

## D3.2: An optimised histological staging system for NAFLD-associated fibrosis

### LITMUS

#### Liver Investigation: Testing Marker Utility in Steatohepatitis

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#### WP3 - Patient Cohorts and Bioresources

<b>Lead contributor</b>	Pierre Bedossa (P1-UNEW) & Dina Tiniakos (P1-UNEW) <a href="mailto:pierre.bedossa@liverpat.com">pierre.bedossa@liverpat.com</a> & <a href="mailto:dina.tiniakos@newcastle.ac.uk">dina.tiniakos@newcastle.ac.uk</a>
<b>Other contributors</b>	Susan Davies (P6-UCAM)
	Johanna Arola (P19-UHEL)

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## 1 Publishable Summary

Fibrosis is the most important and independent prognostic factor in NAFLD. So far, the NASH CRN staging system has been used as the reference. While this score is very accurate for early lesions, advanced fibrosis is collapsed into just 2 stages which include a large range of lesions that might have different prognosis and clinical outcome.

We describe here a 7-tier score of fibrosis that we initially proposed as part of the EPoS project (EPOS staging system) that is linear along the spectrum of fibrosis with similar precision in early and advanced stages. Within LITMUS, we further validated the robustness of this system by assessing inter-observer variability between 9 liver pathologists reviewing a set of 45 NAFLD biopsies both on physical support (glass slides) and digital images (e-slides). Inter-observer reproducibility of the 7-tier scoring system was excellent (Kappa score > 0.8) independent of the type of support used. Furthermore, e-slides appear to be as appropriate as glass slides since the Kappa score was also excellent when pathologists scored the same biopsy on both glass and e-slides.

In conclusion, the 7-tier EPOS staging system that covers the full spectrum of liver fibrosis in NAFLD is very robust. There is no difference in fibrosis staging using either glass or e-slides.

## 2 Introduction

Liver fibrosis stage is the most important independent prognostic factor in Non-alcoholic Fatty Liver Disease (NAFLD). To date, biopsy is considered the best standard to stage liver fibrosis.

The NASH CRN staging system (Kleiner, *Hepatology*, 2005) has been widely used for the evaluation of fibrosis in NAFLD tissue biopsies in natural history studies and in clinical trials. It is a robust staging system but insufficiently accurate in advanced stages (stages 3 and 4) which are most strongly linked to liver-related outcomes.

The glass slide is the physical support used to assess liver biopsy under the light microscope. Strong technical efforts have been put into transferring images from histological glass slides to digital slides (e-slides). E-slides can be easily accessed and interpreted from anywhere without the need of the physical support (glass slides), thus facilitating sharing of slides especially for consult review or clinical trials.

For deliverable D3.2, we aimed to validate a 7-tier histological staging system for NAFLD-associated fibrosis using digital slides. The EPOS staging system is a 7-tier system for semi-quantitating the extent of fibrosis that has been created and tested by the EPOS Histopathology Group, members of which are included in the LITMUS Histopathology Group – LHG (Bedossa, *J Hepatol* 2018).

## 3 Methods

### 3.1 *The EPOS Staging System*

#### 3.1.1 Evaluation of fibrosis using the EPOS staging system

The EPOS staging system includes 7 stages (0 to 6). Compared to NASH CRN, stages 1a, b and c are grouped together while stages 3 and 4 are each expanded into 2 further stages. Stage 0 includes minimal zone 3 sinusoidal fibrosis or minimal portal fibrosis that would be scored as stage 1 using the NASH CRN system.

Schematically, the novel 7-tier fibrosis staging system is shown in Figure 1. In column 1 of the diagram, the NASH CRN stages are shown on the left and the equivalent EPOS stages on the right.

In column 2 (Comments), details for the evaluation of fibrosis are described and rationale for lumping together the NASH CRN stage 1 sub-stages a, b, c is given.

Figure 2 shows representative histological images from each one of the 7 stages (0-6) of fibrosis according to EPOS.






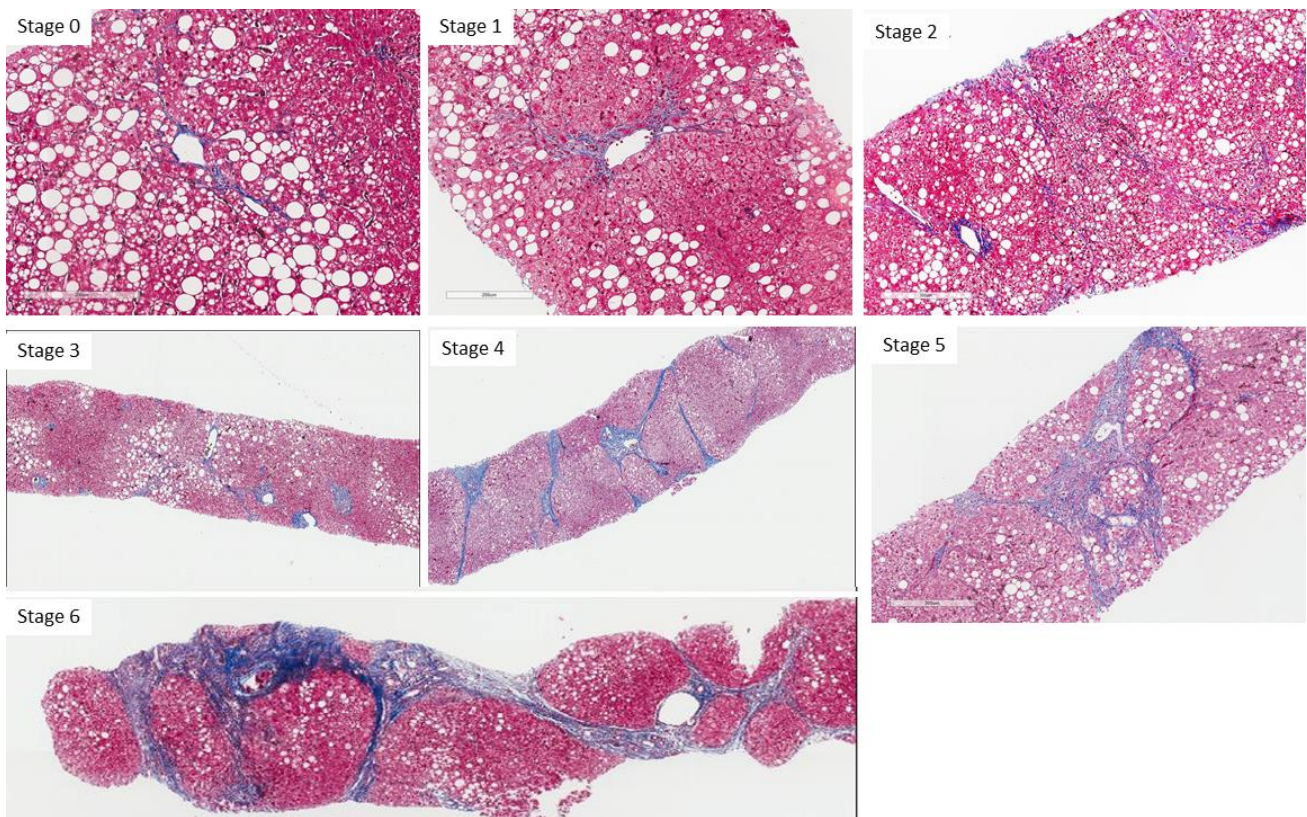
NASHCRN		EPOS	Comments
1a 1b 1c		1	<b>Lumping together</b> because: - Poor <u>reproducibility</u> , <u>Sampling error</u> - No <u>clinical relevance</u>
2		2	<b>Changing definition :</b> central or portal <u>fibrosis</u> + <u>lobular fibrosis</u> or portal + central <u>fibrosis</u>
3		3 4	<b>Increase granularity:</b> Few <u>septa</u> (no more than 2 /10mm length of biopsy)  <u>Many septa</u> (> 2....) <u>without nodule</u>
4		5 6	<b>Increase granularity:</b> <u>Many septa with few nodules</u>  <u>Annular fibrosis with complete nodulation</u>

Figure 1: Description of the EPOS fibrosis staging histological system and its differences from the NASH CRN staging system for NAFLD.



*Figure 2: The stages of fibrosis according to EPOS staging system for NAFLD (Masson trichrome stain: collagen fibres are shown in blue, hepatocytes in red, fat droplets within hepatocytes are white).*

### 3.1.2 Design of the study

In a first step, a group of 9 European liver pathologists, members of the EPoS Histopathology Group, including the leaders (Pierre Bedossa-UNEW, Dina Tiniakos-UNEW) and 2 members of the LITMUS Histopathology Group (Johanna Arola-UHEL, Susan Davies-UCAM) jointly reviewed a set of 45 NAFLD liver biopsies to define consensually the various stages of the new EPOS score of fibrosis.

Then, each participant reviewed independently the same set of 45 NAFLD biopsies (glass slides) with the EPOS staging system to evaluate inter-observer variability.

Three months later, the glass slides were digitized (e-slides) and re-evaluated electronically by each pathologist, blinded to their first assessment on glass slides.

The inter-observer agreement between the liver pathologists was evaluated using Kappa score.

## 4 Results

Table 1 shows the inter-observer variation of the expert liver pathologists when using the EPOS staging system on glass slides of 45 NAFLD cases. The mean kappa score (7-tier) was 0.84 (almost perfect agreement).

Table 1: Kappa scores by pair of observers after assessment of **glass slides** of 45 NAFLD biopsies using the EPOS 7-tier staging system.

EPOS	OBS 1	OBS 2	OBS 3	OBS 4	OBS 5	OBS 6	OBS 7	OBS 8	OBS 9
<b>OBS 1</b>		0,88	0,86	0,85	0,87	0,87	0,77	0,78	0,89
<b>OBS 2</b>	0,88		0,89	0,85	0,89	0,92	0,79	0,83	0,92
<b>OBS 3</b>	0,86	0,89		0,86	0,92	0,9	0,81	0,8	0,91
<b>OBS 4</b>	0,85	0,85	0,86		0,82	0,91	0,87	0,83	0,89
<b>OBS 5</b>	0,87	0,89	0,92	0,82		0,89	0,75	0,75	0,88
<b>OBS 6</b>	0,87	0,92	0,9	0,91	0,89		0,84	0,88	0,93
<b>OBS 7</b>	0,77	0,79	0,81	0,87	0,75	0,84		0,78	0,82
<b>OBS 8</b>	0,78	0,83	0,8	0,83	0,75	0,88	0,78		0,86
<b>OBS 9</b>	0,89	0,92	0,91	0,89	0,88	0,93	0,82	0,86	

Table 2 shows the interobserver variation of the expert liver pathologists when using the EPOS staging system using e-slides of 45 NAFLD cases. The mean kappa score (7-tier) was 0.80 (excellent agreement).

Table 2: Kappa scores by pair of observers after assessment of **e-slides** of 45 NAFLD biopsies using the EPOS 7-tier staging system.

	OBS 1	OBS 2	OBS 3	OBS 4	OBS 5	OBS 6	OBS 7	OBS 8	OBS 9
<b>OBS 1</b>		0,83	0,8	0,68	0,8	0,84	0,73	0,81	0,84
<b>OBS 2</b>	0,83		0,81	0,76	0,91	0,84	0,84	0,88	0,88
<b>OBS 3</b>	0,8	0,81		0,76	0,77	0,91	0,8	0,86	0,84
<b>OBS 4</b>	0,68	0,76	0,76		0,79	0,75	0,83	0,79	0,82
<b>OBS 5</b>	0,8	0,91	0,77	0,79		0,84	0,81	0,88	0,89
<b>OBS 6</b>	0,84	0,84	0,91	0,75	0,84		0,82	0,91	0,88
<b>OBS 7</b>	0,73	0,84	0,8	0,83	0,81	0,82		0,83	0,83
<b>OBS 8</b>	0,81	0,88	0,86	0,79	0,88	0,91	0,83		0,85
<b>OBS 9</b>	0,84	0,88	0,84	0,82	0,89	0,88	0,83	0,85	

The intra-observer variation was minimal using the EPOS 7-tier staging system on glass slides and e-slides, as Kappa values of agreement ranged from 0.79 to 0.89 (mean kappa coefficient 0.85), as shown in Table 3 below. The intra-observer agreement when using the 5-tier NASH CRN system on glass and e-slides was lower with a mean Kappa score of 0.70.

Table 3: Kappa score for each pathologist comparing evaluation on **glass slides vs e-slides** of 45 NAFLD biopsies using EPOS staging system.

	OBS 1	OBS 2	OBS 3	OBS 4	OBS 5	OBS 6	OBS 7	OBS 8	OBS 9
<b>K SCORE</b>	0,80	0,85	0,83	0,79	0,85	0,86	0,82	0,86	0,89

## 5 Conclusions

- The novel 7-tier EPOS fibrosis staging system is an expanded scoring system useful to assess fibrosis for research purposes (natural history study, clinical trial setting). Its usefulness in routine practice remains to be evaluated.
- The inter-observer reproducibility of the EPOS fibrosis staging is excellent between expert liver pathologists.
- E-slides provide material as suitable as glass slides for staging fibrosis, as shown by the high reproducibility of the 7-tier EPOS staging system scores for each observer (high intra-observer reproducibility).

## 6 References

- Bedossa P, Arola J, Davies S, et al. The EPoS staging system is a reproducible 7-tier fibrosis score for NAFLD adapted both to glass slides and digitized images (e-slides). *J Hepatol* 2018; 68 suppl 1:S553
- Kleiner D E, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; 41:1313-1321.