Grant Agreement no. 634413

EPoS

Elucidating Pathways of Steatohepatitis

INSTRUMENT: Research and Innovation Action

TOPIC: PHC-01-2014

D1.3: Final reports summarising recruitment into the EPoS database, biobanked samples availability, and longitudinal outcome studies

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1. Introduction

This document is a report on the establishment and current recruitment status of the EPoS European NAFLD Registry.

At the start of the project, EPoS capitalised on extant NAFLD patient databases and biobank resources established during our previous FP7 project *FLIP* ('Fatty Liver – Inhibition of Progression', Health-F2-2009-241762) and additional cases recruited at consortium member centres (UNEW, ICAN, UHEL, UMC, UNITO). An early enabling action collated these resources to produce a cohort of histologically proven NAFLD/NASH that formed the nucleus of the initial EPoS European NAFLD Registry cohort. These patient data and samples were key to timely initiation of the mechanistic studies conducted within EPoS.

Recognising the importance of the Registry as a resource for the successful ascertainment of the goals of EPoS, and as a foundation for future collaborative NAFLD research in Europe, members of the consortium committed to maintaining and expanding this Registry through further prospective patient recruitment. This process has been ongoing throughout the duration of EPoS. This action has produced a substantial increase in prospectively recruited cases, establishing the EPoS European NAFLD Registry cohort of histologically proven NAFLD/NASH defined according to a standardised set of inclusion and exclusion criteria.

An important project goal was to double the existing NAFLD cohort from approximately 900 cases with histologically proven NAFLD/NASH and/or NASH-related hepatocellular carcinoma. **In fact, prospective recruitment has been highly successful and so the initial target has been exceeded. In total, 3,352 well-characterised cases from EPoS partners are recorded in the Registry at the close of the EPoS project and many remain under longitudinal follow-up.**

2. Enabling Actions

To support the prospective recruitment into the Registry across all partners, two enabling tasks were completed:

1. **Registry Infrastructure:** A secure and highly resilient, purpose-built web-based data portal was developed at UNEW to allow investigators across Europe to: i) enrol patients into the back-end relational database; ii) allocate study identifier numbers; iii) record clinical data (linked-anonymised) according to predefined criteria. Systems have been developed at UNEW to regularly monitor and validate existing and new Registry data as it is gathered.

   Data collection has included standardised data on anthropometrics, comorbidities, drug therapy, clinical biochemistry, activity and dietary habits. In addition, patients have completed validated patient reported outcome measures and questionnaires (CLDQ, IPAQ, EPIC-FFQ and Mediterranean Diet Score). Taken together, these provide a rich phenotypic dataset on each prospectively recruited case.

2. **Biobank Sample Collection:** As described in Deliverable D1.1, samples were collated into the Central Biobank at UNEW. To ensure standardisation of samples, the *EPoS Investigator Handbook & Laboratory Manual* (confidential document, current version v1.3 09-09-2016) was developed at UNEW to support these activities. It provides detailed instructions for how all samples should be handled and prepared for storage, crucially minimising pre-analytical variation.

Sample collection includes frozen liver tissue, formalin fixed (paraffin embedded) liver tissue, DNA, serum, plasma, urine and faeces. A summary of the samples collected at recruitment is shown in Figure 1.

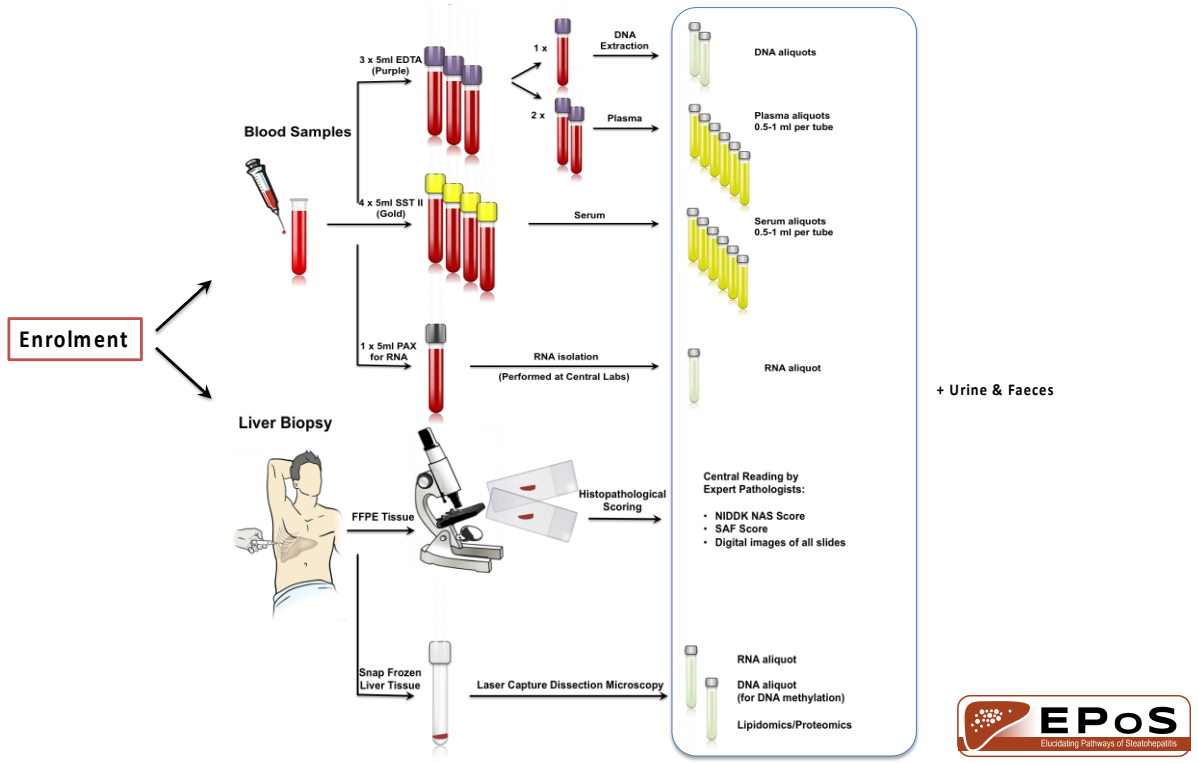

Figure 1: Summary of Samples Collected at Recruitment Baseline Visit.

3. Recruitment of patients into the EPoS Database

Task 1.4: Prospective recruitment of patients into the EPoS Database and EPoS Biobank
Lead Partner: ICAN; Participants: UNEW, UNITO, UHEL, UMC, UCAM

A major advantage of the EPoS Registry infrastructure is that it is highly scalable. This has allowed the EPoS consortium to widen the scope of patient recruitment to centres outwith the immediate consortium membership, operating a "hub and spoke" model to maximise patient recruitment (Figure 2). This has allowed the EPoS European NAFLD Registry to develop towards becoming a key enabler of collaborative research in NAFLD across Europe.

Despite initially slow recruitment, the mitigation strategies put in place by the consortium have proved to be highly successful. The total number of prospective cases recruited during EPoS by consortium members and partners from the previous FLIP consortium was 3,352 (summarised in Table 1). However, the Registry has continued to expand and so, as of October 2019, the Registry contained a total of 7,186 cases (6,413 with histology data available), this figure includes data on additional cases entered by external collaborators. **This makes the Registry the largest international cohort of histologically-characterised NAFLD patients in the world. However, this report will focus on the cases specifically attributable to EPoS recruitment.**

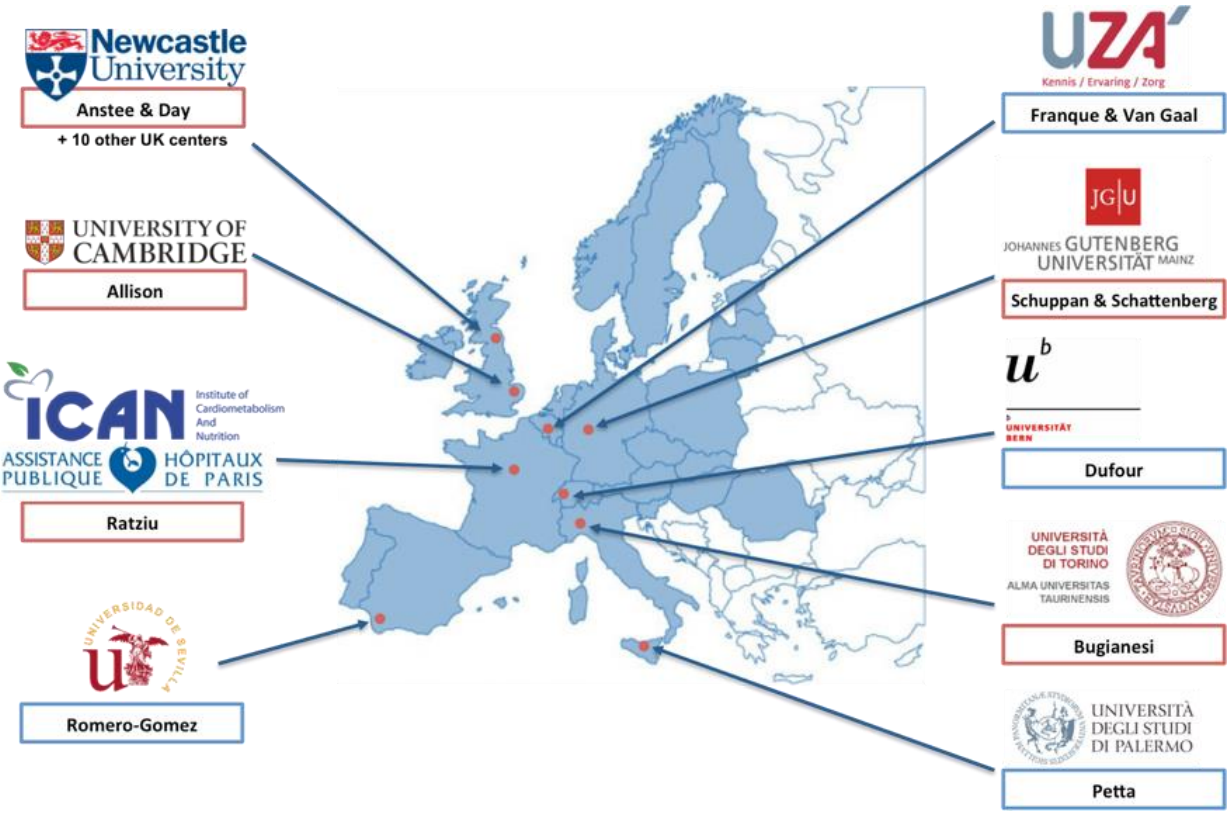

Figure 2: EPoS Registry Recruitment Centres.

Table 1: EPoS Registry Recruitment by Site to October 2019.

Centre	Total Recruitment
UNEW *	458
ICAN *	466
UMC *	673
UCAM *	63
UNITO *	258
UNIBE	101
UA	138
UNOT	36
UNIPA	733
SAS/HUV	149
UHEL *	277
Total	**3352**

* EPoS partners

The mean age of patients in the EPoS partner patient cohort is 52.2 years (range 18 – 93). The gender split of the cohort is 45% female, 55% male (Figure 3).

Close attention within the data is paid to the presence of features of the metabolic syndrome as NAFLD is strongly associated with type 2 diabetes mellitus (T2DM), hypertension, obesity and dyslipidaemia. The percentage of participants with a diagnosis of T2DM is 44%.

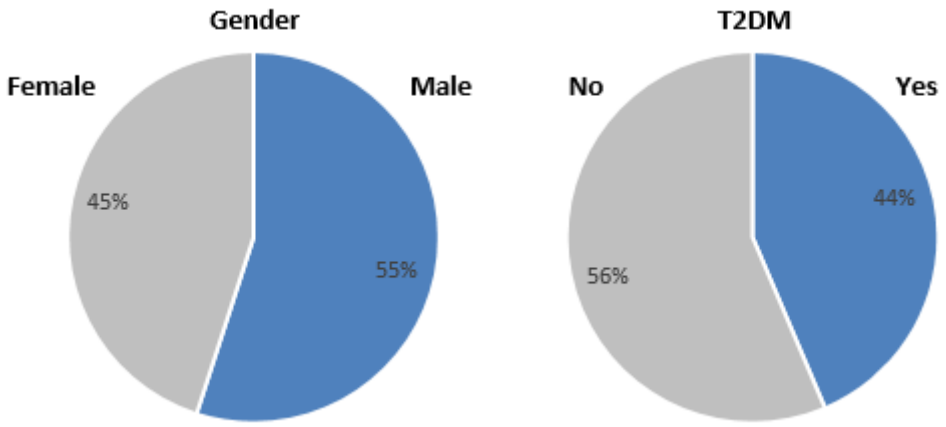

Figure 3: EPoS Cohort by gender and T2DM diagnosis.

2,905 biopsies were histologically categorised and 125 cases were clinically cirrhotic and therefore without biopsy. To standardise interpretation, the majority of liver biopsies have been reviewed in UNEW by the central study pathologist (Dr Dina Tiniakos). The fibrosis stage distribution of the cases with histological readings is shown below in Figure 4. The spread of disease severity has enabled the 'omics' studies to explore the full range of histological disease and the transitions between grades of disease activity.

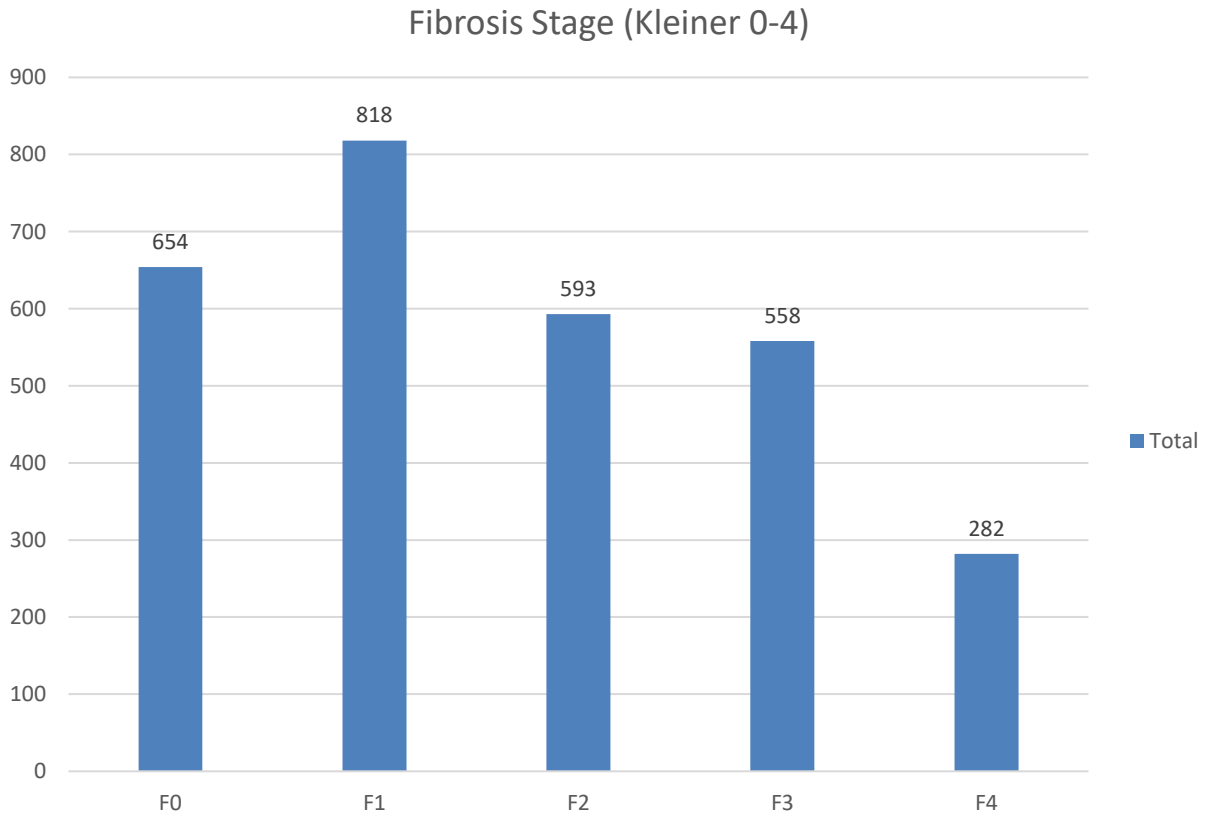

Figure 4: Fibrosis stage distribution.

4. EPoS Biobank

Task 1.5: Collation of previously collected samples and prospectively collected samples into the EPoS Biobank. Lead Partner: ICAN; Participants: UNEW, UNITO, UHEL, UMC, UCAM

As of October 2019, the Central EPoS Biobank had received a total of 16,197 samples from 19 different sites and from nearly 2,000 cases (Table 2). These samples have been inventoried and aliquoted into smaller volumes for use where necessary.

Table 2: Samples received by EPoS Central Biobank up to October 2019.

Sample Type	Baseline	Events	Total
DNA*	554	142	**696**
Liver tissue (frozen)	470	115	**585**
Liver tissue (formalin fixed)	4211	0	**4211**
Plasma	1628	2052	**3680**
Serum	2753	3285	**6038**
Stool	274	41	**315**
Urine	518	154	**672**
Grand Total	6197	**5789**	**16197**

* Excludes DNA from cases already held at UNEW from FLIP

A total of 5,875 aliquots derived from ~1,450 cases have been used for analysis to date. These have been analysed for specific purposes related to EPoS (Table 3) across a number of partners: UNEW, ICAN, NB, ORU, UMC, UNITO and CNR. Use of these samples has underpinned the multi-omics analyses (genetics, transcriptomics, lipidomics, metabolomics, metagenomics) and biomarker discovery actions within EPoS.

Table 3: Samples analysed by EPoS Partners up to October 2019.*

Sample Type	Quantity
Liver tissue (frozen)	504
Liver tissue (formalin fixed)	1180
Plasma	592
Serum	3190
Stool	18
Urine	0
Grand Total	**5875**

* Excludes DNA

5. Longitudinal Follow-up

Task 1.6: Longitudinal follow-up of recruited patients to determine natural history and disease outcomes. Lead Partner: ICAN Participants: UNEW, UNITO, UHEL, UMC, UCAM

As of October 2019, there have been 1,845 follow-up records generated at EPoS partner sites (when additional collaborating sites are included, this exceeds 2,200 records). These are broken down by partner as shown in Table 4.

Table 4: No. of follow-up records introduced by EPoS partners up to October 2019.

Centre	Total Follow-up Records
UNEW	**917**
ICAN	**217**
UMC	**213**
UCAM	**0**
UNITO	**221**
UNIBE	**164**
UA	**12**
UNOT	**13**
UNIPA	**33**
SAS/HUV	**9**
UHEL	**0**
Total	**1845**

In order to better understand the long-term outcomes of NAFLD, EPoS sought to gather not only cross-sectional but also longitudinal data. To facilitate this, if patients consented to participate in the longitudinal aspects of the study, data were collected alongside their routine care on a semi-annual basis. At the end of the study, 1,845 follow-up records were present in the Registry across recruitment sites. These covered 1,038 histologically characterised cases with at least one follow up record (mean number of follow-up visits 1.8/case) and a median duration of follow-up of approximately 7-years from the time of index biopsy of half those enrolled.

The no-cost extension to the project provided the opportunity to further increase patient follow-up duration, increase the number of cases will follow-up data available and also to recruit more cases. For patients with cirrhosis, this led to an increase from 331 patient-years in April 2019 to in excess of 631 complete patient-years of follow-up by October 2019, a 90% increase in duration of follow-up. As an example of the value of this follow-up, the subset of patients included in the RNASeq liver transcriptome analysis now have approximately 63 months (range 20-137 months) follow-up from time of biopsy, with mean follow-up exceeding 5-years. To date, a total of 92 deaths have been captured amongst patients enrolled into the Registry. Beyond the end of the EPoS project, further analysis will continue to explore trends in mortality and to examine how these key clinical outcomes correlate to the biomarker and ‘omics’ data generated within the project.

6. Conclusions

Through EPoS we have established a high quality cohort of patients. The samples and data have underpinned the work of EPoS. The European NAFLD Registry therefore remains an important exploitable resource that will be used going forward to support the work of the IMI2 LITMUS consortium project, which includes all EPoS partners, and will be a valuable resource to support future collaborative research into NAFLD.