Development and evaluation of a baseline-event-anticipation score for hepatitis delta

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Received October 2013; accepted for publication January 2014

SUMMARY. Hepatitis delta is considered the most severe form of viral hepatitis, but variables associated with disease progression are poorly defined. This study aimed to identify risk factors associated with worse clinical outcome in patients with hepatitis delta and to develop a clinical score to determine their risk of experiencing liver-related morbidity or mortality. We followed 75 HBsAg-anti-HDV-positive patients with hepatitis delta for up to 16 years (median 5 years). The baseline-event-anticipation score (BEA score) was developed based on variables associated with the development of liver-related clinical complications. Age, region of origin, presence of cirrhosis, albumin, INR, hyperbilirubinemia and thrombocytopenia were all associated with the development of an event in the training cohort. The BEA score included age, sex, region of origin, bilirubin, platelets and INR. Points were allocated according to hazard ratios, and three risk groups were defined: BEA-A mild risk, BEA-B moderate risk and BEA-C high risk. Hazard ratios of BEA-B and BEA-C patients for liver-related clinical endpoints were 9.01 and 25.27 vs BEA-A with an area under curve of the receiving operating characteristic curve of 0.88. The accuracy of the BEA score was confirmed in two independent validation cohorts followed in Barcelona (n = 77) and Düsseldorf (n = 62). Delta hepatitis is associated with a very severe long-term outcome. The BEA score is easy to apply and predicts with a very high accuracy the development of liver-related complications in patients with hepatitis delta.

Keywords: BEA score, clinical score, co-infection, HBV, HDV, hepatitis delta.

INTRODUCTION

Chronic hepatitis delta is caused by persistent infection of the hepatitis D virus (HDV) in hepatitis B surface antigen (HBsAg) positive individuals [1]. An estimated 15–20 million individuals worldwide are thought to be infected with HDV. Hepatitis delta is considered the most severe form of chronic viral hepatitis rapidly leading to end-stage liver disease, hepatic decompensation and the development of hepatocellular carcinoma (HCC) [2]. Severe courses of hepatitis delta were described in initial studies published after the discovery of HDV during the 1980s [3–6] and
early 1990s [7]. Subsequent works reported less severe disease activity but more frequent cases of cirrhosis in patients with hepatitis delta [8,9]. More recent studies have confirmed that HDV infection is indeed associated with a high frequency of liver cirrhosis and liver failure [10–13].

Treatment of hepatitis delta is challenging as hepatitis B virus (HBV) polymerase inhibitors are ineffective against HDV and conventional recombinant interferon alfa has only limited efficacy [14–16]. Pegylated interferon alfa (pegIFN-α) induced clearance of HDV-RNA in 21–28% of patients in two European multicenter trials [17,18]. In addition, a high proportion of the patients may not be treated with pegIFN-α due to contraindications. Thus, there is still no curative therapy available for the great majority of patients with hepatitis delta, and orthotopic liver transplantation (OLT) remains as the only therapeutic option [19].

Specific characteristics associated with a more severe long-term course of HDV infection remain unknown, which is of particular importance considering not only the above-mentioned limited efficacy of interferon alfa treatment, but also its numerous side effects and high cost. The identification of risk factors predicting the development of clinical events would be very helpful in the long-term management of patients with hepatitis delta, in particular to select those individuals who would benefit the most from antiviral therapy. Of note, none of the most frequently used clinical scores to predict the outcome of liver disease such as the Model for End-stage Liver disease (MELD) or Child–Pugh scores have been evaluated in hepatitis delta.

Therefore, the aim of this study was to identify the clinical parameters associated with worse outcome in patients with chronic hepatitis delta and to develop a clinical score to assess their risk of experiencing liver-related morbidity or mortality in hepatitis delta.

PATIENTS AND METHODS

Inclusion criteria

Only patients with detectable HBsAg and either anti-HDV antibodies (anti-HDV Ab) or HDV-RNA for more than 6 months were included. Patients with undetectable HDV-RNA were considered to have chronic hepatitis delta if they repeatedly showed biochemical signs of hepatitis (e.g. transaminases increased 1.5-fold above the upper limit of normal ULN) in the absence of significant HBV replication. Patients were required to have an available follow-up of at least 18 months with a minimum of three visits at our centre and no longer than 4 years between consecutive visits. Those patients who had undergone an OLT before the first observation (baseline) or less than 18 months after baseline were excluded. Additional exclusion criteria were human immunodeficiency virus (HIV) co-infection, active alcohol abuse or drug addiction, evidence of metabolic, autoimmune or genetic liver disease and ongoing therapies that could interfere with either the liver function or the viral replication (e.g. chemotherapy). The system used for patient selection and inclusion is summarized in Fig. 1a.

Cohort description

A total of 75 patients with hepatitis delta were retrospectively followed for up to 16 years (median 5 years) at Hanover Medical School (Fig. 1a). Seventy-one of these patients presented between 1992 and April 2006 and were already included in the cohort of 258 anti-HDV-positive individuals previously published by Heidrich et al. [20]. Another four patients were selected from 106 serum samples that tested positive for HBsAg and anti-HDV Ab between January 2004 and December 2007. The majority were male (69%), aged between 15 and 61 years (median = 39), and most of them came from Eastern Mediterranean or Eastern European countries (30%, 21%, respectively). The modality of transmission was unknown for 56% of the patients, 23% had a family history of HBV, HDV or both, in 12% of the cases it was transfusion related and 9% had previous history of intravenous (i.v.) drug use.

A total of 400 serum samples were available for HBV-DNA testing and 252 for HDV-RNA. At baseline 50 (67%), patients were HDV-RNA-positive. In addition, nine patients had detectable HDV-RNA during follow-up. In 15 patients, HDV-RNA was persistently undetectable; they had, however, biochemical evidence of hepatitis and low or no HBV replication. In seven of these patients, we were able to exclude other causes of increased transaminases such as nonalcoholic steatohepatitis because they had available liver biopsies; moreover, all 15 patients presented a normal lipid profile (cholesterol mean ± SD 4.24 ± 1.1 mmol/l; triglycerides mean ± SD 1.36 ± 0.6 mmol/l), and their average BMI was 24.4 ± 2.5 kg/m². For one patient, there was no quantitative or qualitative HDV-RNA data available. He was nevertheless included in the study, because his alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were repeatedly above twice the ULN in the absence of high HBV replication; his BMI was 26 kg/m² and he had normal lipid levels throughout follow-up.

Eighteen patients (24%) had received antiviral therapy at some stage before baseline. Twelve had been given (pegylated)interferon alfa (pegIFN-α), and the remaining six patients, nucleos(t)ide analogues (NAs). During follow-up, 31 of the 57 treatment-naive patients at baseline received antiviral treatment with either (peg)IFN-α (n = 9) or NAs (n = 22). Overall, 24 subjects were exposed to (peg)IFN-α, 25 were treated with NAs and 26 remained untreated.

Definitions of clinical endpoints

The primary outcome (event) was the occurrence of a liver-related complication, defined as an episode of hepatic decompensation (i.e. ascites, bleeding of oesophageal vari-
ces or hepatic encephalopathy), HCC (confirmed by histopathological evaluation), need for an OLT or liver-related death.

The presence of cirrhosis was defined histologically, clinically or biochemically: (i) For a histological diagnosis, either a liver biopsy (F5 and F6 according to Ishak) or
transient elastography of ≥13.0 kPa [21] was required. (ii) Cirrhosis was clinically assessed when the patients presented with oesophageal varices or had an episode of ascites, gastrointestinal bleeding or hepatic encephalopathy in their past medical history and (iii) A biochemical–ultrasonographic diagnosis was made when at least two of the following features coexisted: platelet count below 100 000/mL, AST/ALT ratio > 1, cholinesterase below the lower limit of normal, international normalized ratio (INR) > 1.5 and/or splenomegaly (spleen size >12 cm). Patients without an event or lost to follow-up were censored at their last visit.

Baseline was defined as the first available visit. Consecutive follow-up visits were recorded in yearly intervals (12 ± 4 months), skipping to the next if no visit was available for the foreseen date. No gap ≥4 years was allowed.

Serology and molecular diagnostics
Virologic testing was mainly performed in the routine diagnostic laboratory of the department of Gastroenterology, Hepatology and Endocrinology of Hannover Medical School. Serum samples were tested for HBsAg, hepatitis B envelope antigen (HBeAg), anti-HBe antibodies (anti-HBe Ab), anti-HDV and anti-hepatitis C virus (HCV) antibodies (anti-HCV Ab) using the Abbot IMX system (Abbott, Wiesbaden, Germany) until June 1996. From June 1996 until February 2005, the platform was changed to Abbott AxSYM system. In February 2005, it was replaced by the Abbott Architect, which could additionally quantify HBsAg [20]. Until 2005, HBV-DNA was detected in most HBsAg-positive samples by in-house nested PCR (lower limit of detection of 50–100 IU/mL). In addition, HBV-DNA was quantified using the Digene hybridization assay (Roche Diagnostics, Mannheim, Germany). After 2005, HBV-DNA quantification was done with the Cobas TaqMan (lower limit of detection of 12 IU/mL; Roche Diagnostics). HDV-RNA was detected by an in-house nested PCR, and stored serum samples were quantified by a novel automated PCR using the Cobas TaqMan platform [22]. HCV-RNA was quantified using the respective versions of the Roche Cobas Amplicor assays. Biochemical liver function tests were assessed by standard laboratory tests.

The baseline-event-anticipation score
To build the baseline-event-anticipation score (BEA score), we first identified variables associated with a higher risk of developing the above-defined clinical endpoints in the anti-HDV-positive cohort by $\chi^2$ tests, univariate Cox regression models and Kaplan–Meier analysis. Points were allocated according to hazard ratio (HR). Three risk groups were defined according to the cumulative risk of developing an event, the accuracy of different point combinations was compared with receiving operating characteristic (ROC) analysis and the combination with the best performance (higher area under curve, AUC) was selected. Cox regression was again applied to confirm survival differences among different risk groups. Sensitivities, specificities, and positive and negative predictive values were calculated comparing the three risk categories against one another.

The baseline-event-anticipation score was validated in two independent anti-HDV Ab-positive cohorts from Barcelona (Spain, n = 77; details of part of this cohort were published previously [13]) and Düsseldorf (Germany, n = 62; [23]) that were followed for up to 24 years (median: 5.8) and 14 years (median: 1.8), respectively. Survival rates and hazard ratios were calculated with Kaplan–Meier and Cox regression analysis.

Ethics
All three studies were approved by their local ethics review board [13,23]. Our project was reviewed and approved by the Ethik Kommission der Medizinische Hochschule Hannover, which considered that no informed consent was necessary, given the retrospective and noninterventional nature of the study, and that patient's data were analysed anonymously.

RESULTS
Baseline characteristics
We identified 75 individuals with hepatitis delta who fulfilled all inclusion criteria for this study. Most of the patients were male (n = 52, 69%); 30 (40%) originated from the Eastern Mediterranean (E.M.) region (Turkey, Iran, Iraq, Syria), 21 (28%) from Eastern Europe or Central Asia (E.E.; Russia, Kazakhstan, Ukraine, Tadzhikistan, Poland), eleven were German (15%) and the remaining 13 (17%) were either born in eastern Asia, Africa or had an unknown country of origin. Anti-HCV Ab were found in 13 patients; however, only three of them (23%) had detectable HCV viremia at some point during follow-up. Fifteen patients repeatedly had undetectable HDV viremia throughout the follow-up period; four of them presented anti-HCV Ab. Thirty-seven patients presented with liver cirrhosis at their first visit (49%). Patients were followed for up to 16 years (median 5 years). Patients’ characteristics at baseline are summarized in Table 1.
Table 1: Demographic and clinical data of the study cohort at baseline

| Variable                        | Median (Range) |
|---------------------------------|----------------|
| Years of follow-up              | 5 (2–16)       |
| Gender: male: n (%)             | 52 (69%)       |
| Age: [years] median (range)     | 39 (15–61)     |
| Region of origin: E.M.:         | 30; 21; 24     |
| E. E.: others: n (%)            | (40%; 28%; 32%)|
| Cirrhosis at baseline: n (%)    | 37 (49%)       |
| Previous therapies: IFN-based;  | 12; 6: 57 (16%; 8%; 76%) |
| NAs: none: n (%)                |                |
| HBV-DNA: positive/total (%)     | 25/69 (42%)    |
| HBV-DNA: [log 10 IU/mL] median  | 0 (0–7)        |
| NA: 31                          |                |
| HBV Genotypes:                  | 1/21/1/2       |
| A/D/E/F: n (%)                  | (4%/84%/4%/8%)  |
| HBeAg: positive/total: n (%)    | 15/58 (26%)    |
| HDV-RNA: positive/total: n (%)  | 59/74 (80%)    |
| HDV Genotype I: n/total (%)     | 17/17 (100%)   |
| Anti-HCV positive: n/total (%)  | 13/75 (18%)    |
| ALT: [IU/mL] median (range) NA: | 69 (8–1440)    |
| Bilirubin: [μmol/L] median (range) NA: | 12 (5–174)   |
| Albumin: [g/dL] median (range) NA: | 41 (23–55)  |
| Thrombocytes: [10^3/mL] median (range) NA: | 131.5 (16–323) |
| INR: median (range) NA: 8       | 1.13 (0.90–1.83)|
| AFP: [ng/L] median (range) NA: | 3 (0–109)     |
| MELD: median (range) NA: 22     | 8 (5–21)       |
| APRI: median (range) NA: 6      | 2 (0–19)       |
| AST/ALT: median (range) NA: 5   | 0.84 (0.04–4.31)|

NA, data not available; E.E, Eastern Europe (Russia, Kazakhstan, Poland, Tadzhikistan, Ukraine and Uzbekistan); E.M, Eastern Mediterranean (Turkey, Syria and Iran). IFN, interferon; NAs, nucleos(t)ide analogues.

*Fifteen patients tested negative for HDV-RNA throughout follow-up with previous HDV-RNA assays but showed biochemical evidence of hepatitis. For one anti-HDV positive patient, there was no available HDV-RNA.

0.5–9 years). From the 56 individuals with a diagnosed liver cirrhosis, 32 (57%) developed liver decompensation after a median time of 3 years (range 0–10; seven patients had a liver decompensation at baseline). HCC was diagnosed in five subjects after a median time of 4 years (range 2–9 years), liver transplantation was performed in 16 individuals (median 4 years, range 2–9 years) and five patients died (median 3 years, range 2–10 years). Only 23 of the 56 patients with cirrhosis (41%) did not undergo any major clinical complication during follow-up. Of the 19 patients who did not progress to liver cirrhosis, one subject died during follow-up (unknown cause), eleven were still alive at the end of this study and seven were lost during follow-up. Of the 15 patients with undetectable HDV-RNA throughout follow-up, two eventually developed HBsAb (2/15, 13%; after 10 and 11 years, respectively), and four underwent a liver decompensation (4/15, 27%), of which two in turn developed HCC (2/15, 13%).

Of note, eight of the 15 patients with persistent undetectable HDV viremia presented cirrhosis at baseline (53%); during follow-up, six of the seven patients with chronic hepatitis at baseline developed liver cirrhosis (86%). Only four of the cirrhotic patients with negative HDV-RNA throughout follow-up suffered a liver decompensation (29%), and two of them subsequently developed HCC (14%). One eventually received a liver transplantation (7%). Interestingly, the only two patients who developed HBsAb belonged to this subgroup.

The clinical long-term outcome of the complete chronic hepatitis delta cohort is summarized in Fig. 1b.

Biochemical outcome

ALT values were normal at baseline in 26 of the 75 co-infected patients (35%). During follow-up, 11 of these patients maintained normal ALT levels while the remaining 15 patients experienced ALT flares. Forty-nine patients had ALT levels above the ULN at baseline including 27 patients with persistently increased ALT levels and 22 subjects with fluctuating values that normalized at the end of follow-up in 19 cases.

Development of the baseline-event-anticipation score

Variables associated with a higher risk of developing a clinical endpoint in the anti-HDV-positive cohort were determined by univariate Cox regression models and Kaplan–Meier analysis. The following factors were associated with the occurrence of an event: age, region of origin, INR, platelet counts, bilirubin, cirrhosis at baseline, previous exposure to interferon and albumin levels (Table S1).

Due to the small sample size and some missing values, no multivariate but bivariate Cox regression models among the above-mentioned factors were applied to identify independent risk parameters. Only INR greater than 1.2 and thrombocytes below 50 000 and between 50–100 000 showed independent association with the development of an event after adjustment for gender, age, region of origin, cirrhosis at baseline and albumin.

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Rationale for selecting specific variables in the BEA score

From all variables that showed a significant association with the development of major liver-related complications, we selected the following for the development of the BEA score. The selection was done with statistical and clinical criteria to avoid the overestimation of some aspects of the disease, to keep the score as easy to use as possible and to ensure its applicability in diversified settings.

Age: in our study, we showed that age at baseline in patients with hepatitis delta is linked to a particularly severe course and early development of liver cirrhosis and its complications. Moreover, in the literature, age has proven to be an essential prognostic factor for many liver conditions, including hepatitis delta [9,24]. For these reasons, we considered age had to be included in the BEA score.

The region of origin defines to some extent the patient’s access to medical care as well as cultural habits such as alcohol consumption or a fatty diet. These factors can strongly modify the prognosis of the liver disease; consequently, we included it in the BEA score.

INR evaluates the extrinsic pathway of coagulation, which depends on factors I, II, V, VII and X, all of which are synthesized in the liver. Hence, INR is a good marker of the liver’s protein production. Furthermore, in our study, INR was strongly associated with the time to event with a P-value of <0.01 and was thus incorporated to the BEA score.

Thrombocytopenia: in time, liver cirrhosis leads to portal hypertension. One of the main consequences of an increased portal pressure is splenomegaly, which implies spleen hyperfunction and subsequently a drop in platelet counts. Thrombocytes are thus a critical, indirect measure of the liver disease progression and were therefore taken into the BEA score.

We also included bilirubin in the BEA score because it is a well-known marker to evaluate the liver breakdown and excretion capacities, which are decreased both in the advanced stages of the liver fibrosis and in hepatitis flares.

Male sex was not significantly associated with a liver-related complication. However, with a P-value of 0.08 and the existing evidence in the literature that sex strongly influences the outcome of several liver diseases and especially viral hepatitis [12,25], we decided to include it in the BEA score formula.

Cirrhosis at baseline was strongly associated with a worse outcome in the first analysis (P < 0.0001). Nevertheless, we decided to exclude it because it was defined as a conglomerate of variables and thus was not an independent factor (for the definition of cirrhosis, see ‘methods’).

Previous exposure to antiviral therapies along follow-up was not included because we considered the access to treatment is biased by country of origin and general health condition of the patient (i.e. IFN is contraindicated in Child C liver cirrhosis).

Albumin levels at baseline appeared to be highly significant with the χ² analysis. However, due to the large number of missing values at baseline, the available data were insufficient to provide a powerful statistical analysis. Moreover, we had already included INR as a measure of liver synthesis capacity, and to avoid overrating this liver function in the BEA score, albumin levels were excluded.

After the variable selection was finished, we assigned them points according to their hazard ratio (HR): age older than 40 (HR = 2.1, P = 0.04), Eastern Mediterranean origin (HR = 3.76, P < 0.0001), INR above 1.2 (HR = 4.56, P < 0.0001), platelet count below 100 000/µL (1 point, HR = 6.65, P < 0.0001) or 50 000/µL (2 points, HR = 11.63, P < 0.0001), bilirubin above the ULN (HR = 2.21, P = 0.04) and male sex (HR = 2.07, P = 0.08) (Table 2, Fig. 2a). Taken together, in the BEA score, we included factors that reflect the patient’s demographics, the presence of portal hypertension, and the liver’s protein production and breakdown functions.

Risk groups and accuracy of the BEA score

Once points were allocated, multiple score distributions were analysed to differentiate three risk groups. The group division with the best ROC performance (area under curve, AUC = 0.818) was considered the most favourable. The three risk categories were defined as follows: mild risk (BEA-A: patients with 0 or 1 point), moderate risk (BEA-B: 2–4 points) and severe risk (BEA-C: patients with BEA scores above four points). Only one of 19 patients (5.3%) with 0 or 1 BEA points, but 16 of 30 individuals (53%) with 2–4 and 9 of 10 (90%) patients with 5–7 points experienced an event. Significant differences among all the three categories were confirmed by Kaplan–Meier analyses and univariate Cox regression analysis (Fig. 3a).

ROC analyses were used to compare the performance of both score and risk categories with already established scoring/classification liver disease assessments. The AUC of BEA score was 0.88 (Table 3, Fig. 2b); corresponding values were lower for Child–Pugh (AUC = 0.72), ALT-platelet-ratio index (APRI) (AUC = 0.83) and MELD (AUC = 0.85) scores as well as for the AST/ALT ratio (AUC = 0.61).

The BEA score also proved its sound performance in a binary classification test with high sensitivity and negative predictive values (NPV) for the BEA-A risk category as compared to BEA-B and BEA-C (A vs B: sensitivity 94%, NPV 95%; A vs C: sensitivity 90%, NPV 95%), and high specificity and positive predictive values (PPV) when comparing BEA-C with BEA-A and BEA-B (A vs C: specificity 95% PPV 90%; B vs C: specificity 93%, PPV 90%). The Table S2 shows the sensitivity, specificity, positive and negative predictive values of the BEA score.

Finally, we created an automated version of this scoring system for open-use online [26].
The BEA score was validated in two independent cohorts of patients with chronic hepatitis delta, followed in Barcelona (n = 77) and Düsseldorf (n = 62). Age, sex, thrombocytes, bilirubin and frequency of developing an event showed no significant differences with our training cohort (Table S3). However, a higher proportion of patients in the Düsseldorf cohort showed normal INR values than in the other two centres (Düsseldorf = 92% vs Barcelona = 62% and Hannover = 68%; P < 0.001, (Table S3)).

Univariate Cox analysis confirmed significant differences among the three risk groups in both validation cohorts as patients with BEA-B and BEA-C were considerably more likely to experience an event (Barcelona: BEA-A/B/C: HR = 1/31.8/288.8; Düsseldorf: BEA-A/B/C: HR = 1/6.1/90.8; Fig. 3b,c). Remarkably, none of the patients with BEA-C in the Barcelona validation cohort survived 5 years after the first visit, and the only patient with BEA-C in the cohort from Düsseldorf experienced an event the first year of observation.

Sensitivity, specificity, positive and negative predictive values were examined and are shown in Table S2.

**DISCUSSION**

For the management of patients with liver diseases, it is important to determine the individual risk of disease progression. This is critical in particular for hepatitis delta given the poor efficacy of the current interferon-based therapy, the numerous and burdensome side effects that it entails and that it is contraindicated for patients with cirrhosis Child B or Child C [17]. Clinical scores to determine the stage of liver damage and to predict the outcome of patients with liver disease have never been systematically evaluated in hepatitis delta. Indeed, the performance of some of these scores was very weak in our hepatitis delta cohort (Table S3). Thus, we aimed to develop an easy to
apply scoring system that would be helpful in daily clinical practice to determine the risk to develop liver-related morbidity in HDV-infected patients.

In addition to well-established parameters to assess liver function such as INR and bilirubin levels or portal hypertension (thrombocytopenia), we found that age above 40 years and Eastern Mediterranean origin were strongly associated with a higher likelihood to develop a clinical event. We also included male sex in the BEA score despite borderline significance in our analyses, as male sex has been associated with disease progression in another delta hepatitis cohort [12] and has proven to be of influence in the outcome of other viral hepatitis [25]. The BEA score proved to distinguish patients with low, moderate and severe risk of clinical disease progression with high accuracy. The risk for BEA-B and BEA-C patients to develop an event was 9.01- and 25.27-fold increased with respect to BEA-A patients in the study cohort (Fig. 3a). In clinical practice, high sensitivities and negative predictive values of clinical scores are needed if treatment shall preferentially be given to patients at risk; this is the case for the BEA score with both values above 90% (Table S2). Thus, the BEA score can sort patients with a high risk of undergoing a liver-related event in the short term – which are the ones who should receive treatment as soon as possible – from those who have lower risk of liver-related event and do not need to be treated immediately – because they can wait until new therapeutic options are available.

Importantly, the performance of the BEA score was tested and confirmed in two independent validation cohorts. It needs to be considered that like our study population, the Düsseldorf cohort also included several patients born in the Eastern Mediterranean region [23] which was not the case for patients recruited in Barcelona. Furthermore, patients in Düsseldorf had less advanced liver disease as only one patient was included in the BEA-C risk category. Despite these differences, the BEA score proved high accuracy in both validation cohorts.

This study has three main limitations. First of all, we applied stringent patient selection criteria, which is essential to obtain a cohort that can be used to develop a

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**Fig. 2** (a) BEA score algorithm. Patients with chronic hepatitis delta receive one point for every single condition met. The baseline-event-anticipation score is calculated by the sum of all points obtained. Patients with a BEA score of 0–1 points are at low risk of developing an event (BEA-A); those with 2, 3 or 4 points have moderate risk (BEA-B); subjects with five or more points are at high risk (BEA-C) of suffering a major liver-related complication. Note: due to missing values of the different variables, only 59 of the 75 anti-HDV patients were included in this analysis. (b) Receiving operating characteristic (ROC) curves of the BEA score and the three BEA risk categories.
clinical score. For this reason, even though more than 300 anti-HDV-positive individuals were screened, only 75 patients could be included in this retrospective follow-up study. However, the data set proved to be reliable as the key findings could be confirmed in two independent cohorts. Secondly, patients included in all three cohorts had genotype I HDV. Moreover, the external validity of this model is restricted to Caucasian patients. To confirm the universality of the BEA score, it should be tested in cohorts with different genotypes (i.e., from South America) and with other racial and socio-demographic contexts of infection. To make this possible, further international collaboration is needed. In this line, a Hepatitis Delta International Network is currently being set up [27]. And finally, patients in all cohorts were followed at academic centres and thus a referral bias may have led to an overestimation of disease progression.

In summary, we show that our novel simple clinical score can be used to identify subjects with a low, moderate or high risk for disease progression. We suggest that the BEA score is useful for the management of hepatitis delta to decide which patients most urgently require antiviral therapy or need closer monitoring [26].

**ACKNOWLEDGEMENTS**

We thank Regina Raupach, Peter Magerstedt, Martina Darnedde and Rüdiger Horn-Wichmann (†) for technical assistance and Dr. Hans L. Tillmann for helpful discussions.

**DISCLOSURES**

This study was supported by the BMBF-funded Integrated Research and Treatment Center Transplantation (IFB-Tx) (funding number 01EO0802), project 37 (H. Wedemeyer and B. Heidrich). The authors declare no conflict of interest.
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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1: Univariate Cox regression analysis of the clinical and biochemical parameters associated with development of an event.

Table S2: BEA score validation: sensitivity, specificity, positive and negative predictive values (PPV and NPV, respectively) in the study cohort and the two validation cohorts.

Table S3: Description and comparison of study and validation cohorts.

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