setting [6]. Moreover, in HCV-infected kidney recipients, small studies comparing the performance of non-invasive tests to liver biopsies have shown inconsistent results [6]. Therefore, the utility of such an expensive and laborious screening test to detect an infection, which does not have any proven clinical consequences is questionable.

In summary, data about the occult HCV infection in liver and/or PBMCs despite negative HCV RNA in serum are conflicting. Further well-designed longitudinal studies with serial analyses for occult HCV infection from different geographic regions are necessary to finally resolve this controversial issue.

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Conflict of interest

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The beginning of an end point: Peak AST in liver transplantation

To the Editor:
In their review on liver graft quality assessment during preservation, Verhoeven *et al.* [1] point out a painful weakness that continues to hamper progress in the field of liver transplantation. The lack of validated markers that reliably predict graft quality and function make comparison of trial results and meta-analysis impossible. There is an urgent need for international guidelines on appropriate end points to assess liver graft quality and function and the authors refer to early allograft dysfunction (EAD) as described by Olthoff *et al.* [2] as a starting point. Indeed, EAD and particularly peak aspartate transaminase (AST), one of the EAD components, are increasingly used as primary end point in liver transplantation trials aimed to improve (early) graft function (e.g. ISRCTN00167887; ISRCTN39731134). When determining the peak of a marker, it is essential that kinetics of this marker and especially the timing of the peak be precisely known so that determination of that peak can be as accurate as possible. Although AST is a well-recognized marker for hepatocyte injury and used as a surrogate to assess preservation and ischemia-reperfusion injury, it is remarkable how little information is available on the kinetics of AST post-reperfusion. It is generally assumed that AST peaks within the first 24 to 72 h post-reperfusion [3,4]. However, considering AST is released quickly from injured hepatocytes and is also quite rapidly cleared, it is not unthinkable that a peak – particularly an early one – might be missed if samples are not precisely taken. We therefore determined the evolution of AST early post-reperfusion, and the timing of its peak. In addition, we compared the peak AST and its timing in timed post-reperfusion samples *vs.* routinely taken samples (that are usually used to determine peak AST in clinical trials).

We analyzed post-reperfusion AST values in 66 adult liver-only recipients (60 years [48–67], 38 males) transplanted between 11/2011 and 11/2013 who had consented for a prospective observational study on kidney injury during liver transplantation (NCT01333319, approved by the Ethics Committee). In this study, plasma samples were taken at the time of incision, 30 min, 2 h, 6 h, and 12 h post-reperfusion. Furthermore, recipients had routine AST determinations with a first AST sample "at arrival on the intensive care unit" and daily morning measures until postoperative day (POD) 5. The post-reperfusion timing of these routinely taken samples was retrospectively determined from the electronic patient records. AST was determined in the central lab of the hospital (coloric method, Hitachi/Roche Modular P). Continuous variables (median [inter quartile range]) were compared between timed and routine samples by the Mann-Whitney *U* test (SPSS version 19).

Donors were on average 57 (44–68) years old and livers were transplanted with a cold ischemia time of 6 h (5–8) and

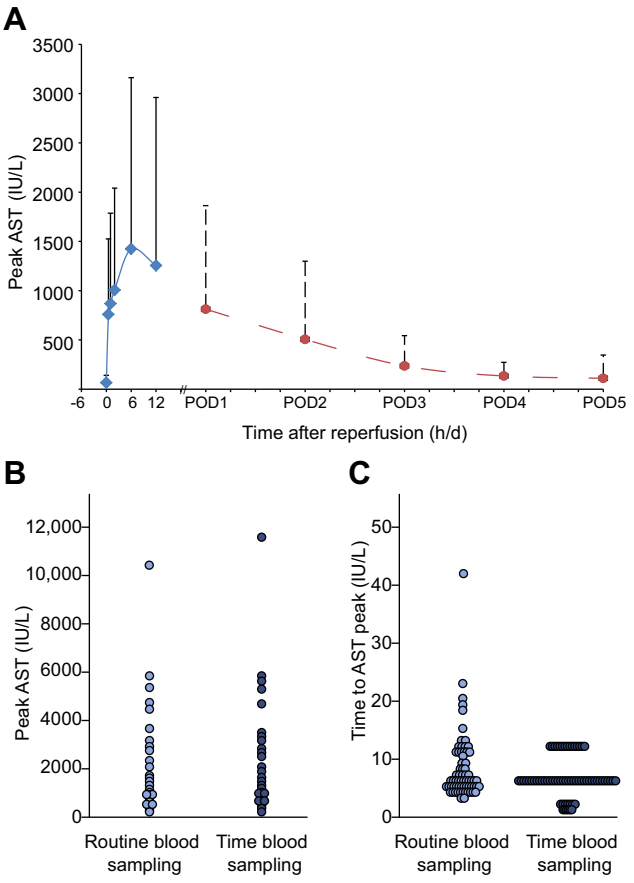

Fig. 1. AST kinetics and timing of peak values after reperfusion. Panel (A) shows the post-reperfusion kinetics of AST (mean ± SD) in 66 liver transplant recipients early after reperfusion (from 30 min until 12 h) and daily until postoperative day 5. Peak AST values measured in routinely taken or timed blood samples are not different in this population ($p = 0.71$), panel (B). The timing of the AST peak is shown in panel (C) and is also similar between routinely taken and timed blood samples ($p = 0.46$).

anastomotic times of 45 min (40–45). Indications for liver transplantation were acute liver failure (n = 7), HCV/HBV cirrhosis (n = 12), cholestatic cirrhosis (n = 13), post-ethyl cirrhosis (n = 14), NASH cirrhosis (n = 5), cryptogenic cirrhosis (n = 4), retransplantation (n = 4) and others (n = 7). In 28 cases a simultaneous hepatocellular carcinoma was present, accounting for the fact that the MELD score at time of transplantation was low (13 [10–18]). Indeed, Eurotransplant awards "exceptional" MELD points in case of hepatocellular carcinoma within Milan criteria [5]. Eleven livers were donated after circulatory death with a total warm ischemia time in the donor of 23 min (16–27).

The AST kinetics show that AST increases immediately after reperfusion to peak at 6 h, after which there is a steady decrease with values halved by POD1 (Fig. 1A). Both peak and timing of the AST peak were similar between timed and routinely taken blood samples in this study population (6 h [6–6] *vs.* 6 h [5–11], $p = 071$; 948 IU/L [593–1508] *vs.* 908 IU/L [512–1146], $p = 0.46$; respectively) with 90% of AST peaks detected in the first 14 h (Fig. 1B and C).

In clinical trials with peak AST as primary end point the time window set to determine the AST peak is often wide (up to POD7). Furthermore, the timing of the first AST measurement is rarely predefined and mostly relies on routinely taken samples. In this liver transplant cohort the majority of peak ASTs are detected at 6 h post-reperfusion with a time window between 5 h and 11 h, considerably earlier that what is usually assumed. Therefore, relying on routine blood samples to correctly measure the AST peak should only be done if the first routine sample is taken in this time period. Subsequently, clinical trials using peak AST as primary end point should clearly define the timing of the first blood sample and specify a time window for this sample in the trial protocol. Based on the cohort presented here, this could appropriately be defined as a sample taken anywhere between 5 h and 11 h after reperfusion and in our experience will often be the first blood sample taken in the intensive care unit.

Conflict of interest

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