[6]*Infectious Diseases Unit, University Hospital of Valme, Sevilla, Spain*
[7]*Infectious Diseases Unit, University Hospital of Puerto Real, Cádiz, Spain*
[8]*Infectious Diseases, University Hospital Reina Sofía, Murcia, Spain*
[9]*Infectious Diseases Unit, University Hospital Donostia, San Sebastian, Spain*
[10]*Infectious Disease Unit, University Hospital Santa Lucía, Cartagena, Murcia, Spain*
[11]*Infectious Diseases Unit, University Hospital Reina Sofía, Córdoba, Spain*
[12]*Hepatology Unit, Hospital Carlos Haya, Málaga, Spain*
[13]*Center for Global Health and Tropical Medicine, Microbiology Unit, Institute of Hygiene and Tropical Medicine, University Nova Lisbon, Lisbon, Portugal*
*Corresponding authors. Address: KU Leuven, Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, Leuven, Belgium. LC: Tel.: +32 16 32 11 76 or BV: +32 16 37 44 00.
E-mail addresses: bram.vrancken@kuleuven.be (B. Vrancken) lize.cuypers@kuleuven.be (L. Cuypers)

[#] These first authors contributed equally to this article.

Reply to: "Cross-country migration linked to people who inject drugs challenges the long-term impact of national HCV elimination programmes"

To the Editor:
We appreciate the interest and comments by Vrancken *et al.* They had previously found that the most prevalent HCV genotype in Spain, genotype 1a, was linked to transmission networks outside the country.[1] Specifically, the origin of those strains could be traced to a number of European countries, including France, Germany and Italy among others.[1] In their letter, Vrancken *et al.* expand their data to HCV genotype 3a, most frequently found among people who inject drugs (PWID) in Spain. This is very relevant information. In our paper, we showed that PWID with and without opiate agonist therapy can achieve high sustained virological response rates with direct-acting antiviral combinations. However, the overall efficacy of direct-acting antivirals in active drug users is lower, mainly due to losses to follow-up. Efforts to reduce their risk of abandoning follow-up are needed to reach HCV elimination targets in Spain. Another potential threat for the elimination efforts in Spain is stressed by Vrancken *et al.* An increasing number of HCV genotype 1a[1] and 3a infections are being introduced to Spain from the rest of Europe, essentially through drug use networks. Thus, HCV elimination plans should be developed with a broader perspective than the national level. Otherwise, successful reductions in the burden of HCV infection in Spain could be counteracted by waves of imported infections.

Conflict of interest
Please refer to the accompanying ICMJE disclosure forms for further details.

Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2019.09.002.

References
[1] Pérez AB, Vrancken B, Chueca N, Aguilera A, Reina G, García-del Toro M, et al. Increasing importance of European lineages in seeding the hepatitis C virus subtype 1a epidemic in Spain. Eurosurveill 2019;24.

Juan Macías
Infectious Diseases and Microbiology Unit, Hospital Universitario de Valme, Seville, Spain
E-mail address: jmacias@cica.es

Assessment of liver phenotype in adults with severe alpha-1 antitrypsin deficiency (Pi*ZZ genotype)

To the Editor:
We would like to congratulate Clark *et al.* for their pioneering work characterizing histological liver injury in patients with the classic severe alpha-1 antitrypsin (AAT) deficiency (genotype Pi*ZZ).[1] The Pi*ZZ genotype is seen in 1:3,000 Caucasians and the associated liver disease is greatly understudied despite the fact that it is more frequent than several well-established liver disorders such as autoimmune hepatitis or primary sclerosing cholangitis.[2,3] While Clark *et al.* greatly enhanced our understanding of the clinical, biochemical and histological liver phenotype of these individuals, we would like to further discuss the following topics: (i) a high occurrence of liver steatosis in

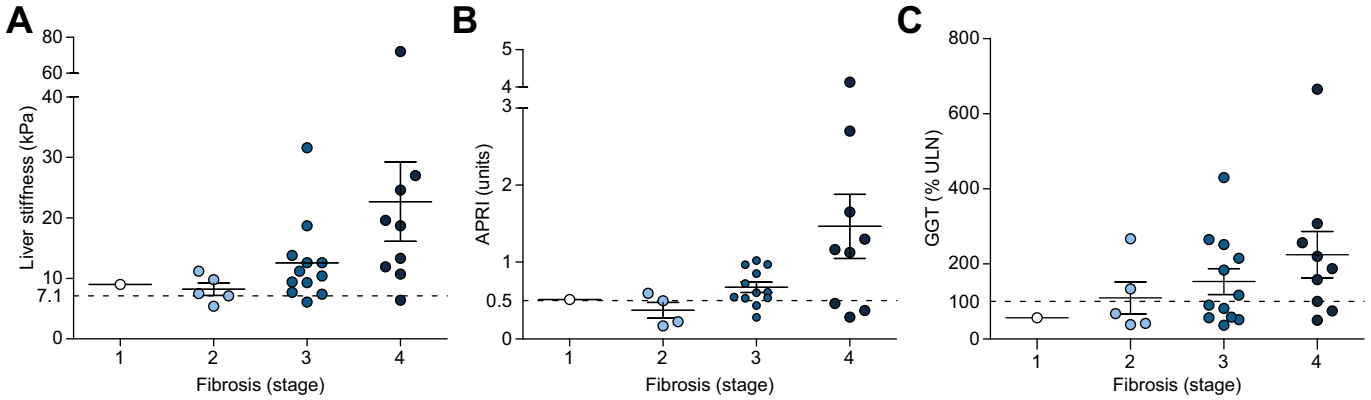

Fig. 1. Comparison of transient elastography-based liver stiffness measurements (A), APRI (B), GGT (C) levels with histologically determined fibrosis scores. Medians and interquartile ranges are displayed. Following cut-off values are highlighted as a dotted line: LSM = 7.1 kPa; APRI = 0.5; GGT = upper limit of normal. APRI, AST-to-platelet ratio index; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; LSM, liver stiffness measurement.

Pi*ZZ carriers; (ii) the usefulness of non-invasive methods to predict liver fibrosis.

In the cohort from Clark *et al.*, 44% of Pi*ZZ individuals displayed liver steatosis.[1] These data mesh well with our own observations, that used transient elastography-based controlled attenuation parameter (CAP) as a non-invasive surrogate of liver steatosis. Our data revealed higher CAP values in Pi*ZZ individuals compared to individuals without any AAT mutation (termed "Pi*MM" or non-carriers) and we suggested impaired hepatic lipid secretion as a potential underlying mechanism.[3] Supporting these data, Pi*ZZ carriers displayed markedly lower serum triglyceride values than non-carriers.[3] Lower triglyceride levels were also reported in the cohort of Pi*ZZ patients by Clark *et al.*, thereby strengthening this observation.[1]

Clark *et al.* assessed the suitability of various non-invasive parameters to predict the histological extent of liver fibrosis. Their data revealed that gamma-glutamyl transferase (GGT) was useful for predicting significant liver fibrosis (*i.e.* fibrosis grade F ≥2; AUROC 0.77), while liver stiffness measurements (LSMs) assessed via transient elastography were most accurate to detect advanced liver fibrosis (*i.e.* fibrosis grade F ≥3; AUROC 0.92). Unfortunately, their cohort contained only 6 individuals with advanced liver fibrosis and patients with cirrhosis were excluded *a priori*. To further test the findings of Clark *et al.*, we evaluated liver biopsies from our own, international Pi*ZZ cohort.[3] Unlike Clark *et al.*, we recruited all comers and biopsied only participants with LSM ≥7.1 kPa or individuals with recurrently elevated liver enzymes. Our analysis is based on 27 individuals in whom biopsies, valid LSM, and serum liver enzyme activities were available. The participants were on average 54 years old and mostly males (78%). We used LSM ≥7.1 kPa as a previously established, aetiology-independent cut-off for the presence of F ≥2 fibrosis (*i.e.*, significant liver fibrosis).[4] All liver specimens were scored by an experienced hepato-pathologist in a blinded fashion using the Kleiner fibrosis score. Notably, 22 out of 23 individuals with LSM ≥7.1 kPa were histologically F ≥2 which suggests that this cut-off is highly predictive of the presence of significant liver fibrosis (Fig. 1A). Interestingly, 3 participants with LSM <7.1 kPa also displayed F ≥2. Two of them had LSM ≥5.45 kPa which corresponds to the cut-off for significant liver fibrosis suggested by Clark *et al.*[1] Since LSM ≥5.45 kPa was found in >50% of Pi*ZZ individuals in our international cohort,[3] this cut-off is not useful for risk stratification. With only 2 exceptions, all individuals with advanced liver fibrosis had LSM ≥7.4 kPa, which together with findings from Clark *et al.* suggest that LSM <7.1 kPa might be useful to exclude the presence of advanced liver fibrosis. Since LSM <7.1 kPa was seen in ~76% of Pi*ZZ carriers, this cut-off appears to be well-suited to rule out the presence of advanced liver fibrosis in the majority of these individuals. Meanwhile, the AST-to-platelet ratio indices (APRI) <0.5 were seen in 5 Pi*ZZ individuals with advanced liver fibrosis (Fig. 1B). In line with the data from Clark *et al.*[1] APRI appears inferior to LSM in ruling out advanced liver fibrosis. Finally, while Clark *et al.* demonstrated that GGT might be useful to indicate significant fibrosis, it does not seem to be beneficial in advanced fibrosis, since 38% of these individuals displayed normal GGT values (Fig. 1C).

Financial support

Our work was supported by the EASL registry grant on alpha-1 antitrypsin-related liver disease, the Deutsche Forschungsgemeinschaft (DFG) consortium SFB/TRR57 "Liver fibrosis" (both to P.S. and C.T.) and the Else Kroener Excellence Fellowship (to P.S.).

Conflict of interest

Please refer to the accompanying ICMJE disclosure forms for further details.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2019.08.011.

References

[1] Clark VC, Marek G, Liu C, Collinsworth A, Shuster J, Kurtz T, et al. Clinical and histologic features of adults with alpha-1 antitrypsin deficiency in a non-cirrhotic cohort. J Hepatol 2018;69:1357–1364.
[2] Silverman EK, Sandhaus RA. Clinical practice. Alpha1-antitrypsin deficiency. N Engl J Med 2009;360:2749–2757.
[3] Hamesch K, Mandorfer M, Pereira VM, Moeller LS, Pons M, Dolman GE, et al. Liver fibrosis and metabolic alterations in adults with alpha1 antitrypsin deficiency caused by the Pi*ZZ mutation. Gastroenterology 2019;157(3):705–719.
[4] Friedrich-Rust M, Poynard T, Castera L. Critical comparison of elastography methods to assess chronic liver disease. Nat Rev Gastroenterol Hepatol 2016;13:402–411.

Julia Kümpers[1,2]
Malin Fromme[1,2]
Carolin V. Schneider[1,2]
Christian Trautwein[1,2]
Helmut Denk[3]
Karim Hamesch[1,2]
Pavel Strnad[1,2,*]
[1]*Coordinating Center for Alpha-1 Antitrypsin Deficiency-related Liver Disease of the European Reference Network on Hepatological Diseases (ERN RARE-LIVER) and the European Association for the Study of the Liver (EASL) Registry Group "Alpha1-Liver", Germany*
[2]*Medical Clinic III, Gastroenterology, Metabolic diseases and Intensive Care, University Hospital RWTH Aachen, Aachen, Germany*
[3]*Institute of Pathology, Medical University of Graz, Graz, Austria*
*Corresponding author. Address: Medical Clinic III, Gastroenterology, Metabolic diseases and Intensive Care, University Hospital RWTH Aachen, Aachen, Germany.
E-mail address: pstrnad@ukaachen.de

Reply to: "Assessment of liver phenotype in adults with severe alpha-1 antitrypsin deficiency (Pi*ZZ genotype)"

Establishing valid cut-offs for non-invasive measures of liver fibrosis to assess liver phenotype in severe alpha-1 antitrypsin deficiency

To the Editors:
We appreciate the interest and comments by Dr. Kumpers[1] *et al.* on our recent work characterizing the clinical and histological findings in patients with severe alpha-1 antitrypsin (AAT) deficiency.[2] To that end, we reviewed how the proposed liver stiffness measurements (LSM) ≥7.1 kPa performed in our cohort. Using that cut-off, the prevalence of clinically significant fibrosis defined as stage ≥2 would be 26%, which is remarkably similar to the 23.6% reported by Hamesch *et al.*[3] in a large study that did not include biopsies. However, our population had fibrosis stage confirmed, and the actual prevalence of fibrosis ≥2 was approximately 35%. The reason for the discrepancy was that an LSM ≥7.1 kPa misclassified the fibrosis stage in a significant number of Pi*ZZ individuals (n = 8 over staged; n = 15 under staged). This highlights the importance of performing a liver biopsy in every patient when evaluating the diagnostic accuracy of non-invasive fibrosis markers. Therefore, it is not surprising that biopsies performed selectively on Pi*ZZ individuals at risk of fibrosis actually confirmed significant liver fibrosis.[1] We agree that an established and validated cut-off (for LSM) would be an important tool in evaluating patients with AAT deficiency. When used to rule in liver disease, an LSM ≥7.1 kPa may be valuable given their cohort contains a population with more advanced disease. Our concern is that this cut-off had a poor negative predictive value when applied to our cohort. The strength in our study was that everyone was biopsied; however, the few individuals with advanced fibrosis limited the ability to establish an LSM that is more in line with established clinical practice. We await better non-invasive markers of fibrosis to rule out advanced disease. Overall, the similarities between our smaller cohort from North America with biopsies and the author's large European cohort with multiple non-invasive measures of fibrosis are notable.[3] What is emerging from these studies is that liver steatosis is a common finding either on biopsy or as measured by controlled attenuation parameter (CAP). We demonstrated that steatosis alone did not predict fibrosis but that PAS + D accumulation and steatosis were significantly associated with metabolic syndrome.[2] Impaired lipid metabolism and its association with alpha-1 accumulation is an interesting hypothesis that deserves further study. The presence of steatosis may be an early sign of cellular stress and/or injury. In the future, measuring steatosis by CAP may be a valuable tool for identifying those at risk.

Conflict of interest
Please refer to the accompanying ICMJE disclosure forms for further details.

Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2019.09.003.

References
[1] Kumpers J, Fromme M, Schneider C, Trautwein C, Denk H, Hamesch K, Strnad P, et al. Assessment of liver phenotype in adults with severe alpha1-antitrypsin deficiency (Pi*ZZ genotype). J Hepatol 2019;71(6):1272–1274. https://doi.org/10.1016/j.jhep.2019.08.011.
[2] Clark VC, Marek G, Liu C, Collinsworth A, Shuster J, Kurtz T, et al. Clinical and histologic features of adults with alpha-1 antitrypsin deficiency in a non-cirrhotic cohort. J Hepatol 2018;69:1357–1364.
[3] Hamesch K, Mandorfer M, Pereira V, Moeller L, Pons M, Dolman G, et al. Liver fibrosis and metabolic alterations in adults with alpha-1 antitrypsin deficiency caused by the Pi*ZZ mutation. Gastroenterology 2019;157:705–719.

George W. Marek III[1]
Mark Brantly[1]
Virginia C. Clark[2,*]
[1]*Division of Pulmonary, Critical Care, and Sleep Medicine, University of Florida, United States*
[2]*Division of Gastroenterology, Hepatology, and Nutrition, University of Florida, United States*
*Corresponding author. Address: Division of Gastroenterology, Hepatology, and Nutrition, University of Florida, United States.
E-mail address: virginia.clark@medicine.ufl.edu