7	−.1±.4	.982	.773	.506	.418
Hb (g/L)	147.1±16.5	147.5±18.6	.4±9.8	148.8±14.6	146.0±16.1	−2.8±11.0	.722	.823	.316	.304
Platelet count ($\times10^9$/L)	240.4±65.1	206.3±55.3	−34.1±41.2	209.5±72.2	212.9±68.9	3.4±30.6	.128	<.001	.652	.002
TBIL (μmol/L)	15.2±6.6	67.0±82.2	51.8±83.1	17.7±6.9	49.3±56.1	31.6±54.0	.211	<.001	.028	.367
DBIL (μmol/L)	5.5±4.1	4.9±2.3	−.6±4.0	5.2±2.1	4.6±1.9	−.6±1.4	.787	.393	.092	.984
Albumin (g/L)	45.8±5.5	44.3±4.0	−1.5±4.6	45.2±5.3	44.0±3.6	−1.1±4.3	.717	.061	.293	.787
ALT (U/L)	94.6±70.0	55.6±49.9	−39.1±75.4	118.0±150.2	33.2±30.5	−84.8±157.4	.445	.004	.041	.160
AST (U/L)	63.3±45.6	44.5±34.5	−18.8±44.8	81.7±74.8	26.2±13.9	−55.5±78.5	.276	.018	.010	.036
AST/ALT ratio	1.6±.7	1.3±.6	−.3±.6	1.5±.6	1.1±.4	−.3±.6	.496	.009	.045	.795
ALP (U/L)	103.5±55.6	89.6±28.3	−13.9±53.9	97.4±20.4	77.0±15.7	−20.4±18.0	.660	.135	<.001	.636
γ-GT (U/L)	82.3±43.5	56.6±37.9	−25.3±43.0	85.9±59.6	34.8±24.0	−51.1±62.1	.805	.002	.004	.088
Glucose (mmol/L)	5.7±1.9	6.0±1.7	.2±1.6	5.4±1.2	5.3±1.1	−.1±1.3	.579	.365	.710	.414
Creatinine (μmol/L)	64.5±12.9	63.8±12.9	−.8±8.4	67.3±15.4	70.5±15.5	3.2±8.7	.501	.596	.148	.120
UA (μmol/L)	374.4±89.1	374.1±104.3	−.3±101.6	464.3±138.7	361.3±162.2	−103.0±165.5	.007	.985	.021	.008
Total cholesterol (mmol/L)	4.9±1.1	4.8±1.1	−.0±.8	5.1±1.1	4.8±1.1	−.2±1.0	.531	.824	.313	.379
Triglycerides (mmol/L)	2.2±1.1	2.1±1.1	−.1±1.4	2.1±.9	1.8±.7	−.3±1.1	.761	.728	.268	.567
HDL-C (mmol/L)	1.0±.2	1.2±.6	.1±.6	.9±.2	1.1±.2	.2±.2	.199	.194	.005	.946
LDL-C (mmol/L)	3.1±1.0	2.8±.8	−.2±.7	3.1±.8	2.9±.9	−.2±.7	.769	.044	.193	.883
FIB-4 index	1.6±1.4	1.9±2.2	.3±1.4	1.8±1.7	1.2±1.2	−.6±.8	.577	.190	.012	.018
MFS	−.5±1.6	.3±1.9	.8±.7	−.2±.6	−.5±.7	−.2±.8	.535	<.001	.228	<.001
HOMA-IR score	6.6±8.7	5.3±3.6	−1.7±9.9	4.8±2.5	6.2±8.5	1.8±9.8	.449	.358	.500	.276
NASH CRN staging, n (%)										
Steatosis										
0	0 (.0%)	1 (2.9%)	−.4±.8	0 (.0%)	6 (35.3%)	−1.1±.9	.960	.098	.004	.006
1	13 (37.1%)	18 (51.4%)		6 (35.3%)	9 (52.9%)					
2	13 (37.1%)	14 (40.0%)		7 (41.2%)	2 (11.8%)					
3	9 (25.7%)	2 (5.7%)		4 (23.5%)	0 (.0%)					
Hepatocyte ballooning										
0	1 (2.9%)	0 (.0%)	.2±.7	0 (.0%)	2 (11.8%)	−.1±.9	.653	.335	.319	.203
1	19 (54.3%)	15 (42.9%)		8 (47.1%)	6 (35.3%)					
2	15 (42.9%)	20 (57.1%)		9 (52.9%)	9 (52.9%)					

(Continues)

TABLE 1 (Continued)

	RLI group ($n=35$)			SLI group ($n=17$)			*p*-value			
	BL (1)	EOI (2)	Δ (3)	BL (4)	EOI (5)	Δ (6)	1 vs. 4	1 vs. 2	4 vs. 5	3 vs.6
Lobular inflammation										
0	0 (.0%)	3 (8.6%)	−.2 ± .8	1 (5.9%)	3 (17.6%)	−.5 ± .8	.438	.296	.053	.198
1	18 (51.4%)	19 (54.3%)		10 (58.8%)	14 (82.4%)					
2	15 (42.9%)	12 (34.3%)		5 (29.4%)	0 (.0%)					
3	2 (5.7%)	1 (2.9%)		1 (5.9%)	0 (.0%)					
Fibrosis stage										
0	8 (22.9%)	5 (14.3%)	.2 ± 1.0	0 (.0%)	4 (23.5%)	−.9 ± .9	.166	.742	.037	<.001
1	15 (42.9%)	18 (51.4%)		8 (47.1%)	10 (58.8%)					
2	7 (20.0%)	5 (14.3%)		3 (17.6%)	0 (.0%)					
3	3 (8.6%)	3 (8.6%)		3 (17.6%)	3 (17.6%)					
4	2 (5.7%)	4 (11.4%)		3 (17.6%)	0 (.0%)					

Note: Data are expressed as means ± SD, or proportions.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BL, baseline; DBIL, direct bilirubin; EOI, end of intervention; FIB-4, fibrosis-4 test; Hb, haemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; MASH, metabolic dysfunction-associated steatohepatitis; MASLD, metabolic dysfunction-associated steatotic liver disease; MFS, MASLD fibrosis score; NASH CRN, Nonalcoholic Steatohepatitis Clinical Research Network; RBC, red blood cell; RLI, routine lifestyle intervention; SLI, strengthen lifestyle intervention; T2DM, type 2 diabetes mellitus; TBIL, total bilirubin; UA, uric acid; WBC, white blood cell; γ-GT, γ-glutamyl transpeptidase; Δ, change from baseline to end of intervention.

of liver fibrosis dynamics reflected that there was a significant fibrosis improvement in the periportal region in the SLI group (−.4288 ± .3281) compared to the RLI group (−.0566 ± .1724, $p=.03$, Figure 2A). As for other liver regions examined, there was no noteworthy difference between the two lifestyle intervention groups. SLI-induced liver fibrosis regression was mainly concentrated in the periportal region.

The representative images from BL to EOI obtained after scanning two individual patients with MASH, respectively, from the two lifestyle intervention groups, are shown in Figure 2B,C. As expected, the fibrosis area in the periportal region decreased from BL to EOI in the selected patients from both lifestyle intervention groups, and this reduction was even more pronounced in the SLI group.

3.3 | Improvement in metabolic parameters

The impact of lifestyle intervention on MASH patients was profound and comprehensive, with significant improvements observed across various clinical metabolic parameters. As shown in Figure 3, the positive trend was particularly pronounced among patients with declining fibrosis stages in the SLI group. Liver histological features showed significant post-intervention improvements in the SLI group, including a reduction in liver steatosis grade and fibrosis stage. Additionally, circulating levels of transaminase and non-invasive fibrosis biomarkers also decreased significantly.

In addition to the amelioration in liver histology features, the beneficial effects of lifestyle intervention extended to islet function and lipid levels in MASH patients, with significant improvements observed in the SLI group. Notably, improvements in various complications associated with MASH, such as obesity, diabetes, and hyperlipidaemia, were predominantly observed in the SLI group.

4 | DISCUSSION

Quantifying liver fibrosis level with qFibrosis may add new information that the current histological fibrosis staging system cannot capture. With enhanced lifestyle intervention, we observed regression of liver fibrosis, particularly in the periportal region, which was not detected with the conventional histological staging of fibrosis.

Lifestyle interventions mainly involve changes in diet and exercise. The fundamental principle of dietary intervention is to change the dietary composition and decrease caloric intake to a more favourable profile, thus reducing liver fat accumulation and lipid toxicity.[36] Compelling evidence indicates that physical activity reduces the risk of MASH beyond the effects of weight loss.[37] Potential mechanisms include regulation of oxidative stress, improvement in mitochondrial function, and attenuation of MASH activity.[38,39] In the context of our study, patients, particularly in the SLI group, demonstrated commendable adherence to lifestyle intervention. We observed that the SLI improved liver fibrosis by 65% from the baseline in patients with MASH. This positive outcome was consistent with weight loss, reduced liver steatosis and serum transaminase levels, and improved insulin resistance.

Within the RLI group, 20% of patients experienced fibrosis regression, a trend generally consistent with the effects observed in the placebo groups of several previous MASH clinical trials.[23,40,41] The potential explanations for this phenomenon may be as follows. Firstly, we have reasonable grounds to believe that every participant

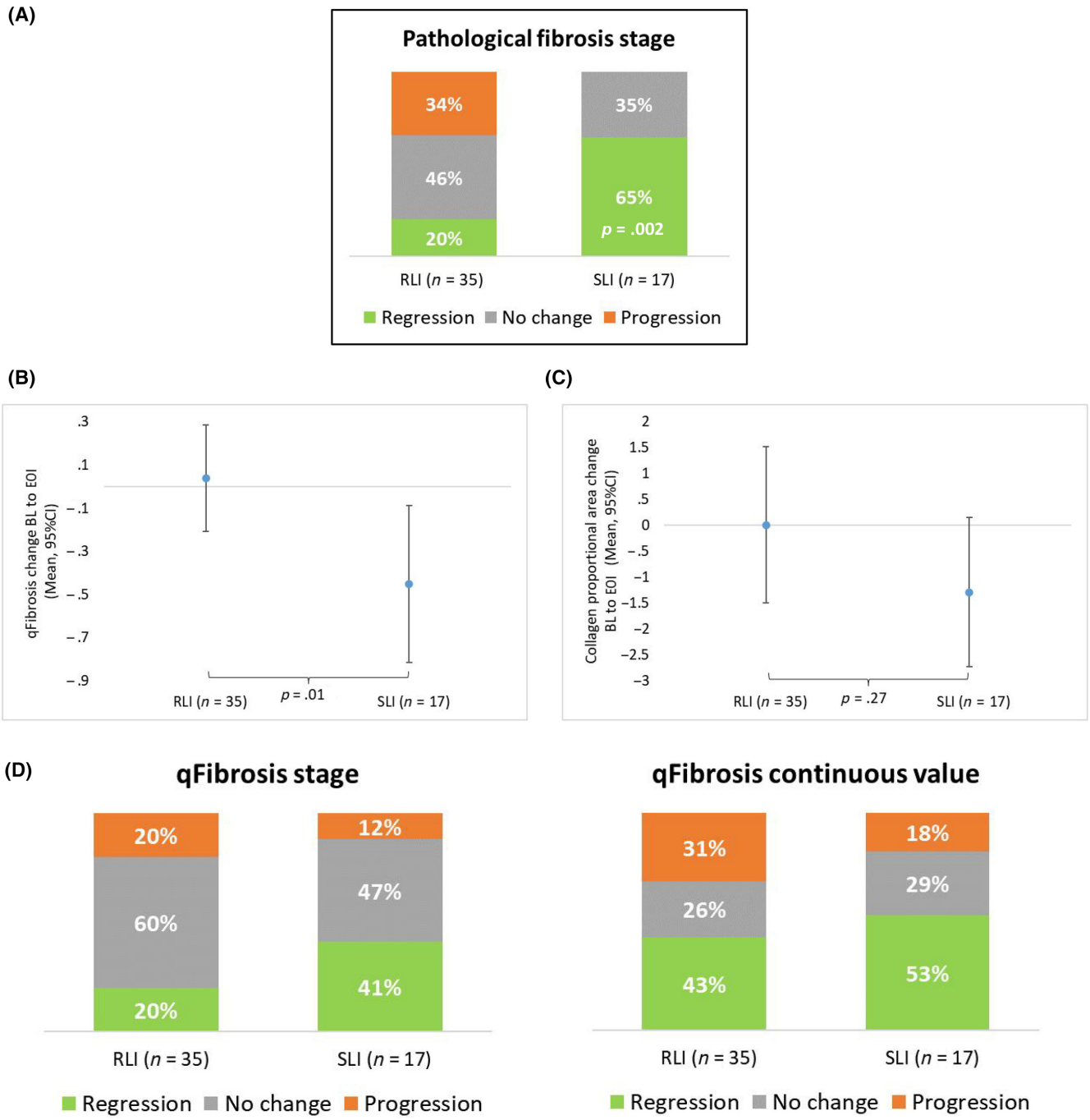
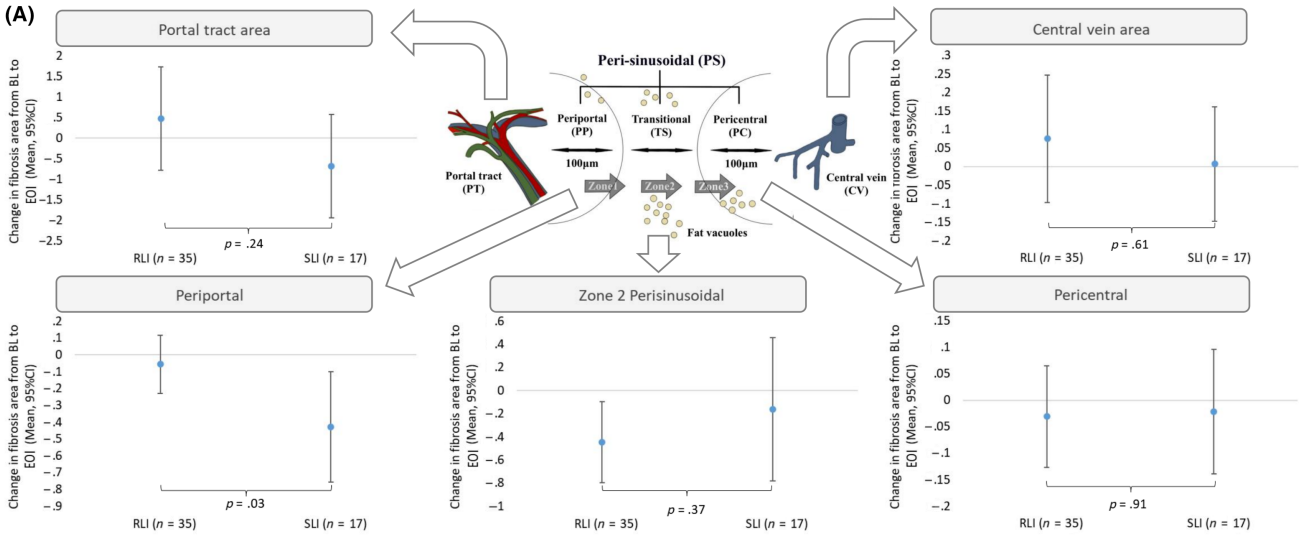
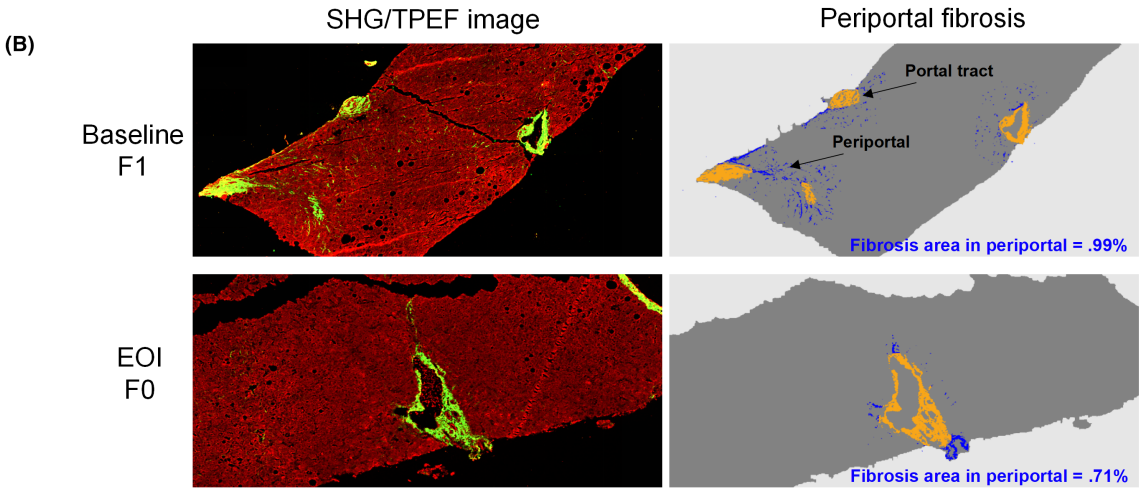


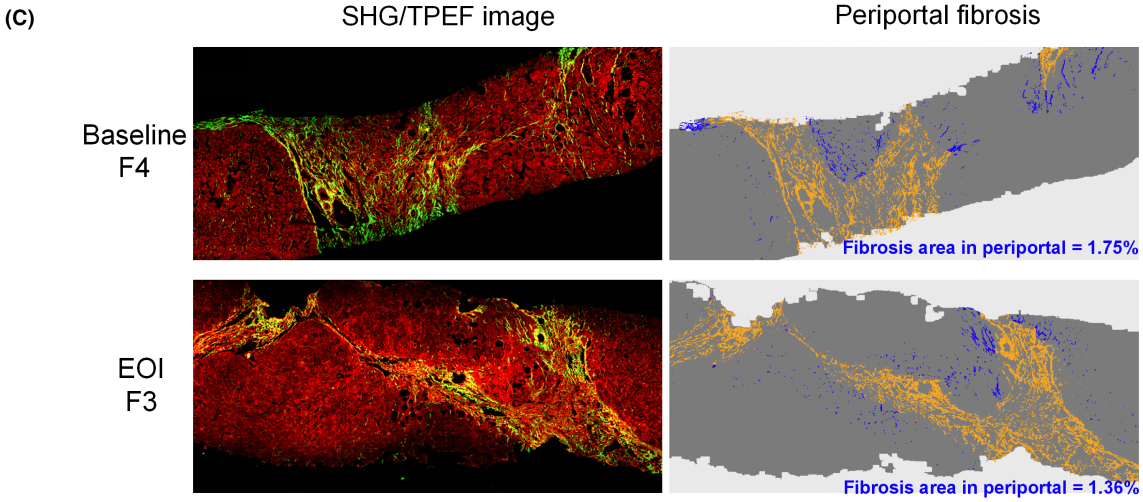
FIGURE 1 Digital assessment of liver fibrosis in patients with MASH undergoing two different lifestyle interventions. (A) Based on the NASH CRN score, P/N/R analysis of liver fibrosis changes from BL to EOI. Changes of qFibrosis (B) and CPA (C) from BL to EOI in the two lifestyle intervention groups. (D) P/N/R analyses of fibrosis changes from BL to EOI, based on the qFibrosis stage and qFibrosis continuous value. BL, baseline; CPA, collagen proportionate area; EOI, end of intervention; NASH CRN, Nonalcoholic Steatohepatitis Clinical Research Network; P/N/R, Progressive/No change/Regressive; RLI, routine lifestyle intervention; SLI, strengthened lifestyle intervention.

in the clinical trial, irrespective of the assigned group, would adopt the lifestyle recommendations. Secondly, patients within the placebo group are likely to modify their behaviour as a response to the attention they receive due to being under observation. Furthermore, as the study progresses, more frequent follow-up visits might lead patients to perceive heightened attention, enhancing the probability of behaviour change. In summary, we consider that the RLI group showed a similar placebo response to that expected in patients in a placebo group in a placebo-controlled clinical trial with awareness of MASH conditions. If the patients take on significant lifestyle changes, the fibrosis regression rate can be as high as 65%, as shown in the SLI group. The AI-based digital pathology showed similar results. Notably, our study suggests the potential to differentiate between RLI and SLI patients within the placebo group in MASH





RLI Patient with fibrosis regression by NASH CRN



SLI Patient with fibrosis regression by NASH CRN

FIGURE 2 Lifestyle intervention-induced fibrosis regression in the periportal region. (A) Zonal fibrosis quantitation assesses the fibrosis area change before and after intervention in each region. Representative cases from BL to EOI in the RLI group (B) and the SLI group (C), as visualized by SHG/TPEF microscopy. On the left is the scanned image with a green colour representing fibrosis. On the right side, the orange colour represents fibrosis within the portal region, while the blue colour represents fibrosis within the periportal region. BL, baseline; EOI, end of intervention; RLI, routine lifestyle intervention; SLI, strengthen lifestyle intervention; SHG/TPEF, second harmonic generation/two-photon excitation fluorescence; NASH CRN, Nonalcoholic Steatohepatitis Clinical Research Network; P/N/R, Progressive/No change/Regressive.

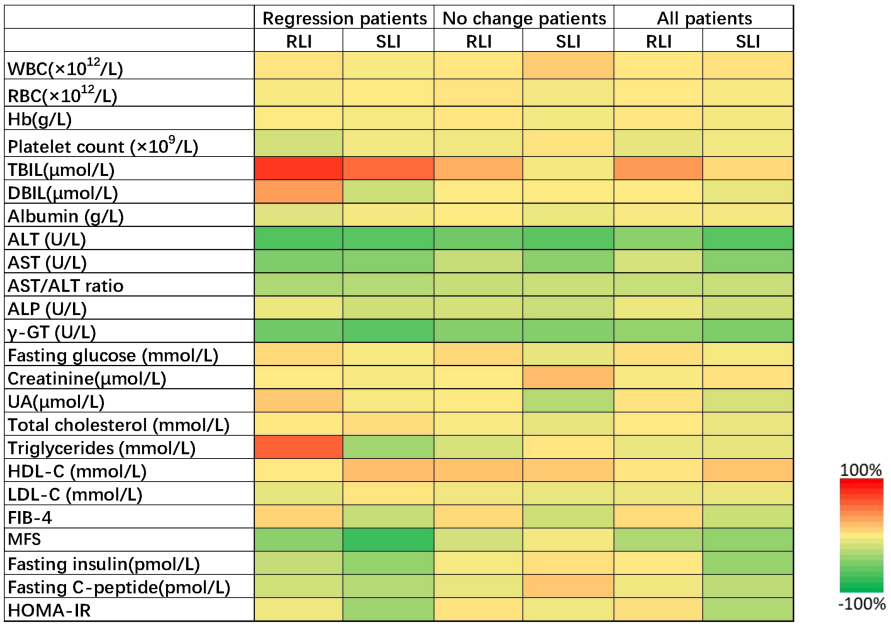


FIGURE 3 Relative changes of clinical biochemical markers' median from BL to EOI within the two lifestyle intervention groups (the RLI and SLI groups) according to liver fibrosis regression, no fibrosis change, and all patients. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BL, baseline; DBIL, direct bilirubin; EOI, end of intervention; FIB-4, fibrosis-4 test; Hb, haemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; MASLD, metabolic dysfunction-associated steatotic liver disease; MFS, MASLD fibrosis score; RBC, red blood cell; RLI, routine lifestyle intervention; SLI, strengthen lifestyle intervention; TBIL, total bilirubin; UA, uric acid; WBC, white blood cell; γ-GT, γ-glutamyl transpeptidase.

clinical trials. This differentiation could be achieved with changes of fibrosis area in the periportal region, using AI-based digital pathology techniques.

Currently, the CRN staging system has characterized liver fibrosis progression, starting from an initial chicken-wire pattern in the perisinusoidal region (F1), advancing to perisinusoidal plus portal fibrosis (F2), bridging fibrosis (F3) and, ultimately, cirrhosis (F4).[42,43] We hypothesized that liver fibrosis regression follows a reverse sequence. In our study, a significant proportion of patients were classified as having fibrosis stage F1, resulting in a more evident liver fibrosis regression in the periportal area of the perisinusoidal region.

The findings of a previous study using digital pathology to investigate liver fibrosis regression induced by tropifexor revealed a more pronounced fibrosis regression in the perisinusoidal region (predominantly in the transitional region), thus showing slight disparities compared with our study.[34] Some hypotheses could explain these apparent discrepancies. Firstly, there might be objective differences in the outcomes induced by these two interventions, which could have implications for guiding therapy. This provides compelling evidence to support the combination of lifestyle intervention with drug treatment for MASH. Secondly, discrepancies between the two study populations might have contributed to the results. The previous study primarily concentrated on MASH patients with F2 and F3 fibrosis stages, whereas most of our patients were classified at the F1 stage.[34] Further research is needed to investigate patients with MASH at different fibrosis stages. In this previous study, 29% of patients with F2 stage and 18% with F3 stage in the placebo group experienced fibrosis regression, mainly in the perisinusoidal region.[34] These results closely paralleled those we observed in the RLI group, substantiating the above conclusions.

While our exploratory study has yielded valuable insights, it is essential to acknowledge certain limitations that may influence the interpretation of results. Firstly, the sample size employed in this study was relatively small, which could potentially diminish the extent of differences between the two lifestyle intervention groups. However, the acquisition of liver biopsy specimens was challenging

for subjects undergoing a lifestyle change intervention. Thus, our study had a sufficient sample size for a lifestyle intervention study with baseline and end of intervention liver biopsies. Secondly, the study was conducted exclusively in two centres in China. Future research endeavours should incorporate a more geographically diverse cohort to enhance the generalizability of the findings. Lastly, the intensity of lifestyle intervention was not precisely quantified. Lifestyle intervention inherently involves multifaceted changes encompassing diet, exercise, and other behavioural modifications, making precise quantification challenging. Consequently, we adopted a pragmatic approach in this study, and patients were categorized into the RLI and SLI groups.

In conclusion, the results of our exploratory study provide new insights into the effect of lifestyle intervention in Chinese adults with biopsy-confirmed MASH. We could detect a more pronounced fibrosis regression with SLI using digital pathology, mainly in the periportal region. The AI-based digital pathology enhances comprehension of lifestyle-induced fibrosis regression in MASH, which is not captured by conventional histological staging. However, it is imperative to stress the necessity for future clinical research support to strengthen and verify these findings in other ethnic groups.

AUTHOR CONTRIBUTIONS

Hai-Yang Yuan, Xiao-Fei Tong, Yang-Yang Li, Sui-Dan Chen, Li-Li Chen, Xiao-Dong Wang, Hong You, and Ming-Hua Zheng contributed to data collection and resource acquisition. Hai-Yang Yuan, Ya-Yun Ren, Xin-Lei Wang, and Ming-Hua Zheng made contributions to the analysis and interpretation of data. Hai-Yang Yuan, Ya-Yun Ren, Xin-Lei Wang, and Li-li Chen worked on visualization of data. Hai-Yang Yuan drafted the manuscript. Giovanni Targher, Christopher D Byrne, Lai Wei, Vincent W.-S. Wong, Dean Tai, Arun J. Sanyal, Hong You, and Ming-Hua Zheng were involved in revising the manuscript critically for important intellectual content. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST STATEMENT

Ming-Hua Zheng has received honoraria for AstraZeneca, Hisky Medical Technologies, and Novo Nordisk lectures and consulting fees from Boehringer Ingelheim. Arun J. Sanyal has served as a consultant to Path-AI, HistoIndex, Fibronest, Biocellvia, Merck, Pfizer, Eli Lilly, Novo Nordisk, Boehringer Ingelheim, AstraZeneca, Akero, Intercept, Madrigal, Northsea, Takeda, Regeneron, Genentech, Alnylam, Roche, GlaxoSmithKline, Novartis, Tern, Fractyl, Inventiva, Gilead and Target Pharmasolutions, has stock options in Genfit, Tiziana, Durect, Inversago and Hemoshear, and receives royalties from Uptodate and Elsevier. His institution has received grants from Intercept, Pfizer, Merck, Bristol Myers Squibb, Eli Lilly, Novo Nordisk, Boehringer Ingelheim, AstraZeneca, Novartis, and Madrigal. Christopher D. Byrne has received an independent research grant for research on MASLD from Echosens, France. Vincent W-S Wong served as a consultant or advisory board member for AbbVie, Boehringer Ingelheim, Echosens, Gilead Sciences, Intercept, Inventiva, Novo Nordisk, Pfizer, Sagimet Biosciences, and TARGET PharmaSolutions; and a speaker for Abbott, AbbVie, Gilead Sciences, and Novo Nordisk. He has received a grant from Gilead Sciences for fatty liver research and is a co-founder of Illuminatio Medical Technology Limited. Christopher D Byrne has received an Independent Research Grant from Echosens. Other authors have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data are not publicly available due to privacy or ethical restrictions. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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