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Direct-acting antiviral treatment outcomes in people infected with endemic compared to epidemic hepatitis C virus subtypes in England

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SUMMARY

Background: Current evidence suggests reduced efficacy of direct-acting antiviral (DAA) treatment among people with endemic Hepatitis C virus (HCV) subtypes rare to high-income countries. We aimed to determine real-world DAA treatment outcomes of people with endemic HCV subtypes in England.

Methods: Data were collected through a national treatment program. People who had their virus subtyped between 2019–2023, were resident in England and had an outcome recorded for their first DAA treatment episode, were included. Subtypes were divided into epidemic and endemic in England; endemic subtypes were confirmed with whole genome sequencing and resistance associated substitutions (RAS) were determined. Logistic regression was used to determine associations between treatment outcome and exposure variables.

Results: In people with an outcome recorded, 93 with an endemic and 8671 with an epidemic HCV subtype were identified, of whom 49.5% (46/93) and 91.8% (7953/8668) achieved a sustained virological response at 12 weeks post end of DAA treatment (SVR12), respectively. In the multivariable model, people with an endemic subtype had 93% (aOR 0.07 95%CI 0.04–0.12, P= < 0.001) reduced odds of achieving SVR12. Treatment with sofosbuvir/velpatasvir or glecaprevir/pibrentasvir was successful for genotypes 1, 2, 4 and 5 (SVR12 100%, n=13) but not 3 (27.3%, n=22) endemic subtypes. Sofosbuvir/velpatasvir/voxilaprevir was successful for GT3 endemic subtypes at retreatment (SVR12 11/12, 91.7%). Treatment failures for genotypes 1, 3 and 4 were likely mediated by naturally occurring baseline NS5A RAS (median n=2).

Discussion: This study provides further evidence that endemic HCV subtypes lead to sub-optimal DAA efficacy, which may impact global HCV elimination.

Abbreviations: DAA, Direct acting antivirals; EASL, European Association of the Study of the Liver; EBR, Elbasvir; GLE, Glecaprevir,; GZR, Grazoprevir; LDV, Ledipasvir; NHSE, NHS England; OR, Odds ratio; ODN, Operational Delivery Network; peg-IFN, Pegylated interferon-α; PI, Protease inhibitor; PIB, Pibrentasvir; RAS, Resistance associated substitution; RBV, Ribavirin; SOF, Sofosbuvir; SVR12, Sustained virological response 12 weeks after treatment end; VEL, Velpatasvir; VOX, Voxilaprevir; WGS, Whole genome sequencing; WHO, World Health Organisation; UKHSA, UK Health Security Agency; UTR, Untranslated region

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Introduction

In 2016, the UK committed to the World Health Organisation (WHO) global target to eliminate Hepatitis C virus (HCV) as a public health threat by 2030, facilitated by the availability and success of direct acting antiviral (DAA) treatments.[1] Prior to 2011, HCV treatment consisted of pegylated interferon-α (PEG IFN-α) and ribavirin (RBV) combination therapy, which had relatively limited efficacy, required long treatment periods (6–12 months) and was associated with a risk of serious and sometimes long-term side effects. The advent of well tolerated oral DAA treatment, however, revolutionised the treatment of HCV, with viral clearance rates of > 95%.[2] HCV is a highly diverse virus with eight genotypes (differing by 30–35% of nucleotide sites) and over 100 subtypes (differing by < 15% nucleotide sites) currently defined. The geographical distribution of these genotypes and subtypes is complex.[3–6] A few of these subtypes are widely distributed across the globe – these account for a large proportion of HCV infections in high-income countries and are often referred to as epidemic subtypes. These epidemic subtypes are thought to have spread rapidly in the period before HCV was discovered, primarily through the use of infected blood/blood products and injection drug use (IDU).[5,7] In contrast, non-epidemic HCV subtypes are highly diverse and found in specific geographical regions, reflecting a long period of endemic infection, including: West Africa (genotype 1), Western and Central Africa (genotype 2), the Indian sub-continent and Asia (genotype 3), Central and East Africa (genotype 4), Southeast Asia (genotype 6) and Central and South Africa (genotypes 5 and 7); a further genotype (genotype 8) has been reported in people with links to India.[3,8,9] Non-epidemic subtypes within these genotypes may be referred to as endemic subtypes.[10] Although often considered rare or unusual in high income countries, in many cases they may be common in some low- or middle-income countries.

The majority of DAA efficacy clinical trials have been carried out in high-income countries, and consequently people with endemic HCV subtypes are underrepresented. Current limited evidence suggests reduced efficacy for several endemic subtypes, including 4r, 1l and 3b, and there are many subtypes with almost no genomic or treatment outcome data available.[11–15] Furthermore, since the introduction of DAA treatments active against multiple genotypes (referred to as pangenotypic DAA regimens), including sofosbuvir/velpatasvir (SOF/VEL) and glecaprevir/pibrentasvir (GLE/PIB), HCV genotyping and subtyping may not always be carried out prior to treatment initiation. In some cases the identification of certain genotypes before first-line therapy is useful for determining a genotype specific treatment; however, even in these cases, assays are often limited to discerning subtype 1a from 1b and genotypes 1–6 (using part of the 5′ UTR plus part of the core or NS5B-coding region).[16] Although information on the prevalence and impact of resistance-associated substitutions (RAS) in endemic subtypes is limited, early data suggest intrinsic resistance to NS5A inhibitors in many of these subtypes may reduce treatment efficacy, including 3b, 3g, 4r and other non-1a/1b subtypes of genotype 1. Additionally, differences in the prevalence and fitness cost of RAS such as S282T in NS5B, and Y93H in NS5A, have been shown compared to epidemic subtypes.[17–21] The European Association for the Study of the Liver (EASL) guidelines acknowledge the challenges of treating people with subtypes infrequently seen in Europe but prevalent in regions of Africa and Asia and suggest that genotype and subtype ideally should be determined before treatment.[2] With the efficacy of pangenotypic DAA regimens against many endemic subtypes with intrinsic drug resistance still unclear, treatment programs may become less effective as the proportion of susceptible HCV subtypes diminishes.

In 2019, the Antiviral Unit at the UK Health Security Agency (UKHSA) developed and implemented a genotype-agnostic HCV whole-genome-sequencing (WGS) assay.[22] Concurrently, socio-demographic, clinical and treatment outcome data for all people receiving HCV DAAs in England are captured as part of a national Hepatitis C treatment registry. The availability of WGS with linked clinical data captures the national landscape of circulating HCV genotypes and subtypes, and associated treatment outcomes. Using this linked dataset, we aimed to determine treatment outcomes in people with an endemic subtype across England, to compare to outcomes among people with an epidemic subtype, and to identify factors associated with treatment outcomes overall. This knowledge could inform treatment programs in lower- and middle-income countries where endemic subtypes are common.

Methods

Study population

This retrospective study included sites across England where HCV treatment data are routinely collected as part of the treatment program delivered by National Health Service England's HCV Operational Delivery Networks (ODNs), alongside laboratory data collected by the UKHSA. Genotyping/subtyping is performed for most people with viraemic HCV in the UK prior to starting treatment, and data for these analyses were selected if the individual's virus was subtyped between 1st August 2019 and 9th August 2023, they were resident in England, and outcome data at 12 weeks following the end of the first DAA treatment event was recorded. Epidemic HCV subtypes were defined as 1a, 1b, 3a, 4a. Subtypes 2a, 2b and 4d were excluded as they could not be identified at subtype level in clinics with routine commercial or in-house assays (line probe, real-time PCR or small fragment genome sequencing assays). Endemic subtypes were defined as all other subtypes. As part of a national surveillance study, ODNs were requested to send a sample for WGS if the virus had been identified locally as an endemic subtype, or an unknown subtype with a clinical profile associated with a non-epidemic subtype (e.g. a person from particular regions of Africa or Asia), was identified.

Outcomes of DAA therapy at 12 weeks post treatment follow-up were initially categorised as: sustained virological response (SVR12), viral breakthrough (achieved viral clearance during treatment but became HCV RNA positive again *during* treatment), viral relapse (achieved viral clearance during treatment but became HCV RNA positive again *post-treatment*), non-response (remained HCV RNA positive throughout and post-treatment), death during treatment or prior to 12 weeks post-treatment, and lost to follow-up (did not attend the 12-week post treatment follow-up appointment).[23] This classification of DAA outcomes ignored any subsequent events (e.g. a death) occurring after the 12-week post-treatment timepoint. For the detailed analyses of predictors of SVR12, we excluded individuals who were lost to follow-up and combined all other outcome categories into a single category who were considered not to have achieved an SVR12.

Variables were collapsed as follows to permit sufficient sample sizes for analyses (see Supplementary material for full classifications): Ethnicity: Asian, Black, Mixed, White and other; Birth region: Europe, Africa, North America, South America, Asia & Australia, Missing; Disease stage: No cirrhosis, compensated cirrhosis and decompensated cirrhosis; Probable infection route: injecting drug use, sexual transmission, healthcare exposure, occupational exposure, and other; ODN region: North, Midlands/East, London, South; Treatment regimen: elbasvir/grazoprevir (EBR/GZR), SOF/VEL, SOF/ledipasvir (LDV), GLE/PIB, SOF/VEL + ribavirin (RBV), SOF/LDV + RBV, EBR/GZR + RBV, SOF/VEL/voxilaprevir (VOX), other (any other combination of DAAs without RBV added) and other + RBV (any other combination of DAAs with RBV); and treatment type: pangenotypic, genotype-specific.

Factors considered for possible associations with SVR12 were: ethnicity, HCV subtype group (endemic or epidemic), age group at first DAA treatment (<25, 25–44, 45–64, 65+ years), sex (men, women), probable infection route, disease stage based on Fibroscan-assessed liver stiffness measurement, recent injecting drug use (current/recent – injected in the last three years, past, never), birth region, UK born, HIV co-infection, previous treatment with pegylated-IFN + RBV +/- a HCV protease inhibitor, ODN region. In addition, information was collected on the name and type (pan-genotypic, genotype-specific) of the treatment regimen, presence of ribavirin in the treatment regimen, and specific treatment regimen.

WGS of endemic HCV subtypes

WGS was performed at UKHSA utilising a genotype-agnostic sequence capture target enrichment technology. In summary, samples underwent RNA extraction followed by generation of DNA libraries. The pooled DNA libraries were then hybridised and captured using 120-nucleotide HCV-specific biotinylated oligonucleotide probes. Following hybridisation HCV DNA bound to the probes was separated to undergo further PCR amplification. Sequencing was carried out using Illumina MiSeq. Detailed methods of the bioinformatic analysis pipeline (including the method for identification of query novel subtypes) and the full sequencing protocol, as well as the clinical validation for seven genotypes and 28 subtypes, can be found here.[22,24]

Resistance associated substitutions (RAS)

Consensus sequences for all endemic subtype samples were submitted to HCV-GLUE [25] for resistance interpretation supplemented by published data.[26] Any mutation characterised as RAS in an epidemic subtype of the same genotype but not as a wild-type in another epidemic subtype of the same genotype or any mutation characterised as RAS in another epidemic subtype of a different genotype was categorised as conferring resistance. Any mutation not characterised as a wild-type or RAS in any epidemic subtype was categorised as conferring possible resistance. Any mutation observed as a wild-type in the sample genotype or another epidemic subtype but not as a RAS in any epidemic subtype was categorised as being susceptible to DAAs. Sample collection date was used to determine the timepoint of sequencing, e.g. at baseline, prior to the first DAA treatment or following a second or third treatment event.

Statistical analyses

All statistical analyses were carried out using R (v4.3.1).[27] Categorised continuous variables were reported as counts and percentages.

Baseline characteristics were compared among those who attended for a 12-week post treatment visit (who were included in subsequent analyses of the predictors of SVR12) and those who were lost-to-follow-up prior to this point using Chi-squared tests. Among the group that had attended for a 12-week visit, univariate and multivariable logistic regression were used to determine the association between an epidemic/endemic subtype and SVR12. For these analyses, a further three transgender individuals were excluded, all of whom had achieved SVR12, due to the very small number in this group. A missing indicator approach was used to ensure that all individuals could be included in analyses, other than where the number of individuals with missing data for a particular variable was too small for analysis (indicated in text). Initial logistic regression analyses utilised standard covariate adjustment to remove the effects of possible confounding factors, with these factors determined a priori using directed acyclic graphs.[28] Potential confounders with a *p*-value ≤0.05 in univariate models were included in a multivariable model as were key variables not believed to confound the association with subtype, but which were of interest (sex, age group at first DAA treatment). To investigate whether these latter factors had a different contribution to SVR12 in those with epidemic/endemic subtypes, interaction terms were added to the model – a significant difference was considered if the p-value for the interaction term was ≤0.05. As the characteristics of those with an endemic versus epidemic subtype were substantially different, which might limit the efficacy of standard confounder adjustment, we also repeated analyses using propensity score confounder adjustment.

Ethics

Under section 251 of the UK NHS Act 2006 and Regulation 3 of the associated Health Service (Control of Patient Information) Regulations 2002, the UKHSA has approval to collect patient-level data for public health monitoring purposes without patient consent, including for the current surveillance study (Reference Number: NIS_CRP_58_2020). This approval is reviewed annually by the UKHSA Caldicott Panel to ensure compliance with information governance policies.

Results

Characteristics of people with endemic HCV subtypes compared to epidemic subtypes

Of the 11,024 individuals, 8761 (79.5%) completed DAA treatment, were tested at 12 weeks post treatment ending, and were included in the analyses of predictors of SVR12. Of these, 93 (1.1%) and 8668 (98.9%) were infected with an endemic and epidemic subtype, respectively. The baseline characteristics of people who were included and those who were lost to follow-up did not differ substantially (Supplementary Table 1).

For those included, the baseline characteristics differed between people with an endemic or an epidemic subtype (Table 1). For example, those with an endemic subtype were more likely to be a woman (48.9% versus 26.6%). The majority of those with an epidemic subtype were of White ethnicity (89.0%) whereas those with an endemic subtype displayed greater diversity in their ethnicities and country of birth. For example, in the endemic subtype group, 39.8% and 32.5% reported a country of birth in Africa and Asia, respectively, with only 25.3% being born in Europe compared to 0.9%, 5.0% and 92.9%, respectively, in the epidemic subtype group. The stage of liver disease also differed by subtype: 31.5% vs 13.9% of those with an endemic and epidemic subtype had compensated cirrhosis, 1.1% vs 3.2% had decompensated cirrhosis. Injecting drug use was the probable infection route for only 11.1% of those with an endemic subtype, with healthcare exposure reported as the probable infection route for 47.2% of this group; however, the majority (81.4%) of people with an epidemic subtype reported IDU as the probable infection route. People with an endemic subtype were less likely to have either a current (1.4% vs 46.5%) or past (5.5% vs 29.9%) history of IDU compared to people with an epidemic subtype, respectively (Table 1).

Treatment characteristics also differed between people with an endemic or an epidemic subtype. Median age at first DAA treatment for people with an endemic subtype was older than that among those with an epidemic subtype (median 54 (interquartile range 44, 63) vs 45 (38, 54) years, respectively). Nearly one quarter (22.8%) of people with an endemic subtype were given RBV as part of their treatment regimen compared to only 4.5% of those with an epidemic subtype. The most used DAA regimens for endemic and epidemic subtype groups were SOF/VEL (31.5%) and EBR/GRZ (38.8%), respectively. Furthermore, 9.8% of people with an endemic subtype received a regimen within the 'other + RBV' category, compared to

Table 1
Baseline demographic and clinical characteristics of people infected with an endemic or epidemic HCV subtype included in analyses of predictors of SVR12.

	N[a]	Overall, N = 8761[b]	HCV subtype category	
			Epidemic N = 8668[b]	Endemic N = 93[b]
Ethnicity	7940			
White		7008 (88.3)	6995 (89.0)	13 (14.8)
Black		162 (2.0)	129 (1.6)	33 (37.5)
Asian		414 (5.2)	385 (4.9)	29 (33.0)
Mixed		86 (1.1)	85 (1.1)	1 (1.1)
Other		270 (3.4)	258 (3.3)	12 (13.6)
Birth region	8082			
Europe		7454 (92.2)	7433 (92.9)	21 (25.3)
Asia		426 (5.3)	399 (5.0)	27 (32.5)
Africa		109 (1.3)	76 (0.9)	33 (39.8)
North America		49 (0.6)	48 (0.6)	1 (1.2)
South America		36 (0.4)	35 (0.4)	1 (1.2)
Australia		8 (<0.1)	8 (<0.1)	0 (0)
UK-born	8082			
Yes		6338 (78.4)	6321 (79.0)	17 (20.5)
No		1744 (21.6)	1678 (21.0)	66 (79.5)
HIV co-infection	8733			
Yes		290 (3.3)	282 (3.3)	8 (8.7)
No		8443 (96.7)	8359 (96.7)	84 (91.3)
Sex	8735			
Man		6386 (73.1)	6339 (73.3)	47 (51.1)
Woman		2349 (26.9)	2304 (26.6)	45 (48.9)
Disease stage	8586			
No cirrhosis		7105 (82.8)	7043 (82.9)	62 (67.4)
Compensated cirrhosis		1212 (14.1)	1183 (13.9)	29 (31.5)
Decompensated cirrhosis		269 (3.1)	268 (3.2)	1 (1.1)
Probable infection route	7160			
Injection drug use		5801 (81.0)	5797 (81.4)	4 (11.1)
Healthcare exposure		397 (5.5)	380 (5.3)	17 (47.2)
Sexual transmission		252 (3.5)	247 (3.5)	5 (13.9)
Occupational exposure		36 (0.5)	36 (0.5)	0 (0)
Other		674 (9.4)	665 (9.3)	10 (27.8)
Recency of drug injecting	7929			
Current/Recent		3651 (46.0)	3650 (46.5)	1 (1.4)
Past		2355 (29.7)	2351 (29.9)	4 (5.5)
Never		1923 (24.2)	1855 (23.6)	68 (93.2)

[a] Column N represents total count with data available.
[b] n (%).

Table 2
Treatment characteristics of people with an endemic or epidemic HCV subtype included in analyses of predictors of SVR12.

	N[a]	Overall N = 8761[b]	Subtype category	
			Epidemic N = 8668[b]	Endemic N = 93[b]
Treatment outcome	8761			
SVR12		7999 (91.3)	7953 (91.8)	46 (49.5)
Relapse		329 (3.8)	300 (3.5)	29 (31.2)
Non-response		170 (1.9)	160 (1.8)	10 (10.8)
Breakthrough		21 (0.2)	14 (0.2)	7 (7.5)
Death during		242 (2.8)	241 (2.8)	1 (1.1)
Age at first treatment, years	8751			
<25		118 (1.3)	116 (1.3)	2 (2.2)
25–44		4051 (46.3)	4029 (46.5)	22 (23.7)
45–64		4041 (46.2)	3992 (46.1)	49 (52.7)
65+		541 (6.2)	521 (6.0)	20 (21.5)
Regimen type	8760			
Genotype-specific		5017 (57.3)	4975 (57.4)	42 (45.7)
Pan-genotypic		3743 (42.7)	3693 (42.6)	50 (54.3)
RBV in regimen	8760			
Yes		408 (4.7)	387 (4.5)	21 (22.8)
No		8352 (95.3)	8281 (95.5)	71 (77.2)
Previous peg-IFN/RBV + PI	8761			
Yes		36 (0.4)	34 (0.4)	2 (2.2)
No		8725 (99.6)	8634 (99.6)	91 (97.8)
Previous IFN/peg-IFN + RBV	8761			
Yes		451 (5.1)	437 (5.0)	14 (15.1)
No		8310 (94.8)	8231 (95.0)	79 (84.9)
ODN region	8761			
North		3174 (36.2)	3147 (36.3)	27 (29.0)
South		2175 (24.8)	2163 (25.0)	11 (11.8)
Midlands/East		2008 (22.9)	1983 (22.9)	25 (26.9)
London		1405 (16.0)	1375 (15.9)	30 (32.3)
Regimen	8760			
EBR/GRZ		3374 (38.5)	3360 (38.8)	14 (15.2)
SOF/VEL		2572 (29.3)	2543 (29.3)	29 (31.5)
SOF/LDV		1382 (15.8)	1371 (15.8)	11 (12.0)
GLE/PIB		1008 (11.5)	993 (11.5)	15 (16.3)
SOF/VEL + RBV		157 (1.8)	153 (1.8)	4 (4.3)
SOF/LDV + RBV		147 (1.7)	143 (1.6)	4 (4.3)
EBR/GZR + RBV		91 (1.0)	87 (1.0)	4 (4.3)
SOF/VEL/VOX		5 (<0.1)	3 (<0.1)	2 (2.2)
Other		11 (0.1)	11 (0.1)	0 (0)
Other + RBV		13 (0.1)	4 (<0.1)	9 (9.8)

Abbreviations: EBR – Elbasvir, GLE – Glecaprevir, GRZ – Grazoprevir, LDV – Ledipasvir, ODN – Operational Delivery Network, PIB – Pibrentasvir, PI – protease inhibitor, RBV – Ribavirin, SOF – Sofosbuvir, SVR12 - sustained virological response 12 weeks after treatment end, VEL - Velpatasvir, VOX – Voxilaprevir.
[a] Column N represents total count with data available.
[b] n (%).

<0.1% with an epidemic subtype (Table 2). Other treatment-related characteristics were similar.

Genotyping of endemic subtypes

Data on initial genotyping performed by local laboratories were available for 92/93 (98.9%) endemic subtypes and when compared to WGS, 9.8% (n=9) were originally mis-subtyped, 7.6% (n=7) were mis-genotyped and 8.7% (n=8) had undetermined genotypes (Supplementary Table 2). Of the 93 people infected with an endemic HCV subtype, a range of subtypes and country of birth were identified with countries in Africa being the most common birthplace for individuals infected with GT1, 2 and 4 (25/50, 50%) and Asia for GT3 and 6 (24/35, 68.6%; Fig. 1 and Supplementary Table 3).

Treatment outcomes and factors associated with SVR12

Overall, 72.5% (7990/11024) of people achieved SVR12, 0.2% (n=21) had viral breakthrough, 3.0% (n=329) viral relapse, 1.5% (n=170) non-response, 2.2% (n=242) died during treatment and 20.5% (n=2260) were lost to follow-up; of this group, 0.1% (n=12) died after achieving SVR12. In comparison to those with an epidemic subtype, those with an endemic subtype were less likely to achieve SVR12 (endemic: 44.7%, epidemic: 72.7%), and be lost to follow-up (9.7% v 20.7%) but were more likely to experience viral breakthrough (6.8% v 0.1%), viral relapse (28.2% v 2.7%) or non-response (9.7% vs 1.5%).

Excluding those lost to follow-up, 91.2% (7990/8761) had an SVR12; 46 (49.5%) of the 93 with an endemic subtype and 7944 (91.6%) of the 8668 with an epidemic subtype (unadjusted odds ratio: OR 0.09, 95%CI 0.06–0.13, p < 0.001) (Supplementary Table 4). Of the other factors investigated, ethnicity (p < 0.001), age at first DAA treatment (p < 0.001), probable infection route (p=0.008), recency of injecting drug use (p=0.01) and whether someone was born in the UK (p=0.03) were each associated with SVR12 (Supplementary Table 4). Specifically, a reduced odds of achieving SVR12 was observed in people of black compared to white ethnicity and in people of older age at first DAA treatment. Additionally, severity of liver disease was associated with the odds of achieving SVR12 (p < 0.001) with those with compensated cirrhosis and decompensated cirrhosis

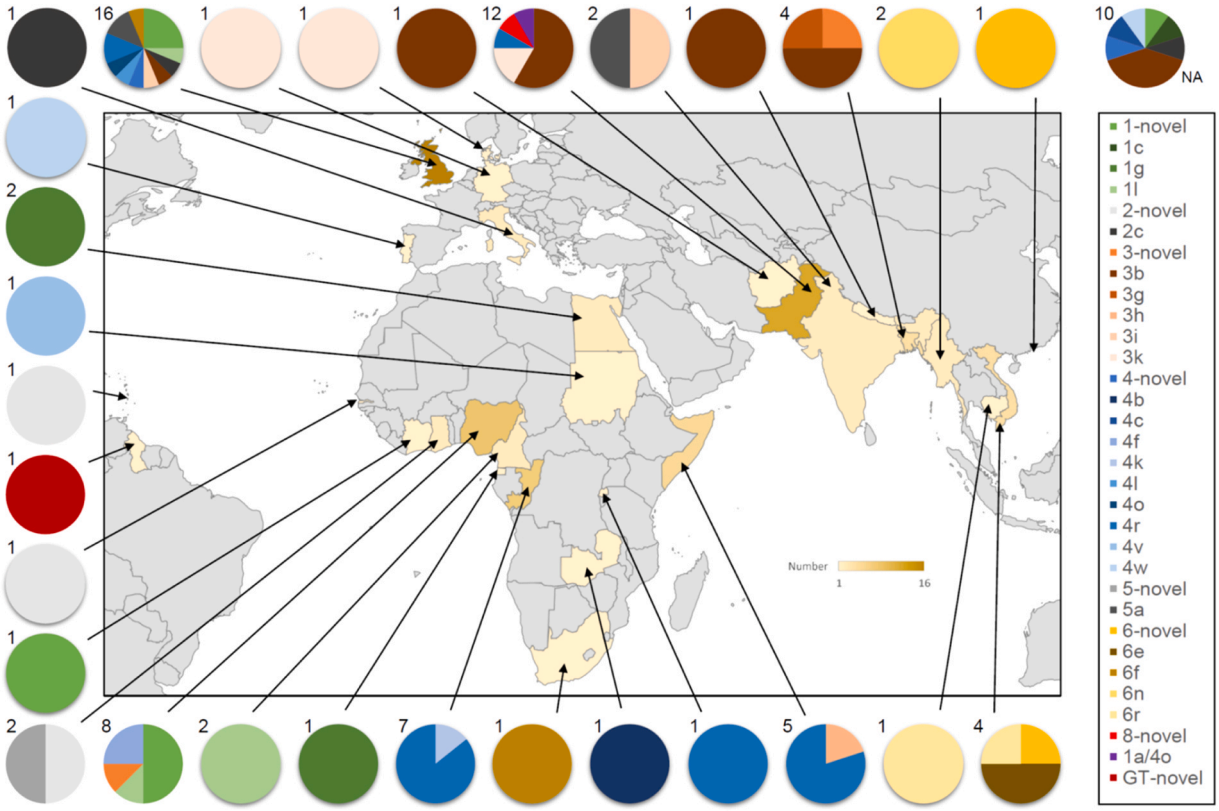

Fig. 1. Global distribution of HCV endemic subtypes by country of birth, represented by gold colour, with intensity corresponding to number of individuals up to a maximum of 16. The subtypes per country of birth are represented by pie charts with total number of sequences in the top left-hand. NA=no country of birth data available.

having a reduced odds of achieving SVR12 compared to those with no cirrhosis. Region of birth was also associated with outcome ($p < 0.001$), with people born in Africa having a reduced odds of achieving SVR12 compared to people from Europe. Previous treatment experience with peg-IFN/RBV plus a protease inhibitor was associated with a reduced odds of achieving SVR12, however this was not the case for previous treatment with peg-IFN/RBV only. Finally, the treatment regimen ($p < 0.001$), ODN region ($p=0.002$) and the inclusion of RBV ($p < 0.001$) were significantly associated with outcome. Those with RBV in their regimen had a reduced odds of achieving SVR12 compared to those that did not. Treatment regimens that resulted in reduced odds of achieving SVR12 were SOF/LDV and SOF/VEL, the same regimens with the addition of RBV, or those treated with another DAA combination plus RBV. In contrast, probable infection route of sexual transmission was associated with an increased odds of achieving SVR12 compared to IDU, and people treated in an ODN within the Midlands/East had increased odds of achieving SVR12, compared to those treated in an ODN in London.

Following adjustment for potential confounders (standard covariate adjustment), subtype group remained significantly associated with outcome, with those infected with an endemic subtype having a 93% (aOR 0.07 95%CI 0.04–0.12, P= < 0.001) reduced odds of achieving SVR12 compared to someone infected with an epidemic subtype (Table 3). Additionally, probable infection route ($p=0.02$), disease stage ($p < 0.001$), sex ($p=0.043$) and age at first treatment ($p=0.010$) each were significantly associated with outcome in the final adjusted model, although birth continent was not.

There was no evidence of an interaction between subtype group and either sex ($p_{interaction}=0.29$) or age at first DAA treatment ($p_{interaction}=0.18$). Furthermore, analyses using propensity score covariate adjustment reached similar findings (Supplementary Table 5).

Treatment outcomes among people with endemic subtypes

For endemic subtypes, the largest groups represented subtype 3b, in which 25% (4/16) of people achieved SVR12, subtype 4r in which 43% (6/14) achieved SVR12, and the genotype 1 novel subtypes in which 50% (5/10) achieved SVR12 (Fig. 2A). Additionally, all people with subtypes 1c (n=1), 3h (n=1), 3i (n=2), 4b (n=1), 4c (n=1), 4o (n=1), 4v (n=1) and 6r (n=2) experienced treatment failure, although numbers were small. The first treatment regimen used, and treatment outcomes, varied among the different subtypes. High SVR12 rates were observed when the pangenotypic regimens SOF/VEL and GLE/PIB were used for GT1, 2, 4 and 5 (100%, n=13). In contrast, reduced SVR12 rates were observed for the non-pangenotypic regimens ELB/GRZ or SOF/LDV at 50% (n=12) and 33.3% (n=18) for GT1 and 4 endemic subtypes, respectively. In addition, reduced SVR12 rates were observed for GT3 endemic subtypes with either SOF/VEL or GLE/PIB (27.3%, n=22) and for GT6 with GLE/PIB (20%, n=5) (Fig. 2B). However, a majority of these were successfully re-treated with SOF/VEL/VOX (91.7%, 11/12) or SOF/GLE/PIB+RBV (100%, 1/1). Detailed information on the proportion of people in each treatment outcome category and liver disease stage for the endemic subtype group are provided in Supplementary Table 6.

RAS at baseline and treatment failure in endemic subtypes

The availability of WGS data for endemic subtypes allowed for analysis of resistance markers in NS3, NS5A and NS5B genes. At baseline, RAS were common in the NS5A gene of endemic subtypes belonging to GT1, 3, 4 and 6 at a median of 2–3 RAS (Fig. 3; Supplementary Figure 1). The median number of RAS increased at treatment failure, up to 4 in those failing multiple therapies, in the NS5A gene of GT1 and 4 and this was accompanied by the emergence of RAS in NS3 and NS5B genes. This phenomenon was directly

Table 3
Results from multivariable logistic regression analysis of factors associated with SVR12.

	N	Multivariable		
		OR	95% CI	p-value
Subtype group				< 0.001
Epidemic	8668	Ref.		
Endemic	93	0.07	0.04, 0.12	
Probable infection route				0.020
PWID	5801	Ref.		
Sexual transmission	252	2.46	1.35, 5.07	
Healthcare exposure	397	1.46	0.97, 2.27	
Occupational exposure	36	1.70	0.51, 10.6	
Other	674	1.18	0.88, 1.60	
Missing	1601	1.01	0.82, 1.25	
Disease stage				< 0.001
No cirrhosis	7105	Ref.		
Compensated cirrhosis	1212	0.68	0.55, 0.84	
Decompensated cirrhosis	269	0.24	0.18, 0.32	
Missing	175	0.84	0.51, 1.52	
Birth continent				0.065
Europe	7454	Ref.		
Africa	109	0.88	0.50, 1.63	
North America	49	1.55	0.55, 6.54	
South America	36	1.42	0.40, 9.11	
Asia & Australia	434	1.66	1.10, 2.58	
Missing	679	1.35	1.0, 1.88	
Sex				0.044
Woman	2349	Ref.		
Man	6386	0.80	0.66, 0.95	
Missing	26	1.19	0.31, 8.17	
Age at first treatment				0.010
25–44	4051	Ref.		
< 25	118	1.18	0.58, 2.88	
45–64	4041	0.85	0.72, 1.00	
65+	541	0.58	0.43, 0.79	
Missing	10	0.56	0.10, 10.6	

Abbreviations: CI – Confidence Interval, OR – Odds Ratio.

observed in people infected with endemic subtypes where samples were available before initiation of therapy and following treatment failure. For example, in a 1-novel subtype (patient #10) there was the addition of RAS 30H and 93N/93H in the NS5A gene, or in a subtype 4r (patient #90), where there was the addition of 31V and 93C in the NS5A gene and 282T in the NS5B gene, or (patient #35) 168V in the NS3 gene (Table 4). However, the median number of RAS appeared to remain constant at 2 in the NS5A gene of GT3 endemic subtypes (Fig. 3).

RAS were more frequent at positions 31 (56%) and 93 (44%) of the NS5A gene of GT1 at baseline and significantly increased at position 30 from 11% to 56% following treatment failure (Fig. 4). In contrast, RAS were more frequent at the NS5A gene positions 30 (88%) and 31 (100%) for GT3 and positions 28 (73%), 30 (91%) and 31 (64%) in GT4 with no significant increase in proportion at these positions or others at treatment failure (Fig. 4).

Discussion

We report DAA treatment outcomes for people with endemic HCV subtypes within a national treatment program, including 30 different subtypes and 29 countries of birth, mostly African and Asian. Only 49.5% versus 91.8% of people with an endemic versus epidemic subtype achieved SVR12, respectively. Those with endemic subtypes had a 93% (aOR 0.07 95%CI 0.04–0.12, P= < 0.001) reduced odds of achieving SVR12. The high prevalence of NS5A RAS (median of n=2) in endemic subtypes of GT1, 3, 4 and 6 at baseline likely contributed to treatment failures. Of note, SVR12 was 100% (n=13/13) when SOF/VEL or GLE/PIB were used for GT1, 2, 4 and 5 endemic subtypes but only 27.3% when used for GT3 subtypes (n=6/22). In particular, the SVR12 for individuals with subtype 3b was alarmingly low (4/16, 25%) despite almost all individuals having received a second-generation pangenotypic DAA regimen. However, it was reassuring that SOF/VEL/VOX cured 8/9 (88.9%) individuals with subtype 3b, either as first-line therapy or after failure. Our study is one of the first to describe a real-world cohort with relatively high numbers of both Asian and African endemic HCV subtypes, in which SVR12 rates were low even compared to similarly diverse cohorts [14,20] and despite half of individuals having received pan-genotypic second-generation dual combination DAAs. Additionally, our study contributes new data on the efficacy of SOF/VEL/VOX for the highly treatment-resistant subtype 3b.

Subtype 3b was the largest group in our study (16/93, 17.2%); of which 12 had known country of birth and for 11 of them this was Pakistan, Bangladesh, Nepal or Afghanistan. SVR12 was only 4/16 (25%), notably lower than that observed in a Dutch real-world study (5/8, 63%) or a phase 3 trial of SOF/VEL (76% (32/42), including 50% (7/14) in those with cirrhosis). Within our study, 9/9 people with subtype 3b and compensated cirrhosis were failed by DAAs, of whom n=7 received SOF/VEL+/-RBV, n=1 GLE/PIB or SOF/DCV/RBV. For those without cirrhosis, 4/7 (57%) achieved SVR12, including 2/3 receiving SOF/VEL and 1/2 receiving GLE/PIB. We observed the NS5A RAS doublet 30K+31M in 16/16 subtype 3b sequences available at baseline and/or post-treatment failure, which confers a highly resistant phenotype to all NS5A inhibitors [19] and likely contributed to the low SVR12. The presence of cirrhosis was an additional adverse factor, as observed in clinical trials.[15,29] Reassuringly, 8/9 (89%) individuals receiving SOF/VEL/VOX, of whom 7 had been failed by SOF/VEL+/-RBV, GLE/PIB, or both, achieved SVR12 following retreatment, which is reassuring for the efficacy of this regimen, and is in keeping with a previous report.[20] Notably, the median number of NS5A RAS (n=2) for GT3 endemic subtypes in our study remained stable after failure, with no emergence of NS3 or NS5B resistance, which is consistent with persistence of naturally occurring high-level NS5A inhibitor resistance and suggests that a regimen with potent NS3 and NS5B inhibitors may be effective both for first-line or post-failure therapy. We also observed 30K+31M in 4/6 non-3b GT3 subtypes (3-novel, 3i and 3k x 2) and SVR12 of 50% (5/10) for people with non-3b GT3 endemic subtypes, of whom eight received SOF/VEL+/-RBV, demonstrating that several other GT3 subtypes are also inherently-treatment resistant. Current estimates are of 14·5 million people with HCV (95% uncertainty intervals 13·2–24·2) in South Asia where these subtypes predominate, highlighting that this region is a key target for HCV elimination.[30] Overall, robust data from clinical trials are needed to understand optimal DAA regimens for subtype 3b and other GT3 endemic subtypes. However, our data provide initial reassurance that SOF/VEL/VOX (and likely other regimens with a potent NS3 and NS5B inhibitor, such as SOF/GLE/PIB) could overcome intrinsic high level NS5A inhibitor resistance, supporting the current approach in EASL recommendations.[2]

The second largest group was subtype 4r (14/93, 15.1%), with only 43% of people achieving SVR12. Eleven of 14 individuals were from Central or East Africa (Democratic Republic of Congo, Somalia and Rwanda) and three were born in the UK. Use of DAAs other than second-generation pangenotypics, including ELB/GRZ and SOF/LDV, resulted in low SVR12 (36.4%, 4/11). Only three individuals received second-generation pan-genotypic regimens, with SVR12 of 66.7% (n=2/3). Our findings of low SVR12 with non-pangenotypic DAAs is consistent with results of the SHARED study, where SVR12 following SOF/LDV for people with subtype 4r in Rwanda was 56% (41%–71%) versus 93% (90%–96%) for non-4r subtypes.[13] An over-representation of subtype 4r virological failures was also seen among individuals in France, treated with different DAA regimens.[18] However, a single-arm trial of SOF/VEL for treatment-naïve patients with diverse GT4 subtypes in Rwanda (SHARED-3), identified SVR12 of 91% (10/11) for subtype 4r.[31] The reduced susceptibility of subtype 4r to DAA is likely mediated by multiple NS5A RAS, with the 28V+30R+31L triplet

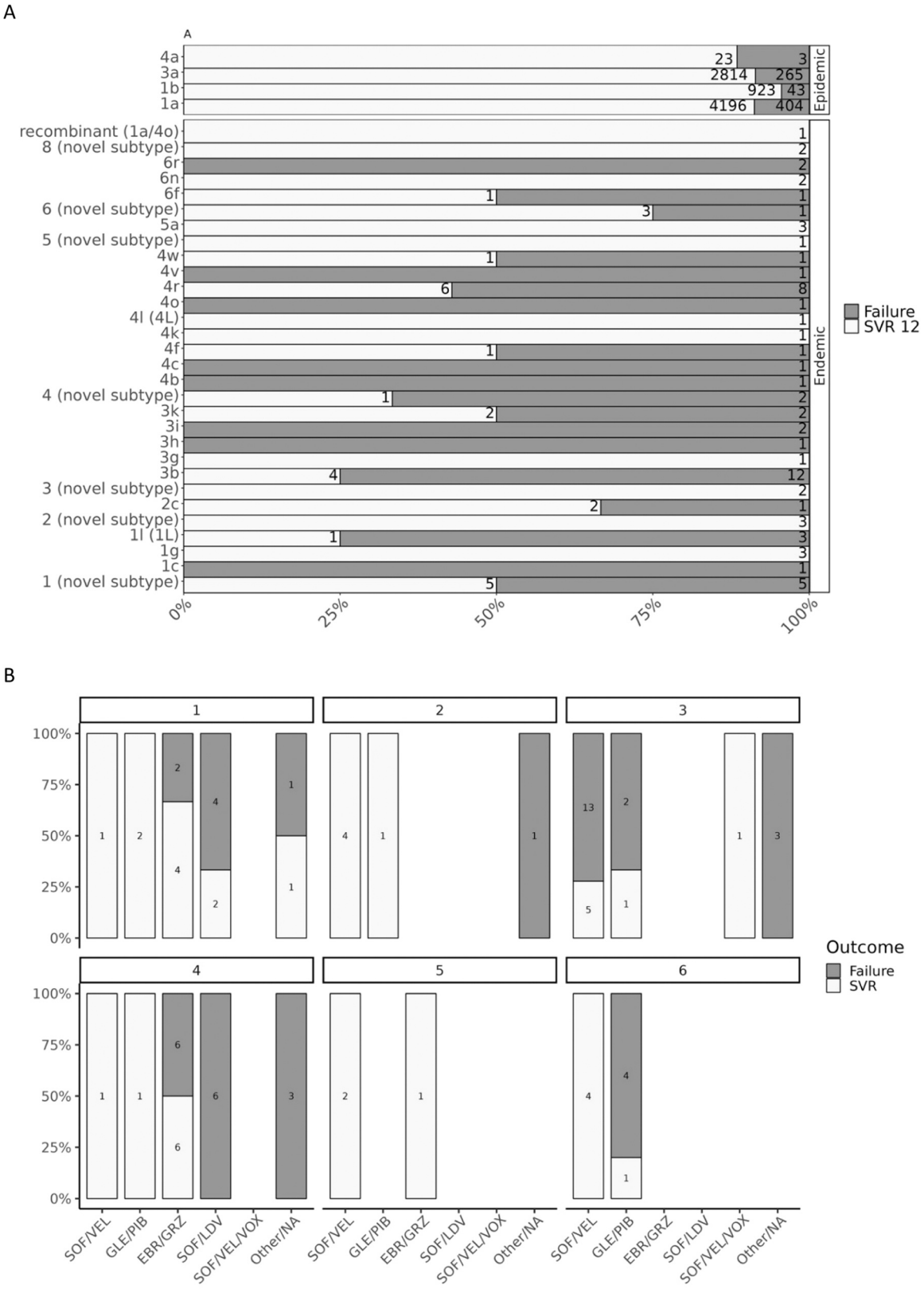

Fig. 2. Bar plot representing proportions and counts of treatment outcome following first DAA treatment, by A) epidemic, and endemic HCV subtypes and B) DAA treatment regimen. Abbreviations: SVR12 - sustained virological response 12 weeks after treatment end, EBR - Elbasvir, GLE - Glecaprevir, GRZ - Grazoprevir, LDV - Ledipasvir, PIB – Pibrentasvir, RBV - Ribavirin, SOF - Sofosbuvir, VEL - Velpatasvir, VOX – Voxilaprevir.

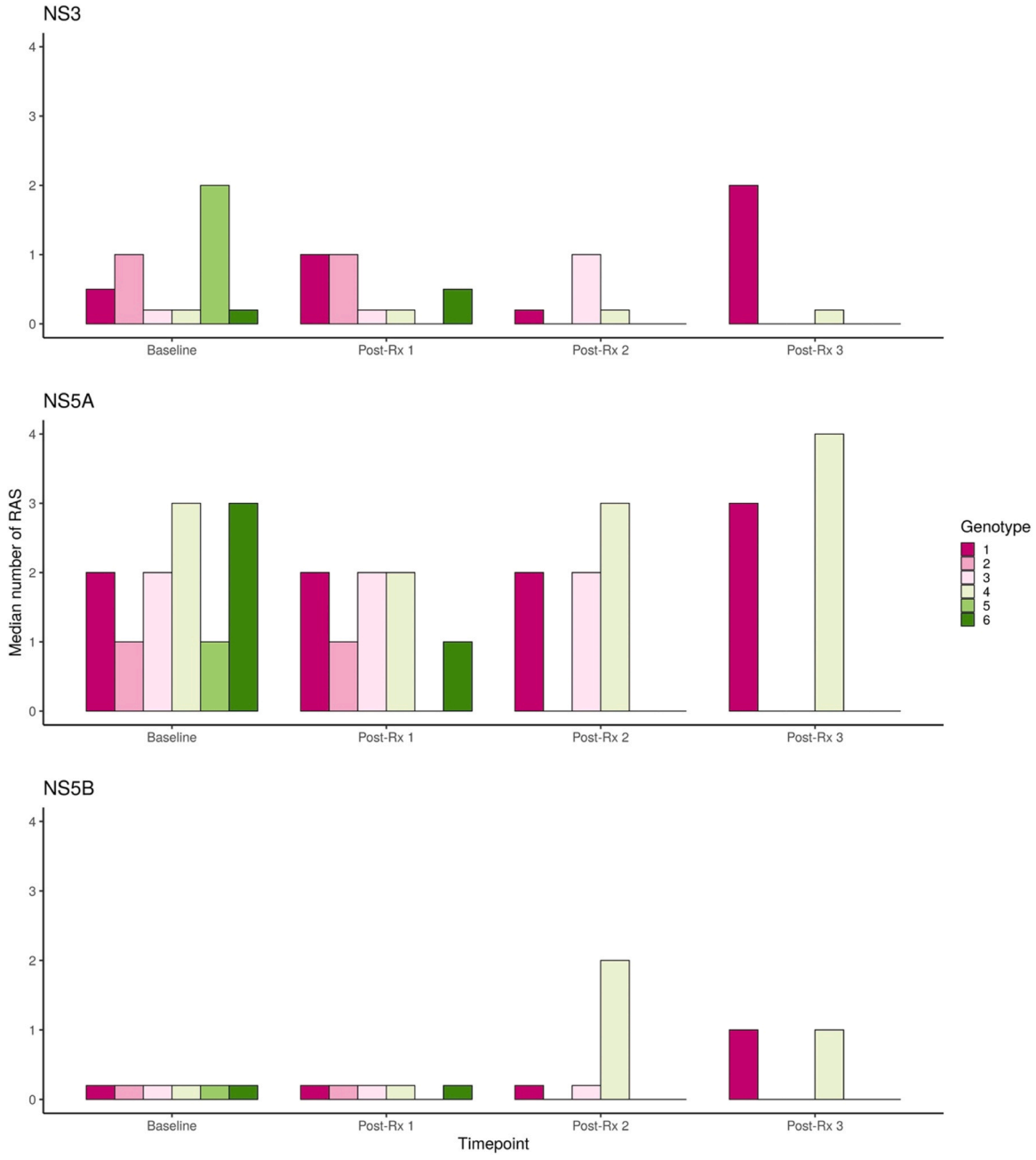

Fig. 3. Median RAS within the NS3, NS5A and NS5B genes for each genotype, at different timepoints of DAA treatment. Treatment timepoints categorised into, Baseline: prior to any DAA treatment, Post-Rx1: RAS post first treatment, Post-Rx2: RAS post second DAA treatment and Post-Rx3: RAS post third DAA treatment.

present in more than 60% of the sequences at baseline in our study, and similarly observed in other reports.[17–19] Interestingly, two individuals in our study with subtype 4r developed the SOF signature RAS NS5B 282T; this included one with virus harbouring NS5A 28V +30R at baseline who was failed by SOF/VEL and subsequently SOF/VEL/VOX and developed both emergent NS5A RAS (28V+30R+31V +93C) and the NS5B 282T. The NS5B 282T mutation is rarely observed after treatment failure in epidemic subtypes, as it impairs viral replication fitness; thus, prolonged exposure to sub-optimal DAA combinations in subtype 4r may select for new mutations that improve viral fitness and favour the persistence of both multiple NS5A RAS and NS5B 282T.

People with GT1 endemic subtypes were mainly from West Africa. SVR12 rates for GT1 endemic subtypes were lower for those receiving versus not receiving second-generation pangenotypic DAAs at 100% (n=3/3) versus 47% (n=7/15) respectively, which is consistent with other real-world cohorts (.[11,12,21] Baseline NS5A RAS for endemic GT1 subtypes were particularly prevalent at positions 31 and 93 at 55.6% and 44.4% (n=9), respectively. The prevalence increased at treatment failure at both positions to 77.8% and 55.6% (n=9), and at position 30, from 11.1% to 55.6%, demonstrating the potential for selecting increasingly resistant viruses with the use of sub-optimal first-line regimens. It is also notable that non-pangenotypic regimens are amongst those recommended in the English National Health Service for all GT1 and 4 subtypes; our findings highlight the sub-optimal efficacy of such regimens against endemic GT1 and 4 subtypes and that people with these subtypes should be offered a pan-genotypic DAA combination first-line.

For the ten people with GT6 endemic subtypes (6-novel, 6f, 6n, 6r), most were born in Southeast Asia and 9/10 (90%) did not have

Table 4
RAS, treatment regimen and treatment outcomes for individuals with samples collected at multiple timepoints.

Identifier	Subtype	Baseline RAS			Rx1	Rx1 Outcome	Post-Rx1 RAS			Rx2	Rx2 Outcome	Post-Rx2 RAS			Rx3	Rx3 Outcome
		NS3	NS5A	NS5B			NS3	NS5A	NS5B			NS3	NS5A	NS5B		
10	1-novel	122T	31M	316N	SOF/ LDV/ RBV	Breakthrough	122T	31M, 93N	316N	GLE/PIB	Breakthrough	122T	30H, 31M, 93H	316N	SOF/ VEL/ VOX	Relapse
29	4b	-	30S, 98H	-	SOF/ LDV	Relapse	-	30S, 93H	-	SOF/ VEL/VOX	SVR12					
33	4r	-	28M, 30R, 31L	316H, 321I	ELB/ GRZ	Non-response	-	28M, 30R, 31L	316H, 321I	SOF/ VEL/VOX	SVR12					
35	4r	-	93S	-	ELB/GRZ	Non-response	168V	93S	-	SOF/ VEL/VOX	SVR12					
58	3b	na	na	na	SOF/VEL	Relapse	-	30K, 31M	-	SOF/ VEL/VOX	Relapse	-	30K, 31M	282T[a]	SOF/GLE/PIB +RBV	SVR12
82	6f	36L, 122T	28T, 30S	-	GLE/ PIB	Relapse	36L, 122T	28T, 30S		SOF/ VEL/VOX	SVR12					
89	4r	-	28V, 30R	-	SOF/ VEL/RBV	Non-response	na	na	na	SOF/ VEL/VOX	Non-response	-	na	na	ELB/ GRZ/RBV	Non-response[b]

Baseline: prior to any DAA treatment, Post-Rx1: RAS post first treatment, Post-Rx2: RAS post second DAA treatment, na: sequence not available
Rx – treatment, RBV - Ribavirin, EBR - Elbasvir, GZR - Grazoprevir, SOF - Sofosbuvir, VEL - Velpatasvir, LDV - Ledipasvir, GLE - Glecaprevir, PIB - Pibrentasvir, VOX – Voxilaprevir.
[a] detected 3 months after end of treatment but had disappeared 3 months later and before initiation of 3rd treatment.
[b] Post-Rx3 RAS: NS5A 28V, 30R, 31V, 93C, NS5B: 282T.

cirrhosis. Notably, SVR12 was lower for GLE/PIB at 20% (n=1/5) than for SOF/VEL (100%, n=5/5). This contrasts with findings from an integrated analysis of phase 2/3 studies reporting treatment outcomes with GLE/PIB for GT6, 59% of individuals with GT6a or GT6e, where SVR12 was 98.4% (123/125).[32] The presence of RAS in NS3 of some GT6 subtypes could be a factor e.g. 56F in both subtype 6r in our study. However, all four cases of 6r in the integrated analysis were successfully treated with GLE/PIB although sequence data is not available. On the other hand, the two subtype 6f, one each in the integrated analysis and our study, were failed by GLE/PIB. A variety of DAA combinations have been used in other real-world cohorts with endemic GT6 subtypes, but it is difficult to draw conclusions as to DAA susceptibility, given the often small numbers of patients for each subtype.[33] Further data are required to understand the optimal regimens and factors for successful treatment of the diverse GT6 subtypes.

There were only small numbers of GT2, 5 and 8 endemic subtypes in our study, but no signal for increased treatment failure was noted. For GT2, 5/6 individuals were cured with pan-genotypic DAAs. For GT5 and GT8, SVR12 was 75% (n=3/4) and 100% (1/1), respectively with pangenotypics used in all but one individual. There was one case of a potential new genotype that was cured with GLE/PIB.

Comparing the endemic and epidemic subtype groups overall, multiple demographic characteristics differed markedly. Ethnicity and country of birth region were more diverse among people with an endemic subtype and, given the origins and distribution of endemic HCV subtypes, it is likely many infections occurred in countries where these endemic subtypes predominate. The most reported probable route of acquisition for endemic subtypes was healthcare exposure including non-occupational contact with blood in a healthcare setting, which also suggests infection acquired overseas. Taken together, our findings suggest transmission of endemic subtypes in England is not currently widespread. Case-finding of people with endemic subtypes and linkage to treatment services should be prioritised to avoid the possibility for treatment-resistant subtypes bridging into high-risk transmission networks. The finding that most epidemic subtypes in England were acquired through IDU is consistent with the literature.[19,34]

A larger proportion of people with an endemic versus epidemic subtype had compensated cirrhosis, suggesting longer periods of infection prior to diagnosis and treatment. This in turn could reflect more limited access to diagnostics and treatment in the LMIC settings where endemic subtypes predominate. Despite the increase in competition from generic manufacturers leading to more affordable generic DAAs, cost remains a limitation for many countries, in addition to access to screening, diagnostics, procurement and distribution.[29] Of note, although DAA treatment was successful for those with an epidemic subtype, the proportion lost to follow up was higher, suggesting a higher likelihood of disengagement from care, particularly among people born within versus outside the UK, even when adjusting for injecting drug use.[35]

In the unadjusted logistic regression, we found that both SOF/LDV and SOF/VEL were associated with reduced SVR12. Whilst it is widely established that LDV is ineffective against many endemic subtypes, the finding for VEL was unexpected. Findings are likely due to a substantial proportion of individuals receiving VEL having been infected with a GT3 endemic subtype, which is inherently treatment resistant to VEL. In the multivariable model, factors significantly associated with reduced SVR12 in our study were male gender and advanced disease stage, which is consistent with previous reports.[36,37] An additional factor was age at first DAA treatment, with poorer outcomes seen for increasing age, particularly age over 65 years. Few studies have examined the association of older age with achieving SVR12, partly because of age limits within clinical trials; however, both age at HCV acquisition and infection duration have been shown to correlate with disease progression.[38,39] In our

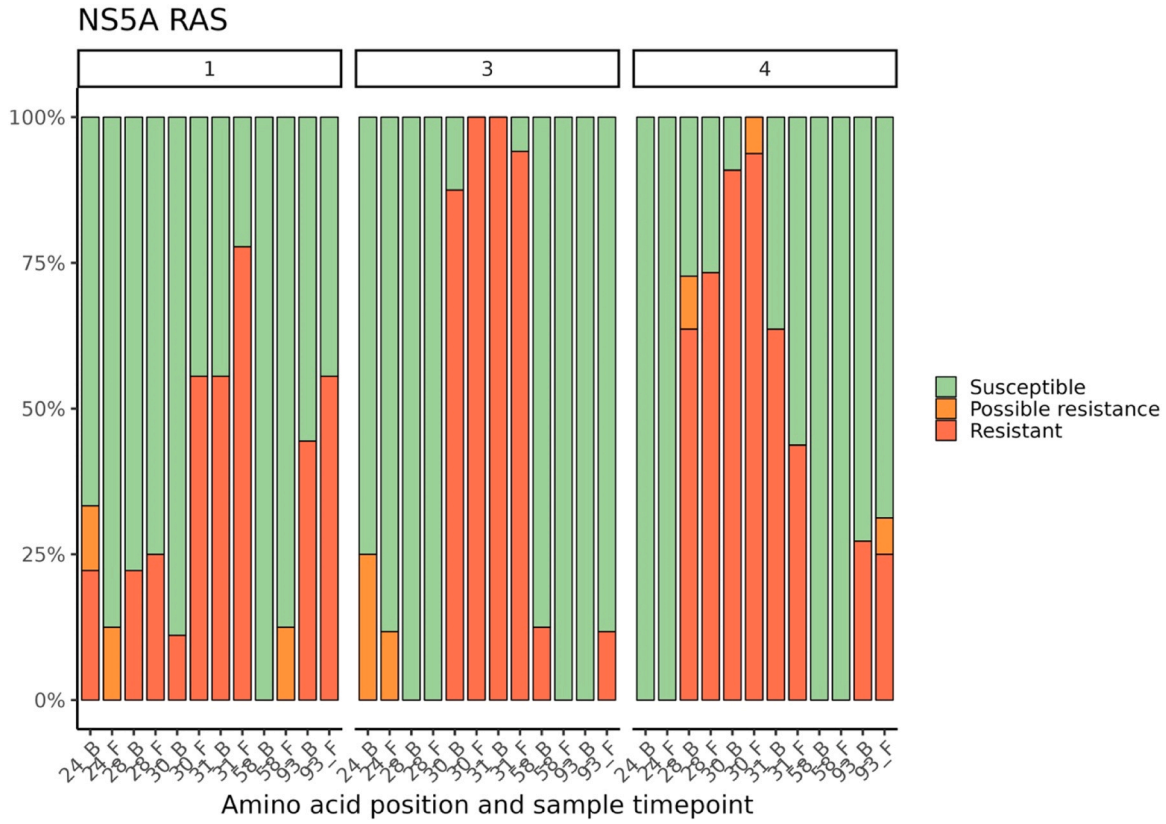

Fig. 4. Proportion of sequences with RAS in key positions of the NS5A gene at baseline (B) and following treatment failure (F), indicating resistance, possible resistance or susceptibility to NS5A inhibitors, for genotypes 1, 3 and 4.

analyses, this association remained despite adjustment for disease stage, thus other factors associated with increasing age such as co-morbidities, which may be increased in those with HCV infection, could be contributing to the reduced efficacy in older populations.[40] A further finding of interest was the approximately 2.5-fold increased odds of achieving SVR12 in those with a probable route of infection reported as sexual transmission, including heterosexual sex and MSM, compared to injecting drug use.

There are limitations to this study. Despite the relatively large sample size, 30 different endemic HCV subtypes were detected, which resulted in small sample sizes for most subtypes. As a retrospective study, interpretation was limited by data completeness, and we cannot discount the possibility of potential confounders being missed that may explain some of the associations seen in the logistic regression. A variety of DAA regimens were used including some non-standard and historic combinations, which may be linked to lower SVR12 rates. Missing data included adherence and engagement data, known to be associated with outcome and which could be influenced by factors such as being non-English speaking, or wariness of health professionals. To generate genomic data for people with an endemic subtype, the additional step of supplying a sample for WGS was required; specimens may have been identified and sent following a poor treatment outcome, rather than due to clinical suspicion of an endemic subtype. This could have resulted in more treatment-responsive endemic subtypes being underrepresented; however, almost half of cases of people with endemic subtypes had a sample taken for sequencing prior to DAA treatment initiation, therefore reducing the likelihood of this potential bias. We were unable to assess the effect of other known factors on treatment outcomes in people with endemic subtypes such as treatment duration or the potential role of RBV.[41] Lastly, the determination of whether a mutation was considered a RAS for endemic subtypes was limited by evidence available and could have resulted in under or over-reporting of RAS.

Overall, we found the odds of achieving an SVR12 in people with an endemic versus epidemic HCV subtype in England were significantly reduced. Whereas second-generation pangenotypic dual therapy DAA regimens were successful for GT1, 2 and 4 endemic subtypes, only the triple combination SOF/VEL/VOX was effective for the major GT3 group, subtype 3b. Our findings highlight the potential risk of inherently treatment resistant subtypes to achieving HCV elimination globally, even where second-generation pangenotypic DAAs are widely available, and call for both genomics surveillance within treatment programs as well as DAA stewardship according to the prevailing subtype distribution within each country.

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Authors contributions

Study concept and design: LTP, DB, CAS, JLM, WMR. Acquisition of data and samples: DB, SP, RS, JLM, Rare HCV Subtypes Group. Analysis and interpretation of data: LTP, DB, CAS, JLM. Writing of the manuscript: LTP. Critical revision of the manuscript: DB, CAS, JLM. All authors reviewed and approved the final manuscript prior to submission.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2025.106465.

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