

29 **# CORRESPONDING AUTHOR**

30 **Email:** philippa.matthews@ndm.ox.ac.uk

31 **Address:** Medawar Building for Pathogen Research, South Parks Road, Oxford OX1

32 3SY, UK

33 **Telephone:** 0044 1865 271973

34

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47

48 **AUTHORS' CONTRIBUTIONS:**

49 • Conceived the study: KJ, EB, PCM

50 • Assimilated/analysed clinical records: LD, SL, JM, MP, SC, KJ

51 • Established the BRC informatics working group: KC

52 • Developed the health informatics (HIC) pipeline: DS, HS, JD, OF, KV, KW

53 • Analysed the data: DS, ALM, MAA, SL, JM, PCM

54 • Literature review: LD, JM

55 • Wrote the manuscript: LD, DS, PCM

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57

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63 .

64 **ABBREVIATIONS**

65	CHB	Chronic Hepatitis B virus infection
66	EPR	Electronic patient record
67	HBeAg	Hepatitis B 'e' antigen
68	HBsAg	Hepatitis B surface antigen
69	HBV	Hepatitis B virus
70	HCV	Hepatitis C virus
71	HIC	Health Informatics Collaborative
72	HIV	Human immunodeficiency virus
73	LFT	Liver function tests
74	LIMS	Laboratory information management system
75	NA	Nucleos(t)ide analogues
76	NIHR	National Institute for Health Research
77	PEG-IFN α	Pegylated interferon alpha 2
78	TDF	Tenofovir Disoproxil Fumarate
79	RBV	Ribavirin

80

81

82 **ABSTRACT**

83 HBsAg and HBeAg have gained traction as biomarkers of control and clearance during monitoring
84 of chronic hepatitis B virus infection (CHB). An improved understanding of the correlates of
85 clearance of these proteins could help inform improvements in patient-stratified care and advance
86 insights into the underlying mechanisms of disease control, thus underpinning new cure strategies.
87 We collected electronic clinical data via an electronic pipeline supported by the National Institute
88 for Health Research Health Informatics Collaborative (NIHR-HIC), adopting an unbiased approach
89 to generating a robust longitudinal dataset for adults testing HBsAg-positive from a large UK
90 teaching hospital over a six year period (2011-2016 inclusive). From 553 individuals with CHB,
91 longitudinal data were available for 319, representing >107,000 weeks of clinical follow-up. Among
92 these 319 individuals, 13 (4%) cleared HBsAg completely. HBsAg clearance rate was similar in
93 individuals on NA therapy (n=4, median clearance time 150 weeks) vs those not on NA therapy
94 (n=9, median clearance time 157 weeks). Those who cleared HBsAg were significantly older, and
95 less likely to be on NA therapy compared to non-clearers ($p=0.003$ and $p=0.001$, respectively).
96 Chinese ethnicity was associated with HBeAg positivity ($p=0.025$). HBeAg clearance occurred both
97 on NA therapy (n=24, median time 49 weeks) and off NA therapy (n=19, median time 52 weeks).
98 Improved insights into the dynamics of these biomarkers can underpin better prognostication and
99 patient-stratified care. Our systematised approach to data collection paves the way for scaling up
100 efforts to harness clinical data to address research questions and underpin improvements in
101 clinical care provision.

102

103 **IMPORTANCE**

104 Advances in the diagnosis, monitoring and treatment of hepatitis B virus (HBV) infection are
105 urgently required if we are to meet international targets for elimination by the year 2030. Here we
106 demonstrate how routine clinical data can be harnessed through an unbiased electronic pipeline,
107 showcasing the significant potential for amassing large clinical datasets that can help to inform
108 advances in patient care, and provide clues that inform new cure strategies. Our cohort from a
109 large UK hospital includes adults from diverse ethnic groups that have previously been under-
110 represented in the literature. Tracking two protein biomarkers that are used to monitor chronic HBV

- 111 infection, we provide new insights into the timelines of HBV clearance, both on and off treatment.
- 112 These results contribute to improvements in individualised clinical care and may provide important
- 113 clues into the immune events that underpin disease control.

114 INTRODUCTION

115 Progression of HBV infection and treatment response is most commonly monitored by
116 quantification of HBV DNA viral load (1). However, viral load measurement is expensive, not
117 universally available; viral DNA levels can fluctuate over time, and quantification can be inaccurate
118 at low levels. Reproducible, automated quantification of other biomarkers such as hepatitis B
119 surface antigen (HBsAg) and/or e-antigen (HBeAg) are therefore attractive biomarkers for use
120 instead of, or alongside, HBV DNA monitoring.

121

122 Both HBeAg and HBsAg are produced from HBV cccDNA which becomes established as an
123 intranuclear 'mini-chromosome' (Fig 1A). Two different pathways can lead to the disappearance of
124 HBV DNA from serum. First, inhibition of viral reverse transcriptase, through use of nucleos(t)ide
125 analogues (NA's) such as entecavir, tenofovir or lamivudine, prevents synthesis of new HBV DNA.
126 This approach does not have a major influence on HBsAg, which continues to be produced from
127 the cccDNA reservoir (Fig 1B). Second, immune responses (either arising naturally or driven by
128 immunotherapies, including interferon) can eliminate infected hepatocytes, removing cccDNA from
129 the liver, and thereby eliminating HBsAg and HBV DNA from the circulation. This pattern occurs
130 independent of NA therapy, and can lead to the complete elimination of HBV infection ('sterilising
131 cure'), or to cccDNA suppression to the extent that neither HBsAg nor HBV DNA can be detected
132 in the serum ('functional cure') (Fig 1C). Sterilising and functional cure cannot be distinguished
133 clinically, but the difference is important because there is a risk of reactivation following functional
134 cure that does not exist with sterilising cure.

135

136 HBsAg levels are typically highest in the earlier phases of infection and in HBeAg-positive
137 individuals, frequently correlate with HBV DNA levels in chronic hepatitis B (CHB) infection, and
138 are associated with risk of subsequent reactivation (2). HBsAg is a quantifiable risk factor for
139 development of hepatocellular carcinoma (HCC) and chronic liver disease (3), although the
140 relationship is not well defined: in some studies, higher HBsAg levels are associated with lower
141 levels of fibrosis (4–6), while in others, lower baseline HBsAg levels are associated with reduced
142 risk of both cirrhosis and HCC (7). HBsAg levels have also been used to classify individuals into

143 those with inactive carriage (together with HBV DNA <2000 IU/ml and normal ALT (8, 9)) versus
144 active CHB (associated with higher viral loads and the attendant risks of inflammatory liver
145 disease, fibrosis and cirrhosis (10–13)). HBsAg elimination is widely regarded as a marker of
146 immunological clearance ('functional cure').

147

148 HBeAg-positivity is associated with high viral loads and is therefore a marker of infectivity. Loss of
149 HBeAg is usually associated with production of anti-HBe antibody (a marker of immune-mediated
150 control), and typically associated with lower viral loads. However, although these broad patterns
151 have been described, further efforts are required to elucidate and interpret the dynamics of HBsAg
152 and HBeAg, with the potential to develop insights into the timing and patterns of immunological
153 clearance, and to improve patient-stratified clinical management.

154

155 We identified nine studies reporting an annual or cumulative HBsAg clearance rate (summarised in
156 Suppl Table 1). Notably, eight of these were in Asian populations (14–21), with the remaining one
157 based in New Zealand (22). The reported clearance rate of HBsAg ranged from 0.15% per year
158 (20) to 2.7% per year (17) with a maximum cumulative clearance of 3.5% (14). Older age was
159 associated with clearance in two cohorts (16, 22). The role of treatment in clearance is
160 inconsistent, with nucleos(t)ide analogue (NA) treatment associated with clearance in some
161 cohorts (14, 18) but not in others (19).

162

163 HBsAg levels can be used to determine treatment response, although this has been more reliably
164 reported for PEG-IFN2 α treatment than for NAs (23, 24), as it implies reduction or removal of the
165 cccDNA reservoir (Fig 1C). Current UK guidelines recommend quantitative HBsAg and HBeAg
166 measurement before starting treatment and at weeks 12, 24 and 48 during treatment, followed by
167 6 monthly measurement during long term therapy (25). European Association for the Study of the
168 Liver (EASL) guidelines recommend quantitative HBsAg measurement annually in treated patients
169 if HBV DNA is undetectable, as well as using HBsAg levels to inform the decision to stop treatment
170 (1). EASL guidelines also recommend HBeAg measurement as part of the initial clinical

171 assessment, and list HBeAg loss as one of the serological responses to treatment, but do not
172 specify a frequency for follow-up testing (1).

173

174 International targets arising from the United Nations 'sustainable development goals' have set a
175 challenge for elimination of HBV infection as a public health threat by the year 2030 (26).

176 Recognising the multi-lateral approaches that will be required to reach this ambitious goal, we here
177 focus on two inter-related aims:

178 i. We set out to showcase how longitudinal data for HBV-infected individuals can be collected
179 through an unbiased electronic pipeline that collates, cleans and anonymises routinely-
180 collected electronic clinical data, in this case driven by infrastructure supported by the UK
181 National Institutes of Health Research (NIHR) Health Informatics Collaborative (HIC);
182 (www.hic.nihr.ac.uk). The aim is to harness clinical data to drive research and quality
183 improvements in diagnostics, monitoring and therapy of viral hepatitis, and to underpin new
184 questions for basic science. Through developing and testing this system, we have devised
185 an approach that can be rolled-out to incorporate other centres, with substantial gains
186 predicted through the power of large datasets.

187 ii. We analysed data for HBV sourced from a tertiary referral UK teaching hospital, in order to
188 develop better insights into patterns of HBsAg and HBeAg clearance. Through the
189 application of an unbiased approach (agnostic to treatment, clinical stage of disease, other
190 biomarkers, or genotype of infection), we aim to develop a clear picture of the dynamics of
191 clearance. Identifying demographic or clinical characteristics that predict specific disease
192 outcomes, provides opportunity for the investigation of immunological correlates of control
193 and clearance.

194 Collectively, this enterprise provides proof-of-principle for the systematic use of electronic clinical
195 data in informing studies of viral hepatitis, as well as shedding new light on the dynamics of
196 clearance of HBsAg and HBeAg.

197

198 **RESULTS**

199 ***Description of a clinical cohort of chronic HBV infection***

200 We identified 553 individuals who tested HBsAg-positive during the six-year period 2011-2016,
201 inclusive. Of these, 319 met inclusion criteria for further analysis (as shown in Table 1; Fig 2).
202 Characteristics of the cohort are summarised in Table 2 and the complete metadata for these 319
203 CHB patients is available as a supporting data file (Suppl Table 2). We collected longitudinal data
204 for a total of 107,702 person-weeks (range 61-702 weeks, mean 338 weeks (6.5 years) of follow-
205 up per individual, IQR 174-487). The median age at first HBsAg test was 34 years (IQR 29 - 43,
206 range 10 – 71), and males accounted for 191/319 (60%) of cases. HIV co-infection was
207 documented in 9 individuals (2.8%), although we cannot exclude the possibility that the true
208 prevalence of HIV-coinfection was higher due to a proportion of individuals who did not have a
209 recent HIV test result.

210

211 ***Frequency of HBsAg clearance***

212 Exemplar patterns of HBsAg clearance are illustrated in Fig 3 (as per definitions in Table 1). Using
213 the most stringent definition of HBsAg clearance, we documented complete clearance in 13/319
214 (4.1%) individuals (for full details see Suppl Table 2 and clearance trajectories shown in Suppl Fig
215 1). The HBsAg clearance rate for this cohort was 0.6% per year. In only 2/13 cases could we
216 estimate the likely duration of infection prior to clearance, one individual who had been vertically
217 infected (HBS-145) and one with iatrogenic infection related to a blood transfusion in childhood
218 (HBS-113). These individuals were both infected for approximately 25 years before clearing
219 HBsAg.

220

221 We classified an additional 27/319 (8.5%) individuals as ‘potential clearers’ on the grounds of
222 HBsAg trends consistently declining towards clearance (criteria in Table 1; clearance curves
223 shown in Suppl Fig 2). These represent a more heterogenous group, but the clearance trajectory in
224 all cases suggests that they would meet the more stringent clearance criteria if prospective
225 surveillance were to be continued. In contrast, HBsAg curves for non-clearers are shown in Suppl
226 Fig 3.

227

228 ***Characteristics of individuals with HBsAg clearance or potential clearance***

229 Adults classified as completely or potentially clearing HBsAg were significantly older than non-
230 clearers (median age 40 vs 34 years; $p=0.003$; Table 2; Suppl Fig 4). There was no difference in
231 sex or ethnic origin between individuals in different HBsAg clearance categories (Table 2). The
232 majority of those who completely cleared HBsAg were HBeAg-negative throughout the period of
233 observation (10/13, 77%). Among the remaining three with detectable HBeAg, two of these lost
234 HBeAg prior to clearing HBsAg (HBS-197 and HBS-223), while one (HBS-195) cleared HBsAg and
235 HBeAg together (Suppl Fig 1). In three cases (HBS-113, HBS-145 and HBS-195), HBV DNA was
236 cleared at the same time as HBsAg; however, in the other ten individuals (77% of clearers), HBV
237 DNA levels were low (<100 IU/ml) throughout the period of HBsAg clearance.

238

239 ***Rate of HBsAg clearance***

240 HBsAg clearance occurred over a median time of 157 weeks (95% CI 90-239 weeks) (Fig 4A).
241 Comparing individuals on treatment ($n=4$) vs. off treatment ($n=9$) during or in the 12 months prior to
242 HBsAg clearance, clearance occurred over similar time frames (median 150 weeks in those on
243 treatment vs. 157 weeks in those not on treatment; Fig 4B). Among 279 HBsAg ‘non-clearers’,
244 246/279 (88%) had HBsAg levels that were persistently >1000 IU/ml. The remaining 12% had
245 more heterogenous HBsAg dynamics, including transient dips <1000 IU/ml (e.g. HBS-298) and
246 sustained levels <1000 IU/ml but without a trend towards clearance (e.g. HBS-368).

247

248 ***Treatment status of different groups***

249 During the HBsAg clearance phase, or in the 12 months prior, 4/13 (31%) individuals defined as
250 having completely cleared HBsAg were on NA therapy (Fig 4B,C). These individuals had received
251 treatment for a median of 13 months (range 2 months – 8 years) prior to clearance. The other nine
252 (69%) were not on treatment in the 12 months prior to HBsAg clearance, but one had received
253 PEG-IFN α therapy 4 years earlier. In those individuals defined as ‘potential clearers’, 7/27 (26%)
254 received NA treatment, two of whom received TDF as part of an HIV treatment regimen.

255

256 We also reviewed treatment data for the 279 individuals who did not clear HBsAg, and were able
257 to retrieve data for 171 of these (61%). Among these, 131 (77%) had received treatment of some

258 type, and 40 had never been treated (23%). We were not able to determine robust time-frames for
259 most treatment episodes. Based on these data, non-HBsAg clearers were statistically more likely
260 to be on treatment than HBsAg clearers (131/171, vs 4/13 respectively, $p=0.001$ by Fisher's Exact
261 test). This may reflect inherently better immune control in the group who clear HBsAg, meaning
262 they are less likely to meet criteria for treatment than non-clearers. However, these data must be
263 interpreted with caution, as bias is introduced as a result of missing data among the non-clearers,
264 and by different time-lines for follow-up (we assessed treatment cross-sectionally in clearers based
265 on a specific time of HBsAg loss, for which there is no equivalent among non-clearers, thus we
266 may have assessed longer follow-up times in the latter group).

267

268 ***HBeAg status***

269 HBeAg was detectable in 81/319 (25%) of individuals at the start of the observed time period.
270 Among these, 51/81 (63%) were male and the median age was 34. By multivariate analysis,
271 Chinese ethnicity was associated with HBeAg-positive status, with 22/56 (39%) of Chinese
272 individuals being HBeAg-positive ($p=0.025$). We documented HBeAg clearance in 44/81 (54%) of
273 these individuals over the observed time period (Table 3). HBeAg loss occurred over a median
274 period of 54 weeks (95% CI 38-66 weeks) between last positive and first negative HBeAg test (Fig
275 4D). Median clearance was 49 weeks (95% CI 29-59 weeks) for individuals who had received
276 treatment in the year prior to the last positive HBeAg result ($n=24$, 55%) and 52 weeks (95% CI
277 14-133) for untreated individuals ($n=19$, 43%); treatment data were not available for 1 individual
278 (Fig 4E,F). Longitudinal data for individuals classified according to HBeAg clearance are shown in
279 Suppl Figs 5-7. We also reviewed treatment data for those who did not clear HBeAg, and were
280 able to retrieve data for 27 of these (73%). Of these, 24 (89%) had received some treatment whilst
281 3 (11%) were untreated.

282

283 ***Association between HBsAg clearance and ALT***

284 Complete longitudinal ALT data are shown for each individual in Suppl Figs 1-3. We investigated
285 whether there were differences in ALT according to HBsAg clearance (for each of the three HBsAg
286 groups defined in Fig 2). There was no significant difference in ALT at the time of first test between

287 HBsAg clearers, 'potential clearers' and non-clearers (Suppl Fig 8). ALT data were available before
288 and during HBsAg clearance for 11/13 individuals who cleared HBsAg. Among these, three
289 individuals (HBS-162, HBS-195 and HBS-314) had a spike in ALT before clearance which returned
290 to the normal range after HBsAg clearance. Another individual (HBS-230) also had a slightly raised
291 ALT before HBsAg clearance, but this did not normalise after HBsAg clearance. In the 7 other
292 cases, ALT results remained within the normal reference range for the entire period of surveillance
293 (Suppl Fig 1).

294

295 ***Relationship between HBsAg and HBV DNA***

296 In 11/13 HBsAg clearers, HBV DNA was below the limit of detection (<20 IU/ml) throughout; in two
297 cases, HBV DNA was cleared at the same time as HBsAg (Suppl Fig 1). The HBV DNA trajectory
298 of individuals classified as potential clearers was more heterogenous (Suppl Fig 2): 10 individuals
299 had cleared HBV DNA by the time of their last HBsAg test, 9 had negative HBV DNA results at
300 some point but had subsequent detectable viraemia, and 8 individuals had detectable HBV DNA
301 throughout the period of surveillance.

302

303 **DISCUSSION**

304 ***Key findings and novelty***

305 We present a diverse cohort of individuals with chronic HBV infection, showcasing a new
306 algorithmic approach to collating a large longitudinal clinical dataset from multiple electronic
307 sources. The HIC infrastructure provides a foundation for future initiatives that collate diverse
308 clinical data. While this current dataset pertains to a single centre only, in the longer term, we
309 propose to adopt this approach to unify datasets from different clinical and geographic settings,
310 generating bigger datasets that have more power to inform both clinical practice and research.
311 This is a powerful approach, as following the initial investment in setting up the infrastructure and
312 software, minimal further effort is required in order to assimilate extended clinical datasets over
313 time.

314

315 HBsAg clearance in CHB is an uncommon event, and large cohorts are therefore required to
316 describe the characteristics of individuals who clear, and to determine the specific dynamics of
317 serological changes. We have here plotted clearance trajectories that are likely to be generally
318 reflective of serological clearance of both HBeAg and HBsAg over time. All previous studies that
319 we identified describing HBsAg loss originate from Asia or Australasia (Suppl Table 1) and are
320 therefore likely to represent HBV genotypes B and C. Undertaking this analysis in a UK-based
321 cohort provides a more diverse mixture of host ethnicities (and by inference, diverse viral
322 genotypes). Unlike some previous studies of HBsAg clearance that introduce bias through a focus
323 on treatment or based on patient recall for follow-up, the approach we took is agnostic to other
324 parameters, thereby providing a more complete picture.

325

326 Our dataset corroborates prior literature in confirming that treatment is not pre-requisite for
327 clearance, and that either functional or sterilising cure, leading to immunological clearance of
328 HBsAg and HBeAg can occur independent of antiviral therapy (Fig 1C) (16, 27). Due to small
329 numbers, we did not have statistical power to determine whether there was a significant difference
330 in the time taken to clear either HBsAg or HBeAg in individuals on treatment compared to an
331 untreated group. However, the comparable speed of clearance on vs off treatment suggests that
332 clearance trajectories are similar irrespective of NA treatment. We found that NA treatment was
333 more common among non-clearers, which may genuinely reflect a higher proportion of this group
334 meeting treatment criteria, but may also be biased by the incomplete nature of our treatment data.
335 Further prospective studies are needed to study the relationship between clearance and treatment
336 in more detail.

337

338 Based on the epidemiology of HBV infection in this cohort, in which a substantial proportion of
339 individuals are likely to have been infected at birth or in early childhood, it is intriguing that HBsAg
340 and HBeAg clearance occur apparently at random in middle adulthood. In the case of HBsAg,
341 clearance is associated with older age as has been previously reported (16, 22). HBeAg clearance
342 occurred over a median period of 54 weeks, substantially more quickly than HBsAg clearance
343 which was documented over a median period of 157 weeks, perhaps indicating different underlying

344 mechanisms at play (28–30). Further studies are needed to determine the relevant immune
345 responses that underpin this clearance, and to identify possible triggers for clearance.

346

347 ***Relevance of HBsAg and HBeAg for clinical practice and research***

348 While some guidelines recommend monitoring of HBsAg levels (1, 31), there is a lack of consistent
349 understanding about how to interpret individual or longitudinal measurements. Developing better
350 insights into the prognostic information that can be captured from this biomarker could be relevant
351 to predicting patient outcomes and providing stratification of therapy. In this study, we did not have
352 routine access to HBsAg levels >1000 IU/ml, but as these data progressively become available,
353 future studies will have the opportunity to develop a better picture of HBsAg distribution across the
354 whole range of CHB infections. Advocacy is required to provide more universal access to platforms
355 that quantify HBsAg, and to improve clinical practice through interval measurements of HBsAg in
356 chronically infected patients.

357

358 The picture we have developed here suggests that the majority of individuals who develop a
359 sustained pattern of HBsAg decline below 1000 IU/ml are likely to go on to clear HBsAg, consistent
360 with previous longitudinal surveillance suggesting that baseline HBsAg levels may be a more
361 accurate prognostic marker than HBV viral load (21, 22). Prospective studies of large HBV cohorts
362 are likely to be needed to identify individuals on a clearance trajectory; enhanced surveillance of
363 these individuals is a promising future route to understanding the immunological correlates of
364 HBsAg clearance.

365

366 ***Caveats and limitations***

367 Clinical data, particularly when collected retrospectively, present significant challenges for analysis.
368 Despite the automated, integrated approach we used, heterogeneity arises from a wide range of
369 factors, all of which potentially limit or distort analysis (summarised in Table 4). We set stringent
370 standards for the quality of data to be included in this analysis, but consequently excluded a
371 substantial proportion of our overall dataset from the record. Careful consideration is needed to
372 balance between inclusion of potentially erroneous or misleading data with the additional power

373 that can be gained from maximising the overall size of datasets. As larger datasets are amassed,
374 the significance of individual errors will be diluted.

375

376 ***Future questions***

377 Prospective surveillance is important in order to provide the opportunity for studying relevant
378 immune responses during the clearance phase. As we have shown that clearance is a relatively
379 long process, occurring over a median of 54 weeks for HBeAg and 157 weeks for HBsAg, this
380 provides a window of opportunity for sampling and follow-up. There is an important distinction to be
381 made between functional cure (sustained loss of HBsAg) and sterilising cure (loss of cccDNA
382 integrated into hepatocyte nuclei); further work is needed to develop biomarkers that can detect
383 cccDNA in order to distinguish between these two different outcomes.

384

385 Studies of both host and viral genetics are required to underpin a better understanding of the
386 mechanisms of clearance, including new approaches to generating full length deep sequencing of
387 HBV, and unbiased methods to study host genetic polymorphisms that impact on disease
388 outcome. In order to power such studies sufficiently to detect relevant signals, large collaborative
389 multi-centre studies may be required. As we improve our insights into the dynamic changes of
390 serological markers, opportunities arise for improving prognostication and providing better patient-
391 stratified care.

392

393 **MATERIALS AND METHODS**

394 **Clinical cohorts and data collection**

395 Our HBV cohort was collected from the records of a large UK teaching hospital in Oxford
396 (<http://www.ouh.nhs.uk/>), which provides 1 million patient contacts per year and receives
397 laboratory samples from the community and four inpatient sites. We retrospectively identified
398 individuals aged ≥ 18 at time of database interrogation (26-Mar-2018) with chronic HBV infection
399 (defined as positive HBsAg on ≥ 2 occasions ≥ 6 months apart) based on laboratory data collected
400 between January 2011 and December 2016. Inclusion criteria and other case definitions are set
401 out in Table 1.

402

403 Our cohort was initially defined by an electronic search of the microbiology laboratory information
404 management systems (LIMS) to identify individuals with a positive HBsAg test. Individual subjects
405 were allocated a pseudo-anonymised ID prefixed 'HBS', these ID numbers are included in the text
406 to allow relevant results to be identified from within our metadata table (Suppl data table 2). We
407 generated a data dictionary of search terms (Table 5) to define the data set. A data product was
408 then created using the Oxford Clinical Informatics Groups research data warehouse. The data
409 warehouse (Fig 5) receives data from operational systems within the hospital, such as electronic
410 patient records (EPR) and LIMS, and maps this data to individuals. Each item in our data
411 dictionary was retrieved and used to create a pseudo-anonymised data product for each individual.
412 These data were cleaned and individuals not meeting inclusion criteria (Table 1) were removed.

413

414 We devised classification criteria for HBsAg and HBeAg to sort each individual into a category
415 based on the dynamics of these serologic markers (Table 1). For HBsAg and HBeAg 'clearers' and
416 HBsAg 'potential clearers', data which were not captured electronically or were not available from
417 the data warehouse e.g. (most recent transient elastography score and HBV treatment status),
418 were retrieved from the patient's written clinical record or from dictated letters from the viral
419 hepatitis clinic.

420

421 **Ethics**

422 The NIHR HIC Viral Hepatitis database was approved by the NRES Committee South Central-
423 Oxford C on 6th October 2015 (REC reference: 15/SC/0523).

424

425 **Statistical analysis**

426 We cleaned and analysed data using R and the data.table package (32). The clearance rate was
427 calculated as $\frac{\text{Number of patients who cleared}}{\text{Total patient years}} \times 100$. Plots were created using ggplot2 (33), and
428 survival analysis and Kaplan-Meier plots created using the survival and rms packages (34). We
429 used Wilcoxon or Kruskal Wallis tests for mean comparison of continuous variables, Fisher's exact
430 test for comparison of categorical variables, and logistic regression for multivariate analysis. Code

431 used for this analysis is available in the attached HBsAg_Final_Analysis.html file included in the
432 supplementary information. To define HBsAg clearance time-frames, we measured from the time
433 of the last HBsAg result of >1000 IU/ml (or the result closest to 1000 IU/ml) to the time point at
434 which HBsAg first became undetectable. For analysis of ALT, we used the result corresponding to
435 the time of the first HBsAg test result.
436

437 **TABLES**

438 **Table 1: Summary of criteria used to confirm inclusion in the analysis and to classify**
 439 **individuals according to HBsAg dynamics and HBeAg dynamics**

Category	Criteria
Inclusion in cohort for analysis	<ul style="list-style-type: none"> • Unique electronic record available • Age ≥ 18 at time of data interrogation • Longitudinal laboratory data available • No ambiguous data points^a • HBsAg detectable at ≥ 2 timepoints ≥ 6 months apart (HBsAg > 20 IU/ml) • ≥ 1 further HBsAg reading (either positive or negative) with a total surveillance period of ≥ 12 months
HBsAg categories	
HBsAg clearer	<ul style="list-style-type: none"> • HBsAg initially detectable, but subsequently falls below the limit of detection (< 20 IU/ml) • HBsAg does not rebound to ≥ 20 IU/ml • ≥ 2 consecutive HBsAg readings < 20 IU/ml
Potential HBsAg clearer	<ul style="list-style-type: none"> • HBsAg falls < 1000 IU/ml on ≥ 2 independent occasions, • HBsAg does not rebound to > 1000 IU/ml • HBsAg not below the limit of detection for two consecutive readings
Non HBsAg clearer	<ul style="list-style-type: none"> • All individuals who are not classified as HBsAg clearer or potential clearer
HBeAg categories	
HBeAg persistently positive	<ul style="list-style-type: none"> • HBeAg above the limit of detection (≥ 20 IU/ml) for all timepoints.
HBeAg persistently negative	<ul style="list-style-type: none"> • HBeAg below the limit of detection (< 20 IU/ml) for all timepoints.
HBeAg clearer	<ul style="list-style-type: none"> • HBeAg detectable at ≥ 2 independent timepoints and subsequently falls below the limit of detection for ≥ 2 consecutive timepoints • HBeAg does not rebound above the limit of detection
Non HBeAg Clearer	<ul style="list-style-type: none"> • All individuals who are not classified as persistently HBeAg positive, negative or as an HBeAg clearer

440 ^a Records with free text or uninterpretable data were removed from analysis

441

442

443 **Table 2: Baseline characteristics of 319 individuals with chronic HBV infection recruited**
 444 **through a UK cohort and classified according to pattern of HBsAg clearance over time.**
 445

	Whole cohort	HBsAg clearers and potential clearers	HBsAg non-clearers	p-value (uni-variate analysis)	p-value (multi-variate analysis)
Number of individuals	319	40	279	NA	NA
Median age in years at time of first HBsAg test	34	40	34	0.0034*	0.0081*
Sex (%)				0.605	0.605
Male	191 (60)	26 (65)	165 (59)		
Female	128 (40)	14 (35)	114 (41)		
Self-reported ethnicity (%)					
White	92 (29)	15 (38)	77 (28)	0.649	0.632
Mixed	18 (6)	0 (0)	18 (6)	0.991	0.989
Asian or Asian British	52 (16)	7(18)	45 (16)	0.641	0.705
Black or Black British	46 (14)	5 (12)	41 (15)	0.697	0.638
Chinese	56 (18)	8 (20)	48 (17)	0.902	0.914
Any Other Ethnic Group	7 (2)	0 (0)	7 (3)	0.992	0.994
Not Stated	48 (15)	5 (12)	43 (15)	NA	NA
HBeAg positive status at baseline (%)	81 (25)	6 (15)	65 (23)	0.3105	0.131
Median elastography score, kPa (based on most recent value)	5.3	4.5	5.5 ^a	0.18	NA
Number of patients receiving treatment (%)	142/211 ^b (67)	11/40 ^c (28)	131/171 ^b (76)	NA	NA

446 ^a Elastography data available for 42 individuals in the non-clearance group, as data not routinely recorded
 447 electronically.

448 ^b Treatment data were missing for 108 individuals among the HBsAg non-clearers, as data not routinely
 449 recorded electronically.

450 ^c Treatment in the 12 months before the last positive HBsAg test

451 NA = not applicable

452

453 **Table 3: Baseline characteristics of HBeAg positive individuals classified according to**
 454 **HBeAg clearance over the observed time period**
 455

	HBeAg clearers	HBeAg non-clearers	p-value (uni-variate analysis)
Number of individuals	44	37	
Median age in years at time of first HBsAg test	34	35	0.75
Sex (%)			1
Male	29 (66)	25 (58)	
Female	15 (34)	12 (32)	
Self-reported Ethnicity (%)			
White	12 (27)	10 (27)	0.959
Mixed	4 (9)	4 (11)	0.819
Asian or Asian British	8 (18)	3 (8)	0.427
Black or Black British	6 (14)	0 (0)	0.991
Chinese	8 (18)	14 (38)	0.330
Any Other Ethnic Group	1 (2)	2 (5)	0.512
Not Stated	5 (11)	4 (11)	NA
Median elastography score, kPa (based on most recent value)	5.5	4.55	0.24
Number of patients receiving treatment ^a (%)	24/44 (55)	24/27 ^b (89)	NA

456 NA = not applicable

457 ^a Treatment in the 12 months prior to the last positive HBeAg result

458 ^b Treatment data were missing for 10 individuals among the HBeAg non-clearers as data not
 459 routinely collected electronically.

460
 461

462 **Table 4: Factors influencing the analysis of retrospective clinical HBV data**

Category of influence	Examples of the effect on data integrity
Patient factors	<ul style="list-style-type: none"> • Many individuals with CHB infection globally are not diagnosed; those with data available for clinical analysis represent a distinct minority group who have been able to access healthcare and follow-up (35). • Patients are lost to follow-up or move between regions. • HBV diagnosis rarely occurs in acute infection, so the duration of infection prior to clearance is unknown. • HBsAg clearance is a relatively infrequent event and thus patient numbers for analysis are small. • Description of a changing cohort is challenging e.g. age changes over time, patients start and stop therapy.
Healthcare factors	<ul style="list-style-type: none"> • Different assays are not always requested simultaneously, thus limiting the correlation between variables (e.g. HBV DNA vs HBsAg). • Follow-up occurs over a variety of different time frames, with different intervals between follow up visits; clearance durations may therefore be over-estimated due to infrequent sampling. • Treatment can alter the dynamics of biomarkers (e.g. ALT, HBV DNA).
Laboratory factors	<ul style="list-style-type: none"> • Assay platforms change over time, which may alter sensitivity, specificity and limits of detection. • Quantitative assays have upper and lower limits of quantification; values outside the window of detection cannot be analysed. • False positive or false negative tests may occur. • Certain data are not routinely generated or captured (e.g. HBV genotype).
Data factors	<ul style="list-style-type: none"> • Results are captured by a variety of different electronic systems (electronic patient record, electronic laboratory systems, pharmacy systems, hand-written clinical notes, dictated clinic letters). • Different healthcare professionals may not record data consistently and coding is subject to errors. • Free text entries in laboratory reporting can lead to errors or ambiguities (e.g. use of comma vs. full stop for decimal point). Certain parameters are not consistently recorded, e.g. ethnicity. • The electronic pipeline only collects certain pre-defined data (e.g. for HIV, HCV, HDV we were only able to access viral load data, not antibody tests, and therefore we do not know the denominator of total tests performed). • Treatment data may not be recorded electronically (often recorded as part of paper notes, making them more difficult to trace); start dates often not documented for patients on long-term treatment. • Poor continuity of data when patients are transferred between different healthcare providers.

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Table 5: ‘Data dictionary’ of clinical and demographic parameters collected for cohort of individuals with chronic HBV infection

Laboratory Parameter	Data source	Date range (for laboratory parameters)	Assay platform	Notes
HBsAg	Microbiology LIMS (Sunquest)	09/2004 – 03/2018	Centaur; 09/2004 – 12/2014 Abbott Architect i2000SR (Abbott laboratories, Chicago, IL); 12/2014 – 03/2018	Traditionally reported as binary test (positive/negative) but generates semi-quantitative data. Lower limit of detection 0.05 IU/mL.
HBeAg	Microbiology LIMS (Sunquest)	04/1995 - 03/2018	Centaur; 09/2004 – 12/2014 Abbott Architect i2000SR (Abbott laboratories, Chicago, IL) 12/2014 – 03/2018	Traditionally reported as binary test (positive/negative) but generates semi-quantitative data.
HBV DNA	Microbiology LIMS (Sunquest)	03/2009 – 03/2018	Cobas TaqMan assay (Roche diagnostics, Branchburg, NJ)	Lower limit of detection 0.9×10^4 IU/mL. 1 IU/ml is equivalent to 2.5-5 genome equivalents (copies/ml)
ALT	Biochemistry LIMS (LIMS)	02/2013 – 01/2018	Siemens ADVIA 2400; 02/2013 – 01/2015 Abbott Architect c16000 or c8000 (Abbott laboratories, Chicago, IL); 01/2015 – 01/2018	Reported as quantitative value. Normal reference range 10-45 IU/L
Ethnicity	Hospital EPR (Cerner Millennium)	NA	NA	Self-reported according to standardised ethnicity codes
Fibroscan result (transient elastography score)	Hospital EPR (Cerner Millennium) / Clinic letter database (Manual)	NA	EchoSens, Paris	Most recent recorded elastography result
HBV treatment status	Hospital EPR (Cerner Millennium) / Clinic letter database (Manual)	NA	NA	Treatment guidelines changed over time, so use of different agents applied across the timespan of the cohort.

469 LIMS = Laboratory information management system; EPR = Electronic patient record

470
471

472 **FIGURE LEGENDS:**

473 **Fig 1: Cartoons depicting key pathways in HBV replication cycle to illustrate targets that**
474 **may bring about control or clearance.**

475

476 **A: Pathways relevant to maintenance of HBV infection.** HBV viral DNA is released in the
477 nucleus, and cccDNA is formed by covalent ligation of the two DNA strands. A stable mini-
478 chromosome is formed, allowing persistence of the virus over time. The cccDNA acts as the
479 template for mRNA and pregenomic RNA (pgRNA). Viral reverse transcriptase (RT) generates
480 new genomic DNA from pgRNA. Non-infectious sub-viral particules (SVP) form from HBsAg and
481 new infectious virions assemble, for release into the blood stream. HBsAg measurement accounts
482 for both the SVP and infectious virions, whereas infectious virions alone can be measured through
483 HBV viral load (HBV DNA).

484

485 **B: Pathways relevant to suppression of HBV infection by NA therapy:** Inhibition of viral RT
486 suppresses generation of new viral DNA. This means new infectious HBV virions cannot be
487 constructed and HBV DNA is undetectable in plasma. However, cccDNA remains as a persistent
488 reservoir in the hepatocyte nucleus, so HBsAg production can continue and rebound viraemia is
489 likely following cessation of therapy. For this reason, individuals with CHB on successful treatment
490 frequently have an undetectable viral load but remain HBsAg-positive.

491 **C: Pathways relevant to functional or sterilising cure of HBV infection:** Upregulation of host
492 immune responses or therapy with interferon (IFN) leads to elimination of the persistent cccDNA
493 reservoir either through death of the hepatocyte or unknown non-lytic methods. HBsAg and HBV
494 DNA both disappear from the blood stream. In practice, there is no clinical test that can confirm
495 complete ('sterilising') cure, so this group is usually regarded as being at a small risk of relapse
496 (i.e. 'functional' cure).

497

498 **Fig 2: Flowchart showing identification and classification of adults with chronic HBV**
499 **infection from a hospital electronic system.** The figure represents 319 individuals who met

500 inclusion criteria, and divides these into three different categories according to HBsAg clearance,
501 and four categories for HBeAg; (for classification criteria, see Table 1).

502

503 **Fig 3: Exemplar trajectories of HBsAg over time representing adults with chronic HBV**
504 **infection.** Individuals are classified as (A) a complete HBsAg clearer, (B) a potential HBsAg
505 clearer (C) a non-HBsAg clearer; (for classification criteria, see Table 1).

506

507 **Fig 4: Kaplan-Meier curves showing trajectory of HBsAg clearance (N=13) and HBeAg**
508 **clearance (N=43) for selected individuals who met criteria for complete clearance from**
509 **within a cohort of adults with chronic HBV infection.** Data are shown for HBsAg (panels A-C)
510 and for HBeAg (panels D-F), initially for all clearers (panels A and D), and then subdivided
511 according to treatment status (panels B and E). Boxes C and F report the median time to
512 clearance for each group in weeks, with 95% confidence intervals. For HBsAg clearance, the
513 upper confidence interval for treated cases cannot be determined due to small numbers.
514 Treatment of HBsAg clearers and potential clearers comprised TDF monotherapy (n=3), TDF with
515 emtricitabine (n=2), 3TC with ADV or TDF (n=4), 3TC monotherapy (n=1), ETV monotherapy (n=1).
516 Treatment of HBeAg clearers comprised TDF monotherapy (n=10). 3TC monotherapy (n=2) ETV
517 monotherapy (n=5), 3TC with ADV (n=3), IFN with RBV (n=1), IFN monotherapy (n=3), treatment
518 data were not available for one individual. * When no values >1000 IU/ml were recorded, the
519 highest value was used. ** Not enough data to calculate upper CI. § Treatment status not known
520 for 1 individual.

521

522 **Fig 5: Flow diagram to depict collection, storage and output of electronic clinical data from**
523 **a Health Informatics Collaborative data warehouse.**

524 The data warehouse receives data from operational systems within the hospital such as electronic
525 patient records and laboratory information management systems (LIMS) and maps this data to
526 individuals where the identifiers are then stored in the master data store and provides the
527 mappings for data products. De-identified linked data is stored separately and forms the content of
528 data products. Definitions of data items are recorded in the metadata catalogue. Data items for the

529 data product are selected using the definitions in the metadata catalogue the mappings for these
530 are retrieved from the master data store and data retrieved from the integrated data store to create
531 the final data product.

532

533

534

535 **SUPPLEMENTARY DATA:**

536 On acceptance for publication, Supplementary Data will be made available at DOI:
537 10.6084/m9.figshare.7262957.

538 Prior to publication, these files can be accessed using the following URL:
539 <https://figshare.com/s/82db3b5cd1dc5c6dd566>

540

541 **Supplementary Table 1: Summary of studies reporting rate and quantitation of HBsAg**
542 **clearance in individuals with HBV infection** - Listed studies were identified from a PubMed
543 search performed in April 2018 with the search terms: ('hepatitis B' OR 'HBV') AND ('clearance'
544 OR 'seroclearance' OR 'vir* negative'), written in English between 2008 – 2018, and reporting a
545 cumulative or annual clearance rate of HBsAg using a quantitative assay.

546

547 **Supplementary Table 2: Data for 319 adults with chronic HBV infection**

548

549 **Supplementary Figure 1: Longitudinal data for 13 adults with chronic HBV infection who**
550 **completely cleared HBsAg** - Each individual is labelled with a unique anonymised ID number,
551 prefixed HBS. Time is shown in weeks since the first HBsAg positive test. Units (y-axis) are shown
552 in IU/ml, except for ALT which is shown in IU/L.

553

554 **Supplementary Figure 2: Longitudinal data for 27 adults with chronic HBV infection on a**
555 **potential HBsAg clearance trajectory.** Each individual is labelled with a unique anonymised ID
556 number, prefixed HBS. Time is shown in weeks since the first HBsAg positive test. Units (y-axis)
557 are shown in IU/ml, except for ALT which is shown in IU/L.

558

559 **Supplementary Figure 3: Longitudinal data for 279 adults with chronic HBV infection who**
560 **did not clear HBsAg.** Each individual is labelled with a unique anonymised ID number, prefixed
561 HBS. Time is shown in weeks since the first HBsAg positive test. Units (y-axis) are shown in IU/ml,
562 except for ALT which is shown in IU/L.

563

564 **Supplementary Figure 4: Boxplot showing the distribution of age among individuals who**
565 **clear or potentially clear HBsAg (n=40, median age 40) and those who do not clear HBsAg**
566 **(n=279; median age 34).**

567

568 **Supplementary Figure 5: Longitudinal data for 44 adults with chronic HBV infection who**
569 **cleared HBeAg.** Each individual is labelled with a unique anonymised ID number, prefixed HBS.
570 Time is shown in weeks since the first HBsAg positive test. Units (y-axis) are shown in IU/ml,
571 except for ALT which is shown in IU/L.

572

573 **Supplementary Figure 6: Longitudinal data for adults with chronic HBV infection who did**
574 **not clear HBeAg.** Each individual is labelled with a unique anonymised ID number, prefixed HBS.
575 Time is shown in weeks since the first HBsAg positive test. Units (y-axis) are shown in IU/ml,
576 except for ALT which is shown in IU/L.

577

578 **Supplementary Figure 7: Longitudinal data for adults with chronic HBV infection whose**
579 **HBeAg status fluctuates.** Each individual is labelled with a unique anonymised ID number,
580 prefixed HBS. Time is shown in weeks since the first HBsAg positive test. Units (y-axis) are shown
581 in IU/ml, except for ALT which is shown in IU/L.

582

583 **Supplementary Figure 8: Boxplot showing the distribution and median of the closest ALT**
584 **result to the first HBsAg test recorded among adults with chronic HBV infection who**
585 **cleared, potentially cleared, or did not clear HBsAg**

586

587 **Supplementary Code:** html file

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706

Fig 1

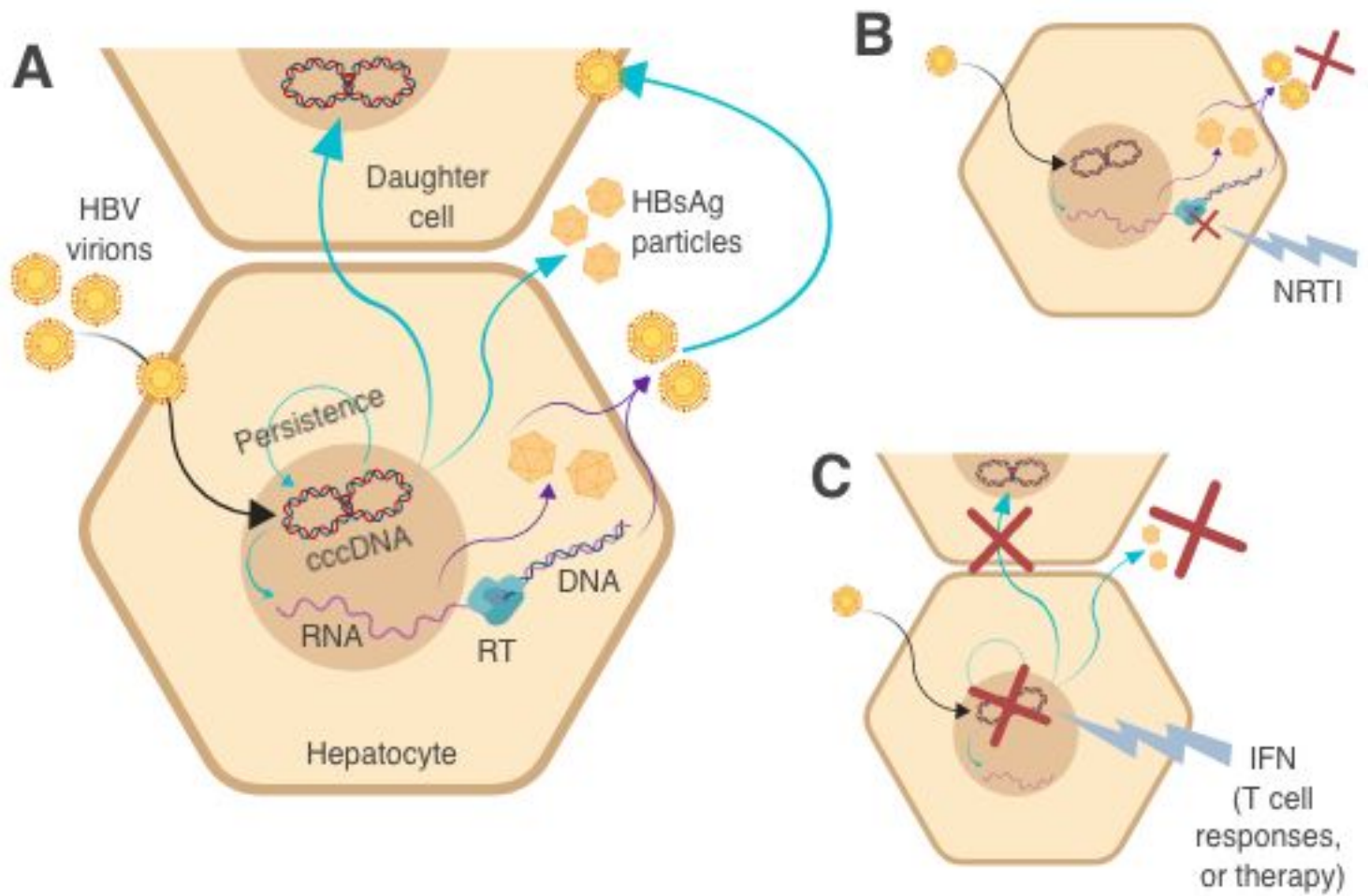


Fig 2

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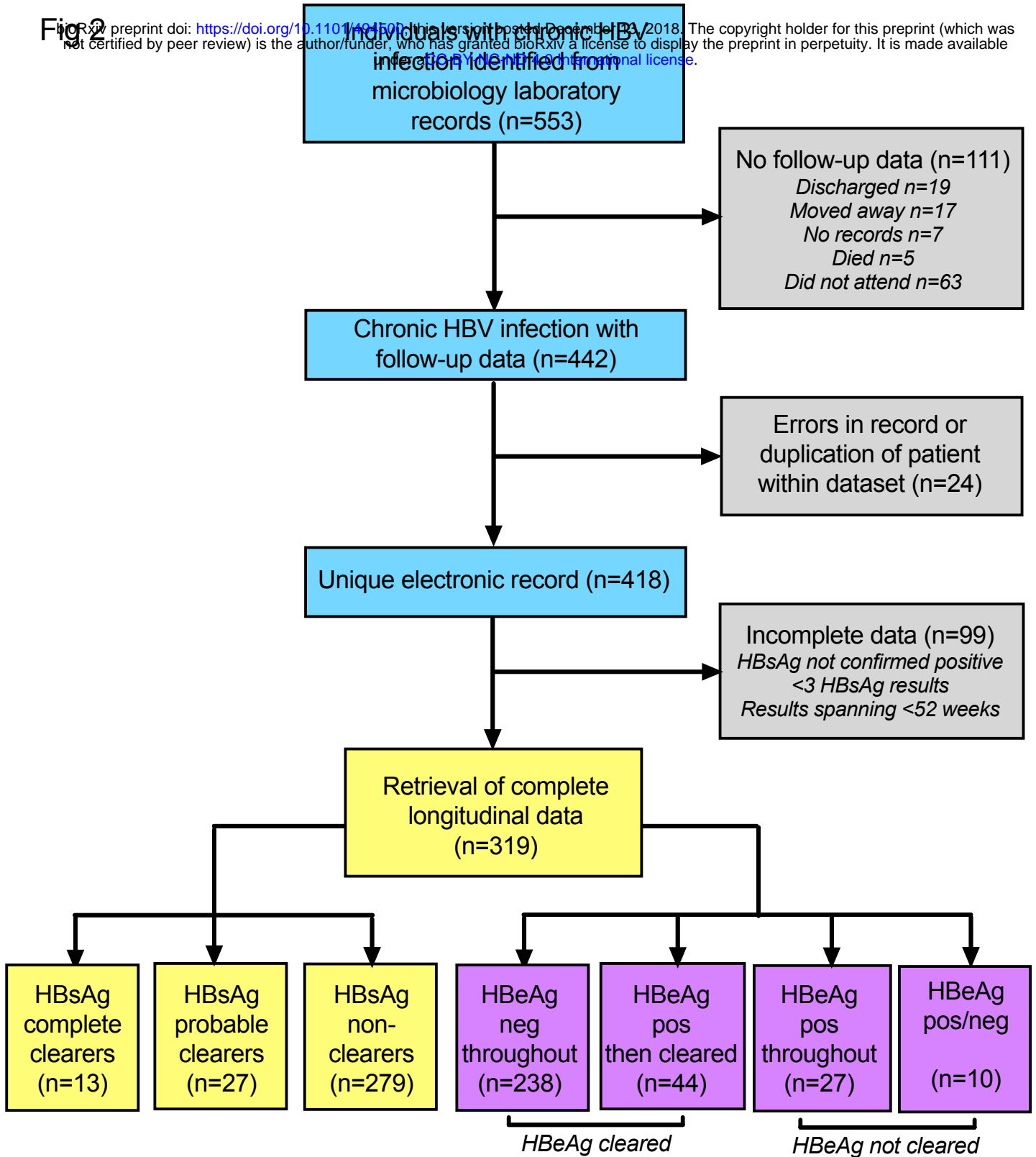


Fig 3

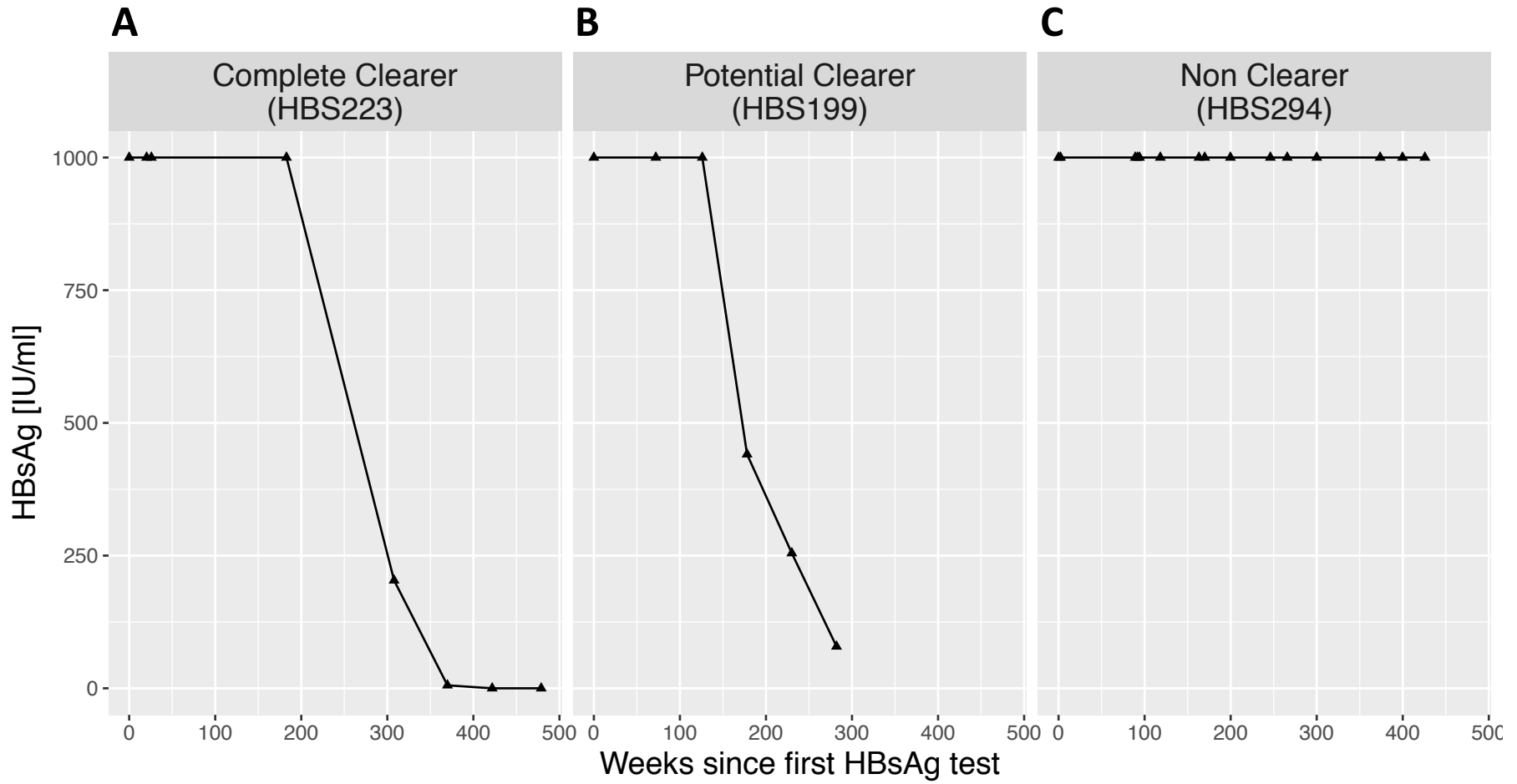
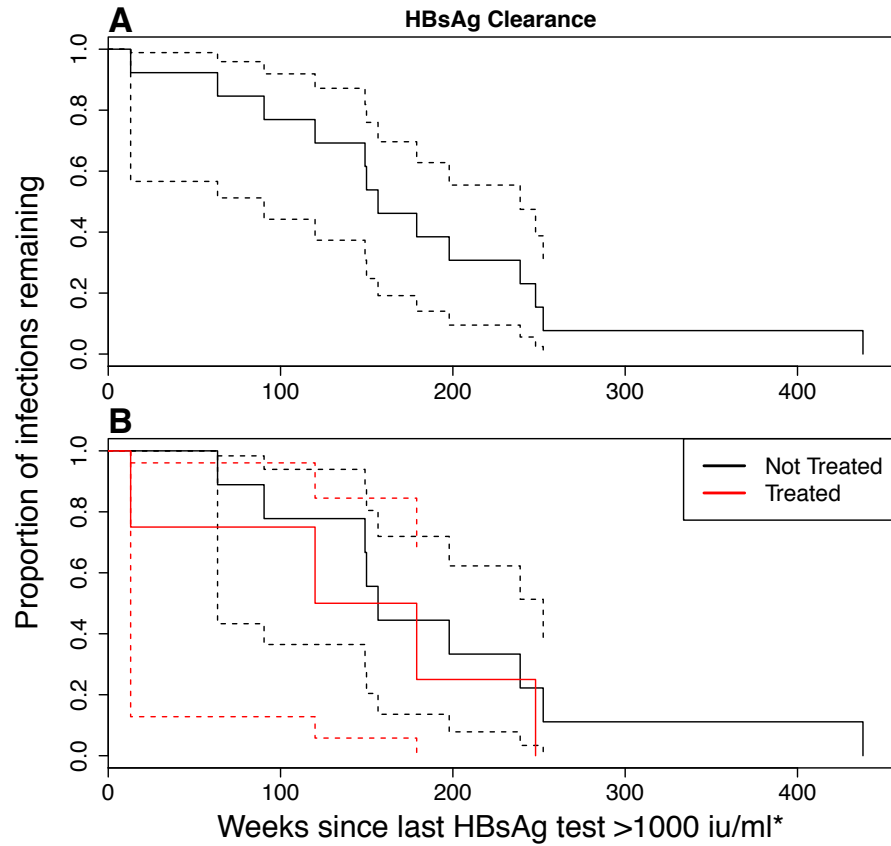
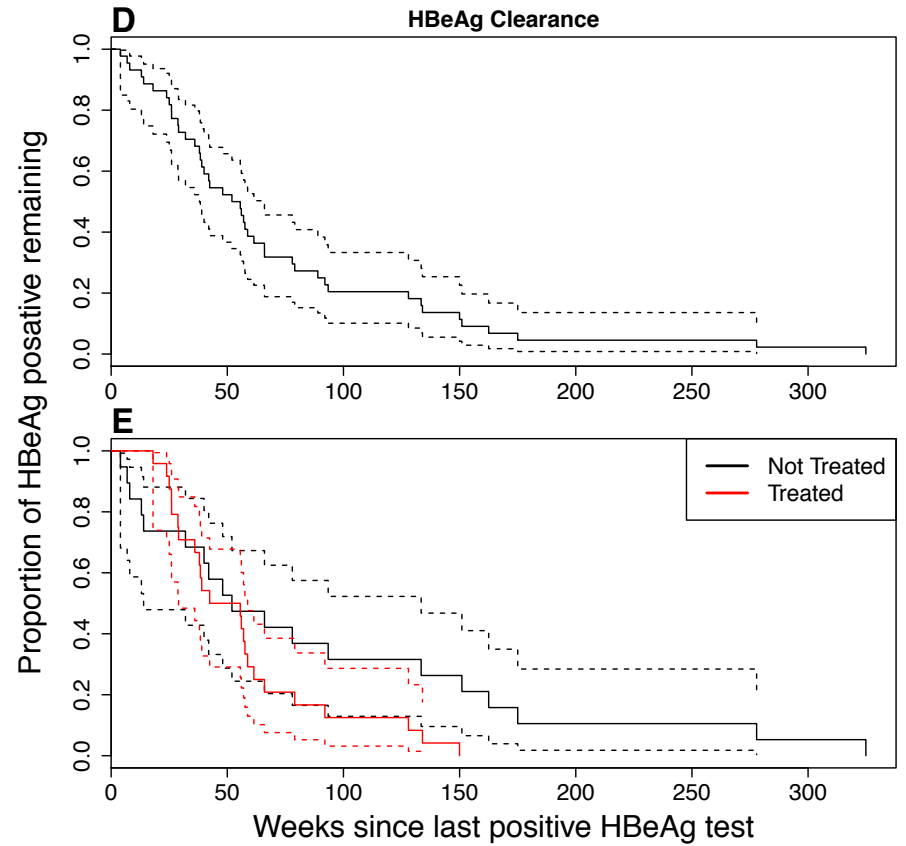


Fig 4



C

Time (weeks)	All cases of HBsAg clearance (n=13)	Untreated cases (n=9)	Treated cases (n=4)
Median	157	157	150
95% Confidence Intervals	90 – 239	63 - 252	13 – NA**



F

Time (weeks)	All cases of HBeAg clearance (n=43)§	Untreated cases (n=19)	Treated cases (n=24)
Median	54	52	49
95% Confidence Intervals	38 – 66	14 – 133	29 - 59

Fig 5

