**Liver Damage Reversal in Severe Obesity: Histological Insights from AI-powered Software**

Short title

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**Background & Aims:** Metabolic dysfunction-associated steatohepatitis (MASH) is a progressive liver disease characterized by steatosis, inflammation, hepatocellular injury, and fibrosis that may lead to cirrhosis or hepatocellular carcinoma. While laparoscopic sleeve gastrectomy (LSG) effectively treats severe obesity and improves liver histology, traditional pathological assessments rely on subjective evaluations. This study aimed to objectively assess LSG impact on MASH histological outcomes using artificial intelligence (AI)-based digital pathology.

**Methods:** We conducted a retrospective cohort study analyzing paired liver biopsies from 44 patients who underwent secondary surgical intervention at Hospital Universitari Sant Joan in Reus due to insufficient weight loss or weight regain after initial LSG. Wedge biopsies were obtained using standardized techniques identical to those employed in the initial procedure. The liver tissue sections were digitized and analyzed using the Liver Explore AI-platform to quantify hepatic cellular features, tissue architecture, and collagen characteristics.

**Results:** LSG-induced weight loss significantly reduced hepatic steatosis (*p* = 4.3×10−10) and hepatocellular ballooning (*p* = 1.4×10−4). Although overall fibrosis scores showed no significant change, specific fibrotic subtypes demonstrated marked improvement, with significant reductions observed in periportal fibrosis (*p* = 9.2×10−5), incomplete septal fibrosis (*p* = 4.6×10−5), and complete septal fibrosis (*p* = 2.5×10−4). Post-surgical changes included increased neutrophil (*p* = 3.7×10−5) and eosinophil (*p* = 0.001) infiltration, with zonal redistribution of immune cells throughout the liver parenchyma, indicating comprehensive reorganization of the hepatic immune landscape.

**Conclusions:** AI-enhanced digital pathology enables objective assessment of hepatic changes post-bariatric surgery. LSG significantly improves key MASH features. The post-surgical increase in neutrophils and eosinophils along with zonal immune reorganization suggests immune-mediated tissue remodeling contributing to hepatic recovery. These findings support LSG efficacy for MASH treatment and demonstrate AI utility in capturing complex liver pathological changes.

Bariatric Surgery, Digital Imaging, Immune System**,** MASH Remission, Liver Zonation.

**Impact and Implications:**

**-**

**-**

**-**

**Highlights (separate editable file):**

**Introduction**

Obesity is a complex and progressive chronic disease that has a growing negative impact on healthcare. In the United States, over 10% of individuals with obesity fall into the severe obesity category, which is defined as having a body mass index (BMI) of 40 kg/m² or higher 1,2. This condition is associated with a higher risk of mortality due to a greater incidence of cardiovascular disease, cancer, liver disease, and other life-threatening metabolic complications 3,4. Effectively managing severe obesity requires a deeper understanding of the risks and mechanisms related to weight-associated diseases. One specific condition, metabolic dysfunction-associated steatotic liver disease (MASLD), is common among these patients and can progress to metabolic dysfunction-associated steatohepatitis (MASH), fibrosis, and complications such as cirrhosis or hepatocellular carcinoma 5,6. The urgent need for timely diagnosis and intervention for patients with MASH highlights the importance of personalized weight loss treatment strategies 7–9.

              Significant weight loss in cases of severe obesity through well-designed, structured, and comprehensive lifestyle intervention programs is often anecdotal. Several medications are now available for the long-term treatment of obesity. Notably, patients treated with Semaglutide can expect to lose an average of 6% to 12% of their initial body weight, with the potential benefit of improving liver histology in patients with MASH 10,11. While bariatric procedures can be effective, they also have potential drawbacks and are typically considered only when other weight-loss options fail to meet therapeutic goals. The most commonly performed bariatric surgeries are laparoscopic sleeve gastrectomy, which accounts for 60.4% of procedures, and Roux-en-Y gastric bypass, which makes up 25.5%. These procedures aim for target weight losses of 25% to 30% and 30% to 35%, respectively 12. Such losses do not resolve obesity but are effective for managing MASH 6–9,13,14. Demonstrating long-term efficacy remains challenging, and real-world data indicate that MASH improvements may not solely depend on weight loss 15. The past decades of research have crystallized in the first drug conditionally approved for treating MASH on March 14, 2024, and weight loss was negligible 16.

Repeated liver biopsies are the main hurdle in MASH drug development and are subject to intensive research and reassessment 17. Regulatory pathways strongly rely on demonstrating MASH resolution without worsening fibrosis and improving fibrosis without worsening MASH 18. Among other challenges, the increase in consistency of the central histology reading process will likely mitigate the impact of inter- and intra-reader variability 19. The current transition from glass to digital methods combines digitization with artificial intelligence (AI) to develop technologies that allow fully automatic diagnosis 20–23.

In this study, we employed these innovative tools for the first time for to evaluate a clinically significant yet understudied cohort: patients with severe obesity requiring two-stage bariatric surgery due to weight regain or inadequate initial weight loss 24. By employing comprehensive histological assessment tools, we provide novel insights into the long-term hepatic outcomes of LSG, addressing a critical knowledge gap in this complex patient population.

**Materials and Methods**

*Study Design and Participants*

This retrospective, longitudinal and observational study was registered under ClinicalTrials.gov number NCT05554224. We obtained paired liver biopsy samples from 44 patients who underwent primary LSG and subsequently required revision bariatric surgery due to insufficient weight loss or weight regain at Hospital Universitari Sant Joan in Reus, Spain.

At the time of the first intervention, all included patients were over 18 years old, had a BMI of 40 kg/m² or higher and presented high-risk comorbidities, such as a diagnosis or treatment for hypertension, dyslipidemia, or type 2 diabetes mellitus. All patients had received positive psychiatric evaluations for bariatric surgery procedure. We excluded patients with clinical or laboratory evidence of chronic or acute inflammation, infectious diseases, severe illness, hepatitis diagnosis or current cancer.

*Sampling and histological procedures*

Hepatic wedge biopsies were formalin-fixed and paraffin-embedded to produce 4 µm slides for histologic evaluation. Routine staining included hematoxylin and eosin (H&E) and Masson's trichrome for assessment of steatosis, hepatocellular ballooning, lobular inflammation, and fibrosis.

Additionally, immunohistochemical (IHC) staining was performed using the fully automated Ventana BenchMark ULTRA system (Roche Diagnostics Corporation, Indianapolis, IN, USA) in combination with the OptiView DAB IHC Detection Kit (Roche, Spain). To detect neutrophils, a specific primary antibody against CD15 was used (CONFIRM anti-CD15 [MMA], mouse monoclonal, Roche). This procedure follows the established routine diagnostic protocol at our hospital. Following antigen retrieval, tissue sections were incubated with the primary antibody, followed by the appropriate secondary antibody. Signal detection was performed using DAB chromogen, and the slides were counterstained with hematoxylin. Positive and negative controls were included in each staining batch to ensure the quality and specificity of the results.

An expert hepatologist evaluated all samples using Kleiner scoring parameters 17. The NAFLD Activity Score (NAS) was calculated by summing scores for steatosis, hepatocellular ballooning, and lobular inflammation. Patients were classified based on their NAS as follows: those with scores of 2 or lower were categorized as non-MASH (n = 1 at the first intervention and n = 27 at the second intervention), those with scores of 3 or 4 were classified as uncertain MASH (n = 4 at the first intervention and n = 10 at the second intervention), and those with scores of 5 or higher were classified as MASH (n = 39 at the first intervention and n = 7 at the second intervention).

Following initial evaluation, slides were digitized using Ventana DP 200 scanner (Roche, Basel, Switzerland) at 40x magnification. H&E and Masson's trichrome slides were analyzed using the AI-based Liver Explore platform (research use only), while IHC slides were processed using the open-source software QuPath.

This study adhered to Good Clinical Practice guidelines and the Declaration of Helsinki, with Institutional Review Board approval under reference number EOMPI2024[244/2024]. Written informed consent was obtained from all participants.

*Digital image analysis*

*Liver Explore* (this paragraph should be written by the PathAI team)

IHC-stained slides were analyzed using QuPath (version 0.5.1), an open-source software for digital pathology analysis. Whole tissue sections were manually annotated as regions of interest (ROIs), and artifacts were excluded through careful visual inspection. Following ROI selection, the Positive Cell Detection tool was applied using a single intensity threshold to identify and classify CD15+ and CD15- cells. After cell detection, quantitative data for each slide was exported for further analysis. Full methodological details, including parameter settings and thresholds used, are provided in the Supplementary Materials.

*Statistical analyses*

All statistical analyses were performed using RStudio (version 2024.09.0.375) and R (version 4.4.2). Continuous variables were described using medians and interquartile ranges (IQRs), while categorical variables were reported as counts and percentages. Because most variables did not satisfy the assumptions of normality, non-parametric statistical methods were employed for comparisons.

Specifically, the Wilcoxon rank-sum test was used for comparisons involving continuous variables between two groups. For categorical data, the Chi-Squared test was utilized. In instances where comparisons were made across more than two groups, the Kruskal-Wallis test was applied. Statistical significance was set at p ≤ 0.05.

Data visualization and graphical outputs were created in R and subsequently refined using Inkscape software (version 1.4.2) to enhance visual presentation quality.

To accurately assess changes in fibrosis proportionate area between interventions, a steatosis correction factor was implemented. This adjustment was necessary to eliminate potential bias arising from the marked difference in steatosis content between first and second interventions. The corrected fibrosis proportion was calculated by dividing the area of each fibrosis subtype by the difference between total tissue area and steatosis area *(Fibrosis Subtype Area / [Total Tissue Area - Steatosis Area])*. This correction ensures a more precise evaluation of fibrosis changes by accounting for the substantial reduction in steatosis following bariatric surgery.

**Results**

1. *Participants*

Table 1 outlines the selected demographic and clinical characteristics of participants from the same cohort, comparing the 1st and 2nd intervention groups. The sample predominantly consisted of female participants. And, as expected, participants in the second intervention group were significantly older than those in the first intervention group, with a *p*-value of 0.024. Similarly, BMI varied significantly between the two interventions, with the 1st intervention showing a higher median BMI (52.4 kg/m²) compared to the 2nd intervention (40.6 kg/m²), with a highly significant *p*-value of 6x10⁻¹³.

Histopathological assessment revealed significant differences in liver damage parameters between the two intervention groups. Steatosis severity was notably higher in the first intervention group, with a greater proportion of participants showing severe steatosis (>33% and >66%) compared to the second intervention group (*p*-value = 9.5×10⁻⁹). Similarly, hepatocellular ballooning was more prevalent in the first intervention group, with a higher number of participants exhibiting many ballooned hepatocytes (*p*-value = 1.7×10⁻⁶).

The analysis of fibrosis patterns revealed distinct distributions between groups, although the overall difference was not statistically significant (*p*-value = 0.077). The first intervention group showed a higher prevalence of bridging fibrosis (F3), while the second intervention group demonstrated greater frequencies of perisinusoidal and periportal fibrosis (F2). Lobular inflammation levels remained comparable between groups (*p*-value = 0.542).

The distribution of MASH diagnoses differed significantly between the two interventions, with a *p*-value of 2.3×10⁻¹¹. Figure 1 illustrates the proportional distribution of participants diagnosed with MASH, non-MASH, and uncertain MASH status across both groups.

1. *Bariatric Surgery Induces Significant Changes in Hepatic Cellular Composition*

Sleeve gastrectomy led to substantial alterations in hepatic cellular distribution (Figure 2). The proportion of normal hepatocytes showed significant improvement, rising from 39% to 48% of the total tissue (*p*-value = 0.019), suggesting a positive restoration of liver parenchyma. Additionally, there was a significant reduction in steatotic hepatocytes, which decreased from 6.7% to 0.3%, with a *p*-value of 4.3x10⁻¹⁰. Similarly, ballooned hepatocytes—typically associated with advanced liver damage 25—declined from 2.53x10-4% to 0% of the total evaluated tissue (*p*-value = 1.4x10⁻⁴). This reduction indicates that surgical-induced weight loss not only alleviates fatty infiltration but also reverses more severe forms of liver cell injury, contributing to a healthier liver phenotype.

The fibroblast population remained stable between the interventions with a *p*-value of 0.309 (Table 2). In contrast, the other hepatocytes—those not categorized as steatotic, ballooned, or normal—showed a significant decrease from 5.1% to 0.03% after the first intervention (*p*-value = 9 x 10⁻¹¹).

Unexpectedly, surgery-induced weight loss led to an increase in the proportion of immune cells, rising from median proportion of 27% to 31% of the total evaluated tissue (*p*-value = 0.019). This change suggests that the immune system may play a key role in the ongoing tissue remodelling process following the initial phase of weight loss, highlighting the complex interplay between inflammation and recovery in the liver. The remaining percentage of the evaluated tissue, accounting for the balance up to 100%, was classified as "other cells".

1. *Zonal Redistribution of Hepatic Steatosis and Ballooning After Bariatric Surgery*

As Figure 3 illustrates, the zone with the highest lipid accumulation prior to bariatric surgery was Zone 3. In this zone, the median proportion of steatotic hepatocytes relative to all cells in the evaluated tissue was 11.4%. Following the intervention, this proportion significantly decreased (*p*-value = 1.5x10⁻⁸), dropping to 0.4%. This reduction marked the most pronounced decrease in steatotic hepatocytes across all liver zones. Zone 2 also exhibited a significant reduction in steatosis, with the initial percentage being 9.5%, which decreased to 0.3% after the intervention (*p*-value = 1.1x10⁻⁹). The zone with the lowest proportion of steatotic hepatocytes prior to surgery was Zone 1, which initially presented 3.9%. After the surgical procedure, the proportion decreased significantly (*p*-value = 9.2x10⁻8), reaching 0.3%—a similar reduction to that observed in Zones 2 and 3. Interestingly, after the intervention, the median percentages of steatotic cells became similar across the three liver zones, indicating a uniform reduction in this liver damage parameter.

Regarding ballooning, the zone with the highest prevalence of ballooned hepatocytes before the LSG was Zone 3, with a mean percentage of 6.54x10⁻3%. After the intervention, this proportion decreased to 0%, which was statistically significant (*p*-value = 0.011). Zone 2 followed in terms of ballooned hepatocyte abundance prior to surgery, with an initial percentage of 4.56x10⁻3%. After the surgical-induced weight loss, this proportion significantly decreased to 1.58x10-3% (*p*-value = 0.014). Interestingly, Zone 1, which initially had the lowest percentage of ballooned hepatocytes (4.53x10⁻3%), did not show a significant reduction after surgery. The percentage decreased to 3.79x10⁻3%, with a *p*-value of 0.058, which did not reach statistical significance. This suggests that while surgical weight loss has a notable effect on ballooning in Zones 2 and 3, its impact in Zone 1 is less pronounced, potentially indicating different pathophysiological processes in the different hepatic zones.

1. *The Immune System Reorganization After Surgery-Induced Weight Loss: The key role of Polymorphonuclear Cells*

Figure 4a depicts significant changes in the immune system microenvironment within the liver following surgery-induced weight loss. The overall proportion of immune cells, increased significantly from 28.9% to 34.3% of total tissue cells, with a *p*-value of 0.019.

Prior to surgery, lymphocytes constituted the predominant immune cell population, followed by macrophages, neutrophils, plasma cells, and eosinophils. Post-surgery, while lymphocytes maintained their predominance, neutrophils emerged as the second most abundant population, followed by macrophages, plasma cells, and eosinophils.

Interestingly, the proportion of macrophages did not change significantly following the initial intervention, with the proportion remaining almost the same—7.3% in the first intervention and 7.1% in the second intervention (*p*-value = 0.463). Similarly, lymphocytes showed no significant change post-surgery, maintaining a consistent 14% with a *p*-value of 0.745. On the other hand, plasma cells exhibited a tendency to increase after the weight loss procedure, rising from 1.9% in the first intervention to 2.5% in the second. However, this change did not reach statistical significance (*p*-value = 0.089), suggesting that the increase in plasma cells may not represent a dominant response to the weight loss due to the sleeve gastrectomy.

In contrast, neutrophils showed a significant increase in proportion after the intervention, rising from 2.9% to 5.1% of the total tissue, with a *p*-value of 3.7x10⁻⁵. Notably, this increase occurred independently of the time elapsed between the two interventions (Figure S1), underscoring their potential role in the liver’s response to surgery-induced weight loss. Eosinophils also showed a significant increase in proportion, rising from 0.3% to 0.5% of the total tissue, with a *p*-value of 0.001.

To further validate the observed increase in neutrophils, we performed IHC staining for CD15⁺ cells in liver tissue samples from both timepoints (Figure 4b). CD15⁺ cells increased from 1.2% to 3.2% (*p*-value = 3.8x10⁻⁶). Additionally, the proportion of CD15⁺ cells strongly correlated with the computationally inferred neutrophil abundance (ρ = 0.68, *p*-value = 7.18x10⁻¹²), supporting the reliability of both analytical approaches and reinforcing the notion that neutrophil recruitment is a prominent feature of the liver's immune adaptation following sleeve gastrectomy.

Although the most significant changes in immune cell quantity are observed in polymorphonuclears, the immune system, as a whole, undergoes notable alterations following surgery-induced weight loss. These changes are evident not only in the overall abundance of certain immune cells but also in their distribution across the different liver zones, suggesting a broad reorganization of the immune landscape (Figure 5).

In Zone 1, the region closest to the portal spaces, there was a notable increase in the proportions of eosinophils and neutrophils following the intervention. Specifically, eosinophils rose from 1% to 1.5% (*p*-value = 0.004), while neutrophils showed a significant increase from 10.3% to 19.5% (*p*-value = 6x10⁻⁶). In contrast, macrophages decreased from 31.4% to 23.1%, with a p-value of 4x10⁻⁶. Lymphocytes and plasma cells did not exhibit statistically significant changes after the weight loss.

In Zone 2, located between Zones 1 and 3, and the one where the most substantial statistically significant changes occur. Neutrophils increase from 11.7% to 20% of the total immune cells (*p*-value = 2.6x10⁻⁵), plasma cells rise from 4.6% to 8.5% (*p*-value = 0.002), and eosinophils increase from 1% to 1.7% (*p*-value = 0.001). In contrast, following the same trend observed in Zone 1, macrophages decrease from 30.2% to 22.6% (*p*-value = 1.8x10⁻⁵), while lymphocytes remain unchanged.

In Zone 3, the region closest to the centrilobular veins, eosinophils and lymphocytes show no significant changes. Neutrophils increase from 12.5% to 18.7% (*p*-value = 0.006), plasma cells also increase from 4.5% to 6.8% in the second intervention (*p*-value = 0.038), and macrophages decrease significantly from 29.5% to 24.5% (*p*-value = 0.023).

At the portal spaces, where blood enters the liver, a significant increase in neutrophils is observed, rising from 12% to 20.2% with a p-value of 0.003. Macrophages and plasma cells significantly decrease from 30% to 26.3% (*p*-value = 0.021), and from 17% to 12.3% (*p*-value = 0.040), respectively. Eosinophils and lymphocytes show no statistically significant changes.

Overall, these findings highlight the dynamic reorganization of the immune system within the liver following bariatric surgery. While eosinophils and neutrophils exhibit prominent increases in the total amount, there are also significant shifts in the distribution of the other studied immune cells. These changes underscore the complex immune responses occurring in the liver after weight loss and suggest that immune cell redistribution may play a significant role in the liver's recovery and reparative processes.

1. *Weight Loss Modulates Fibrosis Distribution*

Pathological fibrosis includes several distinct subtypes, each with varying responses to surgical intervention. Periportal fibrosis occurs around the portal tracts, while perisinusoidal fibrosis affects the space of Disse, the area between liver endothelial cells and hepatocytes. Incomplete septal fibrosis is an early stage of complete septal fibrosis, where fibrotic tissue begins to bridge lobules without forming complete fibrous septa. Complete septal fibrosis signifies advanced scarring, characterized by thick septa of fibrous tissue that disrupt normal liver architecture and can progress to cirrhosis. The most severe form is nodular fibrosis, marked by the formation of regenerative nodules surrounded by fibrous tissue, typically observed in cirrhosis and chronic liver damage 26,27(Figure S2).

Significant changes in fibrosis are observed following weight loss induced by bariatric surgery, as depicted in the upper section of Figure 6. Identifying these changes using conventional H&E staining is challenging; however, the AI-powered overlay algorithm enhances visibility, allowing for a clearer and more accurate assessment of fibrosis. The overall area proportion of pathological fibrosis tends to increase after surgery; however, this decrease does not reach statistical significance, with a *p*-value of 0.88.

Analyzing specific subtypes reveals no significant changes in perisinusoidal fibrosis post-surgery, evidenced by a *p*-value of 0.10, suggesting stability across both pre- and post-surgery samples. In contrast, periportal fibrosis significantly decreases, dropping from 0.06% to 0.02% of the total tissue area, with a *p*-value of 9.2x10-5. Similarly, incomplete septal fibrosis also shows a statistically significant decrease, falling from 0.06% to 0.02% (*p*-value = 4.6x10⁻5), further supporting the trend of fibrosis reduction post-surgery. Among advanced fibrosis subtypes, complete septal fibrosis experiences a marked reduction, decreasing from 0.14% to 0.05% of the total tissue area, with a *p*-value of 2.5x10-4.

Conversely, nodular fibrosis shows a tendency to increase following the intervention. Before surgery, nodular fibrosis accounted for 0.3% of the total tissue area, while after the surgical intervention and subsequent weight loss, this proportion increased to 0.6%, with a *p*-value of 0.18.

Figure S3 illustrates the interactions between various immune cell types and different types of fibrosis. Notably, in the 40μm area surrounding perisinusoidal fibrosis, significant changes in immune cell types were observed. Macrophages and lymphocytes showed a significant decrease (*p*-value = 2.5x10⁻⁵ and *p*-value = 0.026, respectively), while eosinophils, neutrophils, and plasma cells significantly increased (*p*-value = 6.5x10⁻⁵, *p*-value = 2x10⁻⁴, and *p*-value = 0.006, respectively).

In the 40μm area surrounding periportal fibrosis region, significant changes were primarily seen in macrophages, which decreased post-surgery (*p*-value = 0.006), and neutrophils, which significantly increased (*p*-value = 0.003). Adjacent to incomplete septal fibrosis, both eosinophils (*p*-value = 0.013) and neutrophils (*p*-value = 9.3x10⁻³) increased significantly, while macrophages significantly decreased with a *p*-value of 3.8x10⁻⁴.In the area surrounding complete septal fibrosis, macrophages and plasma cells both showed significant decreases (*p*-value = 0.012 and *p*-value = 0.018, respectively), while neutrophils significantly increased (*p*-value = 6.1 x 10⁻⁴). Finally, around nodular fibrosis, following the pattern observed in other regions, macrophages decreased significantly (*p*-value = 0.043), while neutrophils increased (p-value = 1.5x10⁻³). Additionally, there was a significant decrease in lymphocytes with a p-value of 0.039.

Focusing specifically on the fibrotic regions, plasma cells were the only immune cell type to show significant changes in complete septal fibrosis decreasing after surgery with a p-value of 0.024. And, in nodular fibrosis, neutrophils significantly increased post-surgery (*p*-value = 3.8 x 10⁻³), while lymphocytes significantly decreased (*p*-value = 0.020).

**Discussion**

Bariatric surgery improves liver health in patients with severe obesity. Underlying mechanisms remain unknown. While liver biopsy remains the gold standard for evaluating MASH resolution, significant limitations exist at both the procedural and analytical levels that have constrained our ability to characterize post-surgical hepatic changes comprehensively. The invasive nature of the biopsy procedure, coupled with sampling variability and the practical and ethical impediments of obtaining repeated biopsies, creates substantial barriers to longitudinal assessment of hepatic recovery28–31. These procedural challenges are further compounded by the limitations of traditional histological evaluation methods, which, while valuable, are constrained by their subjective nature, inter-observer variability, and inability to capture the complex spatial dynamics and cellular heterogeneity that characterize hepatic tissue remodeling32,33. This dual limitation is particularly significant given that MASH currently affects between 1.5 and 6.45% individuals globally and is projected to become the leading cause of liver transplantation by 203034–37. AI-enhanced digital pathology may represent a valuable tool to address these limitations by providing more objective, comprehensive analysis while maximizing diagnostic yield from available samples.

Our study addresses these knowledge gaps through the application of AI-enhanced digital pathology analysis to paired liver biopsies before and after weight loss induced by LSG. This novel approach provides unprecedented insights into cellular and immune dynamics following bariatric surgery through advanced computational tools that enable precise quantitative assessment while preserving critical spatial context for understanding tissue-level processes. We examined a well-characterized cohort of patients with severe obesity who underwent sequential bariatric procedures, with liver biopsies obtained before and after the initial LSG intervention. Despite achieving significant BMI reductions between procedures, patients remained classified as severely obese after LSG-induced weight loss. Despite this persistent severe obesity classification, patients demonstrated significant improvements across multiple parameters of liver health, most notably in steatosis and ballooning with a *p*-value < 0.001, as described in previous literature.29,38,39

Unlike fibrosis in other organs such as the heart, lungs, or kidneys, hepatic fibrosis has shown remarkable potential for reversibility through mechanisms involving myofibroblast apoptosis, matrix metalloproteinase activation, and collagen degradation40–42. This unique regenerative capacity provides important context for interpreting our fibrosis findings. Although overall fibrosis changes did not reach statistical significance in our study, we observed meaningful improvements in specific subtypes following the intervention. Our AI-enhanced analysis revealed significant decreases in periportal fibrosis, incomplete septal fibrosis, and complete septal fibrosis after surgery. These observed improvements in specific fibrosis subtypes, even in the absence of complete resolution, suggest that bariatric surgery initiates hepatic remodeling processes that may continue to evolve over extended follow-up periods, capitalizing on the liver's inherent capacity for fibrotic tissue remodeling.

A particularly intriguing finding of our study is the post-surgical increase in immune cell infiltration, especially polymorphonuclear cells, which challenges the traditional understanding of liver recovery. While previous research has emphasized inflammation resolution as central to liver improvement43–46, our data reveals a more nuanced process, with elevated neutrophil and eosinophil levels suggesting active immune-mediated tissue remodeling. AI-enhanced digital pathology approach enabled detection of these changes with unprecedented precision, moving beyond traditional pathology's limitation of simply identifying inflammatory foci to characterizing the specific immune cell subtypes and their spatial distribution patterns during post-bariatric liver recovery.

This observation appears paradoxical given neutrophils' established role in MASH progression through ROS generation, proteolytic enzyme release, and pro-inflammatory signaling. However, recent evidence points to their context-dependent functions 47. In the post-bariatric setting, neutrophils appear to transition toward regulatory and reparative roles through several mechanisms: distinct functional subsets emerge48, neutrophil-released matrix metalloproteinases contribute to fibrosis remodeling, and microRNA-223 modulates immune responses by promoting anti-inflammatory M2 macrophage polarization49,50. Furthermore, the process of efferocytosis, described as macrophage-mediated clearance of apoptotic neutrophils, reinforces this anti-inflammatory shift 51.

The spatial distribution of immune cell infiltration across hepatic zones and its relationship to various fibrosis patterns suggests an orchestrated repair process rather than pathological inflammation. These findings align with emerging evidence that controlled inflammatory responses may facilitate tissue repair and regeneration52–54, challenging conventional views about inflammation's role in MASH recovery.

Our study has several limitations. As an exploratory pilot study, our findings require validation in larger and more diverse cohorts. The observational design limits our ability to establish causality between liver tissue changes and surgery-induced weight loss. We cannot definitively determine whether the observed changes are direct effects of the surgical intervention or consequences of weight loss itself. Future studies with larger cohorts and longitudinal sampling will be essential to distinguish between these mechanisms.

In conclusion, this study demonstrates the transformative potential of AI-enhanced digital pathology in understanding liver disease progression and recovery. By applying this innovative approach to paired liver biopsies from LSG patients, we provided detailed quantitative insights into post-bariatric liver recovery that were previously unattainable through conventional histological assessment. Our findings revealed significant improvements in key liver health parameters and, importantly, uncovered an unexpected yet potentially beneficial role of immune cell infiltration in tissue repair. This novel understanding of immune system dynamics suggests promising therapeutic possibilities through targeted immunomodulation strategies. Furthermore, this study show how digital pathology, can overcome traditional histological limitations by enabling precise quantification and objective assessment of complex tissue changes, opening new avenues for both understanding disease mechanisms and evaluating targeted treatments in MASH and other liver conditions.

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