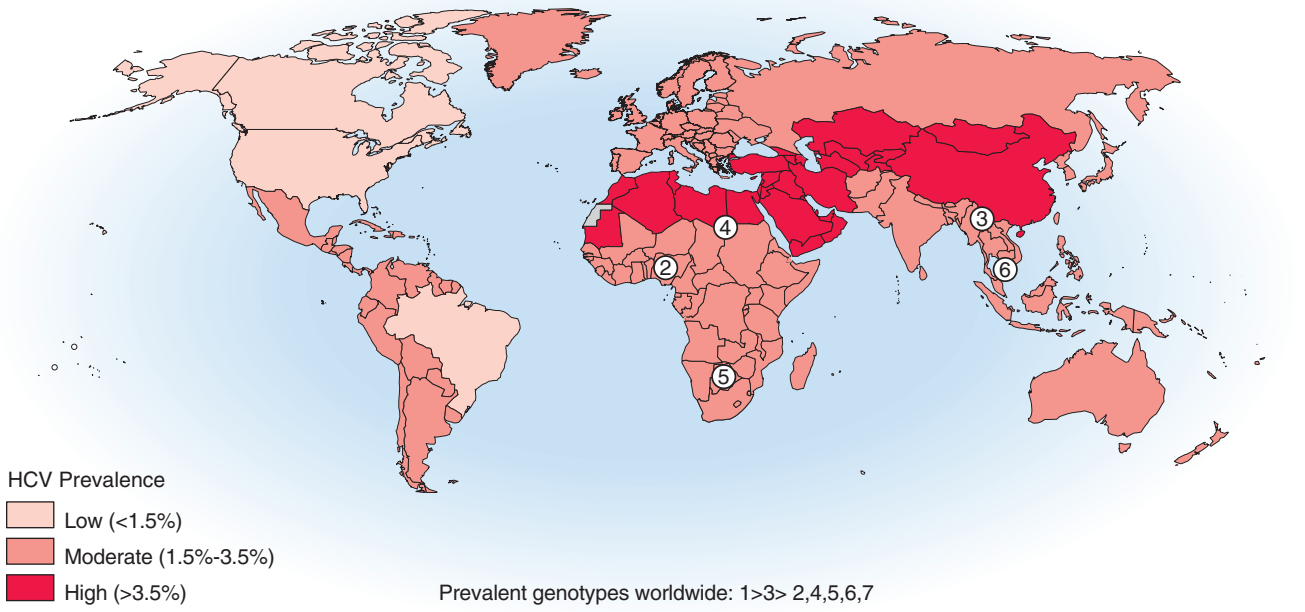

FIG. 154.5 World map indicating prevalence of hepatitis C virus *(HCV)* infection *(shading)* and subtypes *(numerals).* Genotypes 1, 2, and 3 are prevalent with variable dominance worldwide; genotypes particularly diverse (e.g., genotype 2 in Central and West Africa) or associated with a geographic region (e.g., genotype 5 in South Africa) are indicated by *circled numbers. (Prevalence estimates are modified from the Global Burden of Diseases, Injuries, and Risk Factors 2010 study [Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence.* Hepatology. *2013;57:1333–1342.])*

Experimental Models

Autonomously Replicating Viral Replicons and Genome-Length RNAs

Specially constructed subgenomic HCV RNAs ("replicons") and genome-length RNAs have been shown to undergo autonomous replication in certain cultured cells. These model systems have provided important information regarding HCV RNA replication mechanisms and virus–host cell interactions. The first such replicons were dicistronic RNAs derived from molecularly cloned cDNA.[234] The expression of a selectable antibiotic marker from the upstream cistron allowed the selection of stable cell clones supporting replication of the RNA following its transfection into the cells. The downstream cistron, encoding either the NS2-NS5B or NS3-NS5B nonstructural proteins, was placed under the translational control of a picornaviral IRES. Such RNAs demonstrate a surprisingly robust replication phenotype in cultured human hepatoma cells (Huh7 cells and their derivatives), but typically only after the accumulation of adaptive mutations within the HCV sequence (often in the NS5A protein) that enhance in vitro replication capacity.[235] Interesting to note, such mutations appear to substantially attenuate the ability of the virus to replicate in chimpanzees.[236] Some HCV RNAs have been further adapted to growth in HeLa cells and even cells of murine origin.[237]

Because subgenomic replicon RNAs typically lack the sequence encoding the structural proteins (core and E1, E2, and p7), they are not capable of producing infectious particles. However, replication-competent, selectable dicistronic RNAs that encode all of the viral proteins also did not produce infectious particles.[238] Although the first replicons were made from genotype 1b strains of HCV, RNAs from all other major genotypes (e.g., genotypes 1a, 2–6) have been adapted to highly efficient replication in Huh7 cells.[239,240] These subgenomic HCV replicons and genome-length RNAs recapitulate many of the natural mechanisms of HCV RNA replication and have been useful for discerning differences in the susceptibility of different viral strains and genotypes to candidate antiviral compounds and for studying mechanisms of resistance. Further enhancements to these replicons allow for insertion of patient-derived sequences facilitating in vitro phenotypic sensitivity testing to antivirals; however, this process remains largely a research tool.[241–243]

Propagation of Virus in Cell Cultures

The description in 2005 of a complete cell culture system for propagating HCV (designated HCVcc) opened a new era in HCV research, enabling in vitro study of a complete viral life cycle.[9,244,245] Key aspects of this system were the recognition that the JFH-1 (subtype 2a) genomic RNA clone replicated efficiently without the need for adaptive mutations and the isolation of highly permissive derivatives of the Huh7 hepatoma cell line (e.g., Huh 7.5.1) that are defective in some components of the innate immune response, including Toll-like receptor 3 (TLR3) and RIG-I.[246,247] The HCVcc system has been broadened to include chimeras of the JFH-1 strain with portions of other strains (e.g., J6/JFH-1) and derivatives of the subtype 1a strain H77 that are important because of the global importance of genotype 1; more recently recombinants containing nonstructural segments of interest (e.g., NS5A) from all major genotypes.[248–251] Full-length virus clones of other genotypes, which are prevalent globally (e.g., 3a and 6a) and replicate efficiently in vitro, have been established.[252,253]

Animal Models

The chimpanzee, *Pan troglodytes,* is the principal nonhuman animal species that has been demonstrated to be permissive for acute and chronic HCV replication. Percutaneous inoculation of HCV RNA-positive plasma, and in one instance saliva, resulted in HCV infection.[254–256] Also, chimpanzees have been infected by means of intrahepatic inoculation of synthetic genome-length RNA derived from cDNA clones.[147,148] Thus the chimpanzee was a valuable model for hepatitis C infection, although the use of this animal model is now extremely limited.[257] The recent discovery of NPHVs in dogs and then in their natural equine host and in rats offers potential models.[258–260] In particular, rodent hepacivirus (RHV-rn1) has been shown to have a similar genetic organization to HCV, results in chronic infection with gradual fibrosis progression, and responds to HCV antivirals such as sofosbuvir.[261] A more distantly related hepacivirus called GBV-B infects tamarins and marmosets, replicates within the liver of these animals, and can cause both acute and chronic hepatitis with many features resembling hepatitis C.[262] The related *Pegivirus* genus includes HPgV, which was previously called GBV-C and is quite distinct from HCV.[16]

Initially, mouse models of HCV infection were limited to transgenic mice that express transgenes encoding all or some HCV proteins under the control of liver-specific constitutive or inducible promoters.[263–265] Xenotransplantation of human hepatocytes to immunodeficient mice with liver disease resulted in a small-animal model that supports HCV replication.[266] For example, mice with severe combined immunodeficiency (SCID) transgenic for expression of plasminogen activator under control of the albumin promoter (resulting in liver injury) were transplanted with human hepatocytes and then used to test for protection in passive immunization using monoclonal antibodies specific for HCV envelope genes.[190,250] Other models have been generated with different combinations of immunodeficiency and liver injury, resulting in physiologic levels of albumin and lipoproteins.[267]

Immunocompetent mouse models of HCV infection include genetically humanized mice that express human variants of host entry molecules CD81 and OCLN.[144] Although these mice support studies of HCV entry determinants, they do not yet support significant replication.[191,268] Further enhancements to this model by introduction of defects in innate antiviral immunity through cross-breeding have resulted in mice that support completion of the entire HCV life cycle with replication levels suitable for testing of antiviral compounds.[269]

NATURAL HISTORY AND PATHOGENESIS

Viral Persistence

In experimentally infected chimpanzees and in humans, HCV RNA can be detected in plasma within days of exposure, often 1 to 4 weeks before liver enzyme levels rise.[254,270–274] Viremia peaks and may be quite variable in the first 8 to 12 weeks of infection and then plateaus or drops to lower levels and persists (Fig. 154.6).[275] In some instances, plasma HCV RNA becomes undetectable in the first few months and remains undetectable indefinitely (viral clearance); in other instances, viremia is inconsistently detected early and a stable pattern of recovery or persistence is not evident for more than 6 months.[254,273,276–280] Some instances of intermittent viremia may reflect reinfection, which has been observed in active injection drug users.[276] In other cases, rebounding viremia may represent escape from an initially successful immune response.[165,277,279,281] Overall, viremia persists in 50% to 85% of acutely infected persons (see "Acute Hepatitis C").[273,276,282,283]

Host genetic factors influence viral persistence. There are clinical and epidemiologic clues that suggest host genetic factors play an important role in the outcome of acute infection. In common source outbreaks in which persons were accidentally infected with the same HCV inoculum, some recovered and others had persistent infection.[284] In addition, HCV infection more often persists in African Americans than Caucasians and in persons infected with HIV infection compared with immunocompetent persons.[285,286] In one study, 90 persons with plasma HCV antibodies but not HCV RNA were compared with 722 with both HCV antibodies and RNA.[285] Viral persistence was found 5.1-fold more often in blacks compared with nonblacks after adjustment for HIV and other factors. These racial differences and differences in viral persistence after exposure to a common HCV inoculum point strongly to a host genetic basis for viral persistence.

Multiple studies have reported differences in the frequencies of various alleles in persons who have persistent infection compared with those with spontaneous resolution. The most consistent findings map to HLA and genes for λ-IFNs.[287–292] The association of HLA class I with HCV persistence was strengthened when genes for NK receptors were also considered.[287] Specifically, the most inhibitory interactions between *HLA C* and *NKIR* were most strongly linked to viral persistence.

A genome-wide association study compared the frequency of 792,721 alleles in 919 persons with spontaneous resolution of HCV infection with frequencies in 1482 with persistent infection.[293] Marked differences in allele frequencies were found on chromosome 6 for 117 single nucleotide polymorphisms (SNPs) spanning 1 million bp encompassing genes for *HLA* class II (Fig. 154.7). The strongest signals mapped to *HLA DQA1-DQB2* and *DQB1*0301* in particular. The chromosome 19 allele frequency differences mapped to a 55-kb region encompassing λ-IFN 3 (or *IL28B)*. The most significant association was a C/T at rs12979860, 3 kilobases from the *IL28B* start codon. Interesting to note, this allele (but not the chromosome 6 SNP) was initially identified as having predicted the response of persons with persistent HCV infection to IFN and ribavirin.[294] In addition, the global allele frequency correlated closely with the outcome of HCV infection, with the highest prevalences of the favorable allele found in Asia and the lowest in Africa.[290] In the cohort in which the racial difference in HCV persistence was first recognized, rs12979860 accounted for approximately 50% of the racial difference in viral persistence.

rs12979860 is located approximately 3 kb upstream from the gene for IFN-λ3, and a dinucleotide polymorphism was discovered (ss469415590) 367 bp upstream from rs12979860 that was associated with transient production of another protein called IFN-λ4.[291] That dinucleotide polymorphism is highly linked to rs12979860, but in some cohorts appears to be more predictive of both spontaneous resolution and response to IFN and ribavirin treatment, particularly in individuals of African ancestry.[291,295] The mechanisms through which genetic polymorphisms near the genes for λ-IFNs on chromosome 19 and near the HLA class II genes explain viral persistence remain unclear. An SNP (rs4803217) located in the 3′ UTR of IFN-λ3 mRNA was investigated for potential regulation of mRNA translation efficiency.[296] With an in vitro approach, it appeared that rs4803217 induced alterations in gene expression, influencing the global structure of IFN-λ3 mRNA, with the G allele producing a more stable form whereas the T allele mRNA was more dynamic. The hypothesis was that variation in translational efficiency could result in modulation of IFN-λ3 protein abundance and hence HCV clearance based on the genotype. The rs12979860 discovery SNP and rs4803217 were highly correlated with clinical phenotypes. However, in a comparison of rs4803217 and IFN-λ4-ΔG/TT, there was no indication that the rs4803217 allele improved HCV clearance, a finding that argues against this new allele as the causal variant.[297]

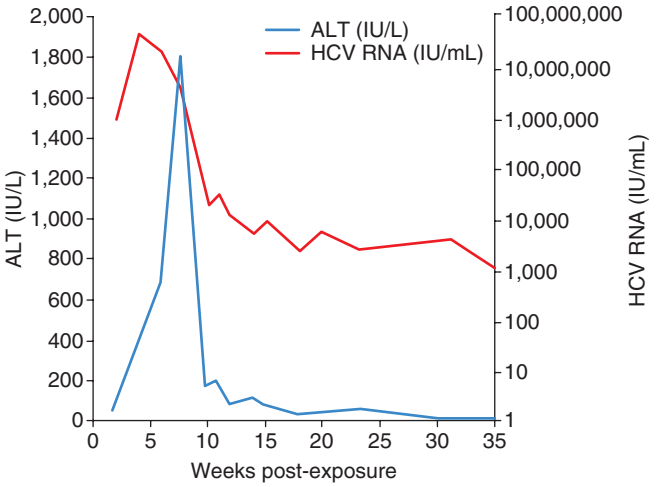

FIG. 154.6 Acute hepatitis C virus *(HCV)* infection in a health care worker following a needlestick accident (time zero). *ALT,* Alanine aminotransferase. *(From Sulkowski MS, Ray SC, Thomas DL. Needlestick transmission of hepatitis C.* JAMA. *2002;287:2406–2413.)*

Innate Immunity

HCV infection induces a wide range of innate host responses, and each may be balanced by one or more viral evasive strategies.[298] At the cell membrane, or more likely in endosomes, an extracytoplasmic PRR such as TLR3 may sense the viral genome from disrupted virions and trigger an antiviral program via the adaptor protein TRIF. Once in the cytoplasm, PAMPs such as the 3′UTR may trigger RIG-I, a cytoplasmic sensor of viral RNA (uncapped RNA that is double-stranded or contains certain homopolymers), and trigger inflammation via the mitochondria-associated membrane adaptor protein MAVS.[299] Signaling via TRIF or MAVS results in nuclear translocation of activated IRF3, IRF7, nuclear factor kappa B (NF-κB), or a combination of these, which trigger transcription of inflammatory genes including IFN-β. IFN-β, through subsequent autocrine and paracrine mechanisms, subsequently stimulates

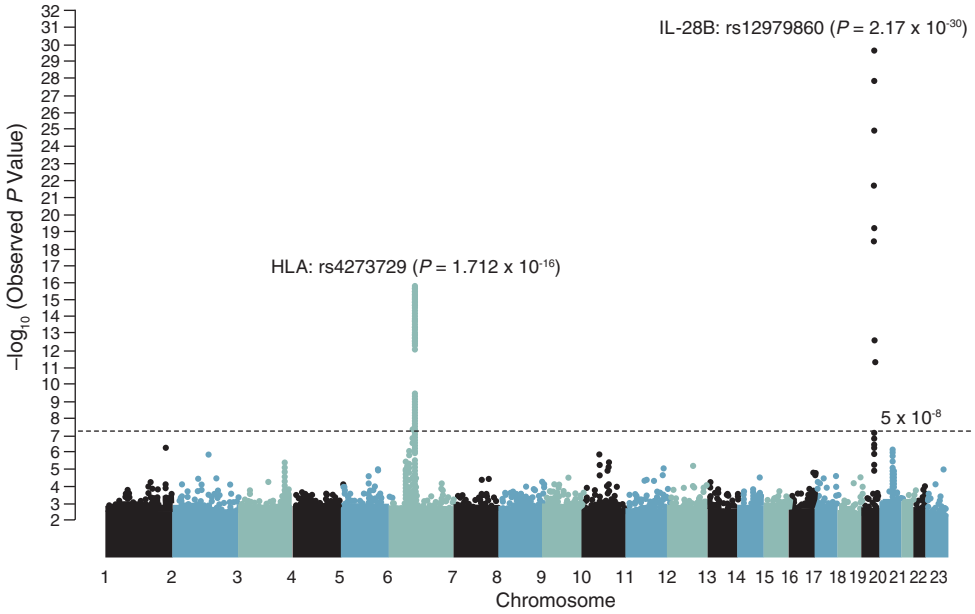

FIG. 154.7 Manhattan plot summarizing genome-wide association study results in 919 persons with spontaneous resolution of hepatitis C virus infection and 1482 persons with chronic hepatitis C virus infection. Each point corresponds to a *P* value from a test of association for an SNP. The $-\log_{10}$ *P* values are plotted by location of the single nucleotide polymorphism (SNP) across the genome for the 23 chromosomes. The *dashed line* represents an accepted level of genome-wide significance ($P = 5 \times 10^{-8}$). SNPs in the *HLA* and *IL-28B* region on chromosomes 6 and 19, respectively, exceed this threshold. *(From Duggal P, Thio CL, Wojcik GL, et al. Genome-wide association study of spontaneous resolution of hepatitis C virus infection: data from multiple cohorts.* Ann Intern Med. *2013;158:235–245.)*

the synthesis of IFN-α and a wide variety of other antiviral cytokines and chemokines that both inhibit viral replication and help to orchestrate the subsequent adaptive immune response.[115] That these responses are important is suggested by the cleavage of TRIF and MAVS, adaptor proteins for TLR3 and RIG-I, respectively, by the HCV protease NS3/4A.[300,301] Gene expression microarray studies have shown convincing, vigorous type 1 IFN responses within the livers of both acutely and chronically infected chimpanzees, indicating that the inhibitory effects of NS3/4A are incomplete.[302,303] This may be due to IFN production by uninfected cells or by recently infected cells in which NS3/4A is not yet active, or another mechanism.

During viral infections, a major source of type I IFNs is the plasmacytoid dendritic cell (PDC), but they are not activated by infectious HCV virions.[304] Further investigation revealed that PDCs are activated in the presence of HCV-infected cells and that this phenomenon required (1) replication, rather than the simple presence, of HCV RNA in the non-PDC, (2) cell-to-cell contact, and (3) expression on the PDC of TLR7. Supernatants from these activated PDCs suppressed HCV replication in an IFN receptor–dependent fashion.[305]

In addition to potentially blocking the induction of IFNs, HCV appears capable of impairing IFN-related effector functions. HCV-related dysfunction in the Jak/STAT signaling pathway in model systems has been ascribed to hypomethylation of STAT1 related to increased expression of protein phosphatase 2A (PP2A), stimulation of IL-8 production, and inhibition of PKR.[130,132,306,307] Confirmation of these findings in model systems expressing HCV proteins in a more physiologic context is necessary before any firm conclusions can be drawn.

NK and NKT cells are abundant in liver and, through production of IFN-γ and other cytokines, prime cellular immune responses.[308] Thus it is important that the binding of the E2 protein to CD81 has been associated with inhibition of NK cell activity.[309,310] Likewise, *HLA Cw*04* and its related haplotypes, which reportedly bind to inhibitory killer immunoglobulin-like receptors on NK cells, have been associated with viral persistence.[287,311] Notably, the least inhibitory *HLA-C-NKIR* haplotypes are most strongly associated with recovery. Furthermore, we have recently learned that these inhibitory effects are reversible with HCV viral suppression. The development of DAA therapy has provided a unique opportunity to understand the reversibility of the NK cell activation and liver inflammation. Because of the immunomodulatory effects of IFN, this was not feasible until the availability of IFN-free regimens. With DAA-mediated HCV viral clearance, there is rapid decrease in NK cell activation and a normalization of NK cell cytotoxic effector functions to levels reported in uninfected controls.[312] Thus the NK cell dysfunction noted in chronic HCV infection is normalized with DAA therapy. Furthermore, a similar study evaluated restoration of HCV-specific $CD8^+$ T-cell function in the setting of DAA-mediated HCV clearance.[313] Together these studies suggest that innate and adaptive immune systems may be in a better spot to respond to reexposure than after IFN-based therapies and may even confer partial protection against reinfection.

HCV may interfere with NK cell activation of dendritic cells (DCs), which facilitate T-cell priming.[314] In some studies, but not others, HCV infection has been associated with measurable impairment of peripheral DC function.[315,316] This impairment is one possible mechanism that might explain the arrested development and then collapse of cellular immune response observed during the transition from acute to chronic infection.[317] Whether or not intrahepatic DCs are infected with HCV is unknown, and studies have suggested that the effects of HCV on DCs do not require viral replication and may be mediated by viral proteins including core protein.[318] In addition, immune responses within the liver may inherently be biased toward tolerance because of the frequent exposure of the intrahepatic environment to antigenic material and cytokines from the gut.[319]

Humoral Immunity

Within months of infection, antibodies to multiple recombinant antigens that correspond to structural and nonstructural genes are detectable in blood.[320] Emergence of HCV-specific antibodies does not correlate temporally with viral recovery. Indeed, although virtually all immunocompetent persons develop antibody responses to some HCV antigens, most infections persist. Viral recovery has also been described in persons with congenital agammaglobulinemia.[321] On the other hand, humoral immune responses can neutralize HCV, at least partially. For example, there were fewer HCV infections in liver transplant recipients who received immune globulin before 1990, when it contained antibody to HCV.[322] Likewise, in a randomized controlled study, immune globulin administration was associated with a reduced incidence of sexual HCV transmission.[323]

Available evidence suggests that HVR1, located at the N-terminus of E2, harbors one or more neutralization epitopes and that it is a site of mutations causing immune escape during acute and chronic infection.[158,324–326] In the chimpanzee model, passively transferred anti-HVR1 antibodies could neutralize HCV infection in chimpanzees, whereas those same antibodies did not neutralize an inoculum collected later that had amino-acid changes in the envelope sequence.[324,327] Postexposure immune globulin administration prolonged the incubation of HCV infection, and in later studies was associated with early termination of infection.[328] Persons with reduced humoral immunity also accumulate fewer amino-acid mutations in the E2 sequence.[162,329] In persons who recover from HCV infection, HCV antibody responses decline, sometimes below the level of detection by commercial assays.[256,276,330] In contrast, $CD4^+$ lymphocyte responses often are maintained (see the following section). Antibodies to HCV envelope proteins appear later and at lower titers compared with antibodies directed against nonstructural proteins, suggesting that neutralizing antibodies may play only a minor role in spontaneous resolution.[331,332] However, negative results may be due to mismatch of poorly conserved envelope antigens.

Several functional expression systems have enhanced studies of humoral responses directed against the HCV envelope proteins, including the use of lentiviral HCV pseudoparticles (HCVpp) in cell culture–based assays for neutralizing antibodies.[332–334] Application of this system to samples from one well-characterized individual suggested that HCV escapes continuously from neutralizing antibody responses in vivo.[158] Studies using HCVpp have shown a relationship between the control of virus infection and a neutralizing antibody response in single-source outbreaks of acute HCV infections.[335,336] These studies showed that a strong and progressive neutralizing antibody response resulted in decreased viremia and that rapid and early induction of neutralizing antibodies was associated with viral clearance. Meanwhile, absent or low-titer neutralizing antibodies in the early phase resulted in chronic infection, regardless of later induction of cross-neutralizing antibodies. Studies using autologous HCVpp have also demonstrated sequential escape from neutralizing antibody responses and that this mechanism drives the high rate of E1/E2 evolution.[159,160,337] Collectively, the available data suggest that the humoral immune responses reduce the fitness of some HCV variants, can cross-neutralize genotypes, and possibly limit the severity or even risk of recurrent infection but are unlikely to be necessary or sufficient for primary viral clearance.[338]

The potential existence of broadly neutralizing antibodies that are not associated with virus escape are critically important in the search for a universal vaccine. Studies in human liver chimeric mice have had success in protecting against HCV infection by delivering neutralizing antibodies through a recombinant adeno-associated virus (AAV) vector.[339] This group also tested the ability of these antibodies to interfere with established HCV infection. With maintenance dosing of the antibodies every 3 days, HCV RNA fell below the limit of detection between 5 and 11 days after the antibody treatment was started.[339] These data support that neutralizing antibodies are able to protect uninfected hepatocytes from becoming infected and allow for clearance of HCV in already infected hepatocytes.

Cellular Immunity

Viral recovery has been associated with a vigorous, broad cellular immune response.[277,340–342] Experiments carried out in chimpanzees indicated that both $CD4^+$ and $CD8^+$ memory T cells play critical roles in protection against reinfection with HCV.[343,344] The control of second infections appears to be linked kinetically to rapid acquisition of cytolytic activity by $CD8^+$ T cells that are resident in the liver and is normally associated with an expansion of circulating $CD4^+$ and $CD8^+$ memory T cells.[344] Most people with acute HCV infection develop vigorous $CD4^+$ and $CD8^+$ T-cell responses in the first few months, such that there is overlap between those who will clear and those who will develop persistent infection.[317] Subsequent evolution to persistence is associated with a rapid loss of multifunctional and proliferative anti-HCV $CD4^+$ T cells.[345] Prolonged viremia is characterized by HCV-specific $CD8^+$ lymphocytes that are unable to produce IFN-γ (a so-called "stunned" phenotype) and often express counterregulatory molecules such as programmed death 1 (PD-1) that could contribute to their inability to eradicate infection.[346–348] Stronger polyclonal cytotoxic T-lymphocyte (CTL) responses in the peripheral blood and liver also have been associated with lower levels of circulating HCV RNA.[349,350] HCV-specific CTLs also have been found in persons who were exposed to HCV but never were known to have had HCV antibody or viremia.[351,352] Theoretically, inhibition of the programmed death pathway may result in reversal of this exhausted phenotype and clearance of HCV infection.[353] Although registration trials of PD-1 inhibitors excluded HCV-infected subjects owing to concern for immune reconstitution syndromes, there are reports of use of these checkpoint inhibitors in patients with HCV-associated liver disease. In one HCC therapeutic study with tremelimumab, a fully human monoclonal antibody that binds to cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), 12 of 14 subjects had a decline in HCV RNA from baseline, but no patients resolved their HCV infection.[354]

Still other mechanisms have been suggested to reduce the susceptibility of infected cells to cytolytic attack by immune cells. The HCV core protein may bind to the cytoplasmic tail of the TNF-α receptor and the lymphotoxin-β receptor, based on ectopic expression studies.[60,61,63] This binding occurs immediately adjacent to the death domain and may modulate signal transduction through the receptor. Although the relevance and biologic effects of this interaction remain controversial, it is possible that it protects the infected cell against TNF-α–mediated apoptotic cell death. Although this has not been observed in HCV transgenic mice, defects in Fas-mediated apoptosis have been documented in HCV-transgenic mice, both in vivo and in hepatocyte explant cultures ex vivo.[355]

Finally, HCV sequence variation and immune escape from both T and B cells may also contribute to viral persistence.[85,356] Mutation within the amino-acid sequence of a critical epitope may allow a new quasispecies variant to evade a previously suppressive immune response, either cellular or humoral.[149,158,326] In several studies, acutely infected persons who developed persistent infection had a more complex quasispecies.[85,356] In agreement with that finding, viral escape in CTL epitopes has been reported frequently in persistently infected persons even when examined during acute infection.[165] Consistent with this notion, probable escape mutations within class I MHC-restricted epitopes were observed in chimpanzees that had an inadequate $CD8^+$ memory T-cell response owing to antibody depletion of $CD4^+$ memory cells.[344] In summary, it appears likely that many factors contribute to HCV persistence and that HCV may have evolved a variety of redundant and overlapping mechanisms of immune evasion to ensure its long-term persistence in the majority of immunologically normal persons who become infected. It is probable that additional mechanisms of viral immune evasion will be identified in the future.

Disease Progression

Although HCV infection leads to hepatic inflammation and steatosis, the major consequence of persistent HCV infection is the development of hepatic fibrosis, which may progress to life-threatening cirrhosis and a greatly increased risk of HCC (see "Clinical Manifestations of Hepatitis C Virus Infection"). These long-term complications occur in persons with persistent infection and generally more than 20 years after the onset of infection, although more rapid progression has been reported in persons infected after the age of 40 years, with immunosuppression from HIV or organ transplantation, or agammaglobulinemia, or in the setting of other comorbidities including HBV coinfection, diabetes, insulin resistance, obesity, excessive alcohol consumption, and hepatic steatosis.[357–365]

There are wide estimates (5%–25%) of the probability of cirrhosis occurring within 10 to 20 years of infection and little information available on progression beyond 30 years (Fig. 154.8).[366,367] One of the longest follow-up studies comes from the Irish anti-D immunoglobulin cohort, wherein over 800 Rh-negative women in Ireland were infected with HCV genotype 1b via contaminated anti-D immunoglobulin from a single donor.[368] Infected in 1977–79, these women have been followed for almost 4 decades. The most recent report of liver disease outcomes included assessments through December 31, 2013. This comprehensive report found that 19% of chronically infected women developed cirrhosis and that the rate of cirrhosis development increased over the successive decades of follow-up. Risk factors associated with more rapid progression

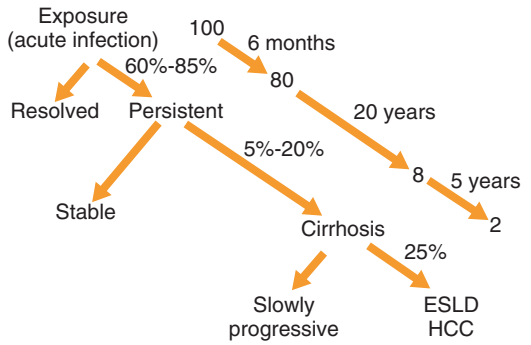

FIG. 154.8 Natural history of hepatitis C virus (HCV) infection. Estimates of the most common outcomes of HCV infection are provided, extrapolated to the hypothetical acute infection of 100 persons (numbers 100, 80, 8, and 2). *ESLD,* End-stage liver disease (e.g., esophageal varices, ascites, or hepatic encephalopathy); *HCC,* hepatocellular carcinoma. See text for expanded discussion.

of fibrosis included diabetes and high alcohol consumption. Once cirrhosis occurs, the rates of progression to liver failure (decompensated cirrhosis) and HCC are approximately 2% to 4% and 1% to 4% per year, respectively.[369–371] In the anti-D cohort, 1.9% and 4.9% of women developed HCC and died of liver-related disease, respectively.

Several studies are instructive for the range of outcomes and disease cofactors. In two separate studies of women infected by contaminated Rh immune globulin, the exact onset of HCV infection could be inferred, and the incidence of cirrhosis during 15 to 20 years of follow-up was very low (<5%); but, as noted earlier, in one of these cohorts with longer follow-up this risk increased over time, whereas in the other it remained low.[372–375] In another cohort of 1667 HCV-infected injection drug users, infected an average of 14 years and followed for an average of 8.8 years, the incidence of liver-related mortality was also low (3 per 1000 person-years).[285] Liver biopsies were performed on a random sample of 210 HCV-infected members of this cohort, and only 10% had evidence of serious liver disease (Ishak modified fibrosis scores of 3–6). There was little progression an average of 3 years later.[376,377] In a follow-up study of 1176 HCV antibody–positive participants in the same cohort approximately 10 years later, an increased risk of cirrhosis was noted and linked with increasing age, alcohol use, hepatitis B coinfection, high body mass index (BMI), and HIV infection.[378] These studies underscore the role of factors such as increasing age, comorbidities, and coinfection with HIV in progression of HCV-related liver fibrosis, and differences in cohorts with similar follow-up and exposures also emphasize unrecognized confounders.

In yet another study, Seeff and coworkers evaluated mortality and morbidity in a cohort of patients a mean of 18 years after development of posttransfusion hepatitis in comparison with control patients who were similarly transfused but had not developed recognizable hepatitis.[372,379] Overall mortality was high, reflecting the severity of underlying conditions, but not increased in HCV-infected patients. Liver-related mortality was slightly higher in patients with posttransfusion hepatitis (3%) than controls (1.5%), and it was estimated that approximately 10% of patients with posttransfusion hepatitis had cirrhosis 20 years later.[372] Other studies of posttransfusion hepatitis have found a higher rate of cirrhosis.[357,359]

This variability and uncertainty in the estimated frequency of life-threatening liver injury relate to limitations in basic research tools and the variable impact of environmental, host, and possibly viral factors for disease progression in different study cohorts (Table 154.1). Studies in tertiary care facilities generally predict higher rates of progression because they include a greater proportion of symptomatic participants (referral bias). Disease stage measurements are also relatively imprecise (see "Liver Fibrosis Staging"). However, much of the variability relates to environmental and host differences.

The leading environmental determinant appears to be alcohol ingestion.[380–382] Although excessive alcohol ingestion and HCV infection independently may cause cirrhosis, combined exposure has a synergistic

TABLE 154.1 Factors Associated With Cirrhosis in Persons With Hepatitis C Virus Infection

FACTOR	IMPACT	COMMENT
Environmental		
Alcohol use	+4	The importance of minimal alcohol ingestion (<20 g daily) has not been established.
Host		
HIV infection	+4	Increasingly important as HIV-related survival improves. May be masked by competing mortality.
HBV infection	+3	Strong effect when HBsAg positive; relatively uncommon.
Age	+4	Strong effect; increases as low as 40 yr. Hard to distinguish from infection duration.
Body mass index	+2	Associated with metabolic syndrome.
Duration of HCV infection	+3	Cirrhosis is rare before 10 yr.
HLA type	+1?	HLA B54 is correlated with increased risk of cirrhosis; DRB1*0301 with lack of cirrhosis.
Viral		
Quasispecies complexity	+1	Cross-sectional studies cannot assess causality, and complexity may be confounded by duration of infection.
HCV genotype 3	+3	Supported by multiple studies in different cohorts. Some are limited by cross-sectional assessment. Increased rates of steatosis with genotype 3 may contribute.
Quantitative measures of viremia (serum or plasma HCV RNA level)	+1	Not always detected or lost in multivariate analysis of age or HIV.

HBsAg, Hepatitis B surface antigen; *HBV,* hepatitis B virus; *HCV,* hepatitis C virus; *HIV,* human immunodeficiency virus; *HLA,* human leukocyte antigen.

effect.[285,380,383] This is especially true with heavy alcohol ingestion (>50–125 g a day), which in one study increased the risk of cirrhosis approximately 100-fold.[380] The mechanism underlying this synergy with an environmental toxin remains obscure, but both alcohol and HCV infection may cause microvesicular steatosis, suggesting a common pathway involving mitochondrial injury.[384,385] Coinfection with HBV may also accelerate disease progression, whereas persistent HPgV (formally called GB virus C or hepatitis G virus) infection is likely not pathogenic and does not appear to affect hepatitis C.[386–390] A novel human virus, human hepegivirus (HHpgV-1), which shares features with HCV and HPgV, was discovered in blood transfusion recipients in 2015.[391] In an HIV-HCV coinfected cohort of people who use intravenous drugs, HHpgV-1 infection was reported in 10.9% of patients.[392] The medical importance of this virus remains unclear, as does whether it has any influence on the natural history of HCV infection. Schistosomiasis coinfection is associated with more rapid progression of HCV-related fibrosis in Egypt.[393] Increased progression of liver disease also has been reported in immunosuppression associated with agammaglobulinemia and transplantation.[394–396]

There is substantial evidence that disease progression is increased in persons infected at older ages, and this factor alone may explain much of the variability in studies because persons infected at older ages through transfusions appear to have the highest rates of progression to cirrhosis.[285,397,398] Although a few studies have detailed the natural history of HCV infection in children, the overall rate of disease progression appears quite slow, with few notable exceptions.[399–401]

Genotype has also been identified as a potential predictor of severe fibrosis. Early studies suggested an association with the genotype 3 infection and greater severity of fibrosis, but these retrospective studies

were limited by small cohorts, variability in patient characteristics such as presence of insulin resistance or BMI, HCV genotype distribution, and inconsistencies in methodology, particularly with respect to the grading of steatosis.[402,403] A retrospective analysis of the Swiss Hepatitis C study cohort ($N = 1189$) reported an independent association of genotype 3 infection with fibrosis.[193] A meta-analysis of 8 single biopsy studies ($N = 2349$) in patients with chronic HCV infection confirmed an association between fibrosis and genotype 3 (odds ratio, 1.52).[404] The same meta-analysis included 8 paired biopsy studies, which were underpowered and did not reveal a similar association. The primary limitation of these studies was that they did not account for steatosis. Studies that have considered steatosis as an independent risk factor for fibrosis have drawn different conclusions. For example, a large meta-analysis of 3068 patients with chronic HCV infection reported that genotype 3 infection was associated with steatosis, not fibrosis, and a multivariate analysis of fibrosis identified steatosis and level of inflammatory activity at histopathologic assessment as independent predictors of disease.[365] There are multiple studies supporting the association of steatosis with higher rates of fibrosis progression and studies supporting the concept that greater liver fibrosis risk is predominantly associated with steatosis in genotype 3 infection.[405]

Since the advent of active antiretroviral therapy, HCV-related liver disease has emerged as a major cause of death in HIV-infected persons.[406–408] In the large D:A:D study, 49,741 HIV-infected persons were followed from March 1999 through February 1, 2011, and 3909 died.[408,409] The primary causes of death were acquired immunodeficiency syndrome (AIDS; 29%), non-AIDS cancers (15%), and liver disease (13%). Although this was a decline in liver-related deaths from second highest to third from the prior publication from this cohort, the absolute decrease was minimal. Overall, all causes of death decreased, highlighting the significant positive impact of antiretrovirals (ARVs) on mortality, with one exception: non-AIDS cancers, which remained stable. Accumulating population data show that liver disease will attenuate the remarkable long-term benefits of antiretroviral therapy in the subset coinfected with HCV (see Chapter 128) until greater penetration of DAAs is seen.[410–412]

Hepatic Fibrosis

Liver fibrosis is a wound-healing response that occurs in the setting of chronic liver injury. Liver fibrosis is the net result of a complex, tightly regulated dynamic process in which collagen and other proteins are deposited and removed from a matrix in the subendothelial space between hepatocytes and the sinusoidal endothelium. Accumulation of liver fibrosis occurs in response to all forms of liver injury, and with viral hepatitis begins in the periportal zone and gradually extends as so-called septae into lobules toward the central veins.[413] As the matrix expands and changes its composition, normal liver physiology can be disrupted and the architecture of the organ altered, although it is not clear whether this process is clinically evident before development of cirrhosis. The hepatic stellate cell (Ito cell) appears to be the chief architect of this matrix, responding to a variety of stimuli and resulting in activation (see Friedman's excellent review[414]).

Cytokines, reactive oxygen species, and other mediators of inflammation can initiate stellate cell activation, which can be perpetuated by autocrine and paracrine stimulation. Kupffer cells can play an important role initiating and perpetuating fibrogenesis, through production of TGF-β metalloproteinases, and reactive oxygen species.[415–417] $CD8^+$ and $CD4^+$ lymphocytes also appear to influence the pathogenesis of fibrosis, chiefly through stellate cell activation. In the CCl_4 mouse model, fibrogenesis is enhanced when Th1/$CD8^+$ lymphocyte responses (in particular, IFN-γ) are depleted and by expression of Th2/$CD4^+$ lymphocyte-derived cytokines such as IL-4.[418] These data are interesting in light of the clinical observation of greater progression of fibrosis in persons coinfected with schistosomiasis in whom Th2-like responses predominated,[393] and the extremely rapid fibrosis progression in persons with congenital agammaglobulinemia.[394] Similarly, evidence suggests that alcohol-related liver injury and fibrosis may be mediated in part by microbial translocation products including lipopolysaccharide, which may trigger TLR4 on stellate cells, resulting in recruitment of Kupffer cells and sensitization of stellate cells to the profibrotic action of transforming growth factor-β (TGF-β).[419] The strong associations between progression of fibrosis and both older age and various forms of immunosuppression are consistently found. In the previously mentioned cohort of injection drug users, even after adjustment for other factors such as alcohol intake, hepatitis B coinfection, and BMI, persons with HIV had liver fibrosis stages comparable with HIV-uninfected persons an average of 9.2 years older.[378] However, it remains unclear whether age and immunosuppression affect liver fibrosis through similar mechanisms.

Likewise, it remains unknown why only some immunocompetent persons with chronic hepatitis C develop cirrhosis. There is little reason to believe that certain HCV variants are more virulent than others, although as previously discussed there are differences in fibrosis progression for some genotypes, in particular genotype 3. These differences in pathogenesis are less likely to be due to "virulence" and more likely related to differences in HCV-related steatosis. In acute infection, the virus replicates at high level for weeks with little or no evidence of liver damage, and large studies have not shown an association between the development of hepatic fibrosis and HCV viremia in chronic infection.[412]

Host genetic factors probably play a role in determining why some infected persons develop cirrhosis. Wiley and coworkers have reported that African-Americans have slower progression of fibrosis compared with Caucasians, a finding that appeared independent of other factors and that has also been noted by others.[420,421] Specific HLA alleles have been associated with differences in the progression of disease, as have polymorphisms in several genes believed to play a role in disease pathogenesis, including TGF-β.[422–424] With few exceptions, however, the link between these polymorphisms and the pathogenesis of disease remains unclear.

A genome-wide association study of the IDEAL study identified two SNPs—the *IL28B* SNP, previously discussed, which was also associated with treatment response and natural clearance, and an SNP in the *PNPLA3* gene—that were associated with steatosis on histopathology.[425] The *PNPLA3* gene codes for a hydrolase against triglycerides and retinyl esters in hepatic stellate cells. The polymorphism, rs738409 GG, was initially identified as a risk factor for nonalcoholic fatty liver disease and was associated with an increased risk of steatosis and fibrosis in the setting of chronic HCV infection.[425] Multiple candidate gene studies have now reported an association of PNPLA3 and severity of liver disease including fibrosis and HCC.[426] The I148M variant has been reported to modulate the fibrogenic phenotype of hepatic stellate cells, the primary cell type in fibrogenesis and matrix deposition.[427] Thus it is possible that the failure of some studies to show associations of polymorphisms and fibrosis is because they do not target the primary pathogenic mechanism, which may be steatosis.

Human Immunodeficiency Virus and Hepatitis C Virus Infection

HIV infection adversely affects all phases of the natural history of HCV, increasing the frequency of viral persistence after acute infection, the level of viremia among persistently infected persons, the rate of progression to cirrhosis, and the proportion of persons who will ultimately develop end-stage liver disease.[428,429] Darby and coworkers[430] studied mortality from liver disease and HCC among 4865 men with hemophilia who were exposed to HCV-contaminated blood products. At all ages, the cumulative risk of liver-related death after the presumed exposure to HCV was 1.4% (range, 0.7%–3%) for HIV-uninfected men and 6.5% (range, 4.5%–9.5%) for HIV-infected men.[430] Goedert[431] also found a significantly increased risk of liver disease in HIV- and HCV-coinfected members of the Multicenter Hemophilia Cohort Study. In studies done before effective antiretroviral therapy use was widespread, the impact of liver disease on mortality in HIV- and HCV-coinfected persons was lower in injection drug users than in patients with hemophilia. Thomas and colleagues[285] did not detect more end-stage liver disease in HIV-infected members of a study of 1667 HCV-infected current and former injection drug users. This apparent discrepancy was chiefly due to high competing causes of mortality in the HIV-infected drug users. In that same cohort, a more recent analysis of 1176 HCV antibody–positive participants, the prevalence of cirrhosis was higher in HIV- and HCV-coinfected persons (19.5%) than in those infected with just HCV (11%).[378] Similarly, Lo Re and colleagues investigated rates of hepatic decompensation among

veterans with HCV infection and found higher rates in patients with ARV-treated HIV and HCV coinfection (hazard ratio, 1.56).[432]

On the other hand, it remains unclear whether chronic hepatitis C affects the natural history of HIV disease. In the Swiss cohort study, Greub and colleagues[433] reported that among 3111 patients receiving antiretroviral therapy, HCV-infected persons had a modestly increased risk of progression to a new AIDS-defining event or death, even among the subgroup with continuous suppression of HIV replication. They also reported that the magnitude of the CD4 cell increase after effective anti-HIV therapy was significantly less than observed in HCV-uninfected persons, suggesting that HCV coinfection may blunt immune recovery.[433] Likewise, an analysis of 9164 HIV-infected persons with known dates of seroconversion, since 1997 when antiretroviral therapy was available, reported higher HIV/AIDS-related mortality among HIV- and HCV-coinfected persons than among those with HIV infection alone.[434] On the other hand, among 1742 patients in a Baltimore HIV clinic, differences in the progression to AIDS or death chiefly appeared to be attributed to lower exposure to highly active antiretroviral therapy among the HCV-coinfected injection drug users.[435] Similarly, Chung and coworkers failed to detect a difference in immune restoration in HIV- and HCV-coinfected participants in a well-controlled AIDS Clinical Trial Group study, suggesting that the effect of HCV infection on progression of HIV is not large.[436]

There is a complex relationship between antiretroviral therapy and liver disease progression in HIV-infected persons. On one hand, antiretroviral therapy can itself be hepatotoxic. Hepatotoxicity occurs in about 10% of persons given a new antiretroviral regimen, more often if the person is HCV coinfected.[437–439] In some cases, antiretroviral therapy–associated hepatotoxicity has been linked to liver failure and death.[440] However, in most cases it manifests with an elevation in liver enzymes (often greater than fivefold normal) in the absence of symptoms. Although there is no reason to withhold antiretroviral therapy from HCV-infected persons, close monitoring is advisable if there is cirrhosis. Treatment of HCV infection also may reduce the incidence of antiretroviral therapy–related hepatotoxicity.[441]

On the other hand, antiretroviral therapy may protect against the adverse effects of HIV infection on liver disease.[442–444] In one recent prospective study, antiretroviral therapy exposure and effectiveness was compared in 174 HIV- and HCV-coinfected persons who underwent at least two liver biopsies between January 1998 and July 2006, and 41 (24%) had significant progression (≥2-point increase in Ishak system score).[445] Little difference was detected in this study in the rate of fibrosis progression according to antiretroviral use between biopsies. However, most persons were already on antiretroviral therapy before the first biopsy, which might have diminished the effect in the relatively short interval between biopsies. Because antiretroviral therapy is widely advocated at all $CD4^+$ lymphocyte counts, the clinical implication of demonstrating whether antiretroviral therapy is beneficial in patients with HCV coinfection has diminished.

The pathogenesis of HIV and HCV coinfection is poorly understood. HIV infection is likely to affect the adaptive immune response to HCV. Similar to experimental depletion of $CD4^+$ lymphocytes in the chimpanzee model, HIV infection of $CD4^+$ lymphocytes activated to combat HCV could diminish $CD8^+$ lymphocyte responses and maturation of humoral immunity.[344] Kim and coworkers correlated the nadir of CD4 lymphocyte depletion with the magnitude of reduction in HCV-specific $CD8^+$ lymphocyte responses in HIV-infected persons (who, it is interesting to note, do not have lower Epstein-Barr virus– or cytomegalovirus-specific $CD8^+$ responses).[446,447] Likewise, Netski[448] found that HCV-specific humoral responses were indirectly correlated with $CD4^+$ lymphocyte suppression. Thus, HIV-related CD4 lymphocyte depletion probably contributes to the clinical observation that HIV infection reduces the likelihood of spontaneous HCV clearance.[285]

Whether HIV replication in hepatocytes, Kupffer cells, or stellate cells occurs and directly affects HCV replication or fibrogenesis is largely unknown. Likewise, HCV replication in monocytes and lymphocytes has been inferred, but only at low level and in a minority of cells.[224,436] Chung and coworkers have reported that HIV proteins such as p24 and virus (produced from other cells) can enhance HCV replication in vitro and increase TGF-β1 expression and that these processes can be blocked by antibodies to the CCR5 or CXCR5 HIV coreceptors.[449] These investigators also showed that HCV and HIV independently induce TGF-β1 through the generation of reactive oxygen species, which in turn induces p38 MAPK, JNK, ERK, and ultimately NF-κB.[450,451] Increased STAT1 activation and Fas ligand expression in HIV-infected persons may lead to increased hepatocyte apoptosis, an effect that appears to be enhanced by both HCV and HIV envelope proteins.[452,453]

HIV infection may also enhance liver fibrosis by increasing translocation of microbial products from the gut. HIV infection of gut lymphoid tissues is associated with enhanced microbial translocation, and these products are taken up near the portal vein by Kupffer cells.[454,455] As has been shown in an experimental model of alcohol-related liver disease, that process may lead to increased Toll cell 4 activation of stellate cells, especially if the Kupffer cell ability to take up bacterial products is diminished by HIV infection.[419] Cirrhosis itself, irrespective of the etiology, increases shunting of blood past the liver and might explain some of these findings.

The interaction between antiretroviral therapy and HCV infection is also poorly understood. Hepatotoxicity in HIV- and HCV-coinfected persons could reflect decreased drug metabolism, HCV-specific immune reconstitution, or increased susceptibility to mitochondrial dysfunction.[385,456–459] Thus, the mechanisms by which these viruses interact and the pathogenesis of coinfection remain important research topics.

CLINICAL MANIFESTATIONS OF HEPATITIS C VIRUS INFECTION

Acute Hepatitis C

Acute HCV refers to the initial 6 months of infection.[460] Although usually not associated with clinical symptoms (present in 15%–30%), acute HCV infection may cause malaise, nausea, and right upper quadrant pain, followed by dark urine and jaundice.[461] Symptoms usually occur within 5 to 12 weeks of the HCV exposure and are clinically indistinguishable in the individual patient from other types of acute viral hepatitis.[461] HCV RNA can be detected in blood within days of exposure (2–14 days), followed by elevations in serum levels of the liver-specific enzyme, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and in some cases bilirubin.[273,276,462] The most detailed information regarding the time course of acute infection comes from studies of HCV-contaminated transfusion recipients. Mosley and coworkers performed a look-back study of 94 persons infected through transfusion.[274] In 67 sera collected between the fourth and eighth days after transfusion, HCV RNA was detected in all but 2, underscoring the rapid onset of viral replication. There was wide variation in incubation (time to elevated ALT or symptoms), which ranged from 6 to 112 days (median, 46 days). Shorter incubation was associated with higher ALT, and jaundice was detected in only 21%.

The early HCV RNA kinetics have been well described and can be useful to determine the probability of natural clearance.[463,464] The early viral dynamics have been characterized in three phases: pre–ramp-up (first week after exposure), ramp-up (second week after exposure), and plateau phase (2–3 months after exposure).[463] Fluctuations in levels of HCV RNA are common (>1 log), and low levels of HCV RNA can be useful in differentiating acute from chronic infection and can help in predicting which patients are more likely to naturally clear the infection.[463–465] Factors associated with HCV RNA dynamics and natural clearance include sex, HIV coinfection, IFN-λ CC genotype, and HCV genotype 2.[466] As noted previously, in approximately 25% of patients, acute HCV infection is followed by spontaneous clearance, whereas in the rest it progresses to chronic infection. In addition to the innate and humoral immune response, host factors play a role in determining the risk of persistence, including sex, HIV coinfection, and genetics.[280,290,467]

Fulminant Hepatitis C

The frequency with which HCV causes fulminant hepatitis is controversial. HCV infection has been associated with 40% to 60% of fulminant NANB in Japan, but it is an uncommon cause of fulminant liver disease in Western countries.[468–470] This discordance might arise from variation in either host factors or viral strains, or both. There appears to be an increased likelihood of fulminant liver disease following acute HAV infection in persons with underlying chronic hepatitis C.[471]

Chronic Hepatitis C

Seventy-five percent or more of persons with acute hepatitis C infection develop persistent infection with long-term viremia, as described earlier (see "Viral Persistence") (see Fig. 154.8). Thus, although only one in six cases of symptomatic acute viral hepatitis is due to HCV infection, HCV is the leading infectious cause of chronic liver disease in the United States. Persistently infected individuals tend to have few symptoms (e.g., fatigue or malaise), resulting in lack of presentation to care and therefore lack of diagnosis. Because of this challenge and of failure of risk-based screening to identify the majority of people living with HCV infection in the United States, the CDC made the recommendation to expand screening to all persons born between the years of 1945 and 1965—that is, birth cohort testing.[472]

Once chronic, HCV infection persists indefinitely. Serum ALT levels typically fluctuate independent of symptoms, whereas serum HCV RNA levels remain fairly constant.[282,357,473,474] The degree of inflammation present at liver biopsies also varies over time.[358,475] Some individuals will develop fibrosis, which typically begins in portal triads but can bridge between triads or central veins and ultimately destroy the hepatic architecture, progressing to nodularity and cirrhosis.[413] There is a poor correlation among necroinflammatory liver injury, serum ALT levels, serum HCV RNA levels, and the extent of fibrosis.[476] The rate of hepatic fibrosis progression has been reported with serial liver biopsies in two large systematic reviews in populations with HCV monoinfection and HIV and HCV coinfection. The mean annual stage transition probabilities have been reported as 0.117 and 0.122 (F0 to F1), 0.085 and 0.115 (F1 to F2), 0.120 and 0.124 (F2 to F3), and 0.116 and 0.115 (F3 to F4), respectively.[428,477] Once established, 10% to 20% of HCV-infected persons with cirrhosis will decompensate clinically within 5 years, as evidenced by bleeding esophageal varices, ascites, encephalopathy, or HCC.[369–371] In a study of 214 persons with chronic hepatitis C and compensated (Child Pugh A) cirrhosis followed for an average of 114 months, 32% developed HCC, which occurred at a rate of 3.9% per annum.[478]

Hepatocellular Carcinoma

Primary HCC is typically a late complication of chronic hepatitis C, primarily occurring in patients with cirrhosis.[479,480] The rate of HCC development in people with HCV-associated cirrhosis is approximately 2% to 4% per year, with higher rates reported in some countries.[481–483] HCV-related liver cancer has been particularly evident in Japan and Italy and often follows 2 or more decades of infection.[480,484] The incidence is increasing in the United States, with new liver cancer cases increasing 38% from 2003 to 2012 and associated mortality increasing 56% during that same period.[485] An increasing incidence of HCC in Western countries has been attributed to prior increases in the prevalence of HCV infection and aging of those cohorts.[486,487] Worldwide, there is considerable variation in the fraction of HCC attributed to HCV infection and variability among HCV-infected persons in the proportion of mortality attributed to HCC versus cirrhosis. These differences probably are due to heterogeneity in the average ages of HCV-infected persons and the relative abundance of hepatitis B or other HCC cofactors.[479,480,488,489]

In Japan and Southern Europe, 50% to 75% of HCC is associated with HCV infection.[484] In one Italian study, HCV infection was found in 71% of patients with liver cancer, and HBV infection in only 15%.[480] In contrast, in a study from Taiwan, only approximately 15% of HCC was attributed to HCV.[490] In Japan, the mortality rate due to HCC increased approximately twofold during the 1980s and is chiefly linked to HCV.[491] Among HCV-infected persons in Japan, the mortality from HCC exceeds that due to cirrhosis. Reports from the Japanese Ministry of Health suggest that this increase in the HCV-related disease burden stems from widespread illicit injection of amphetamines in the 1950s and related spread of HCV within the Japanese population.

Overall, more than half of patients with cirrhosis untreated with antivirals die from cirrhosis complications before a diagnosis of HCC can be made.[492] With increasing access to DAA therapy, with which it is expected that eradication of HCV will decrease the risk of decompensating events, it is likely we will see a shift in HCV-attributable mortality from cirrhosis to HCC.[493] Modeling studies project a decrease in cirrhosis-related mortality by 21% to 27%.[493] This increased lifespan also returns a longer lifetime risk of developing HCC. These findings are based on the assumption that HCC incidence is not modified by DAA therapy, yet there is already emerging data that DAA will affect HCC incidence.[494] A meta-analysis of 18 studies from the IFN era suggested that sustained virologic response (SVR) was associated with a reduced risk of HCC (relative risk, 0.24; 95% confidence interval, 0.18–0.31).[495] Whether this remains true in the DAA era is an emerging area of investigation. Early studies suggested either minimal or no decrease in risk of HCC after DAA therapy, whereas others actually reported an increased risk of HCC after DAA exposure.[496–499] However, more recent and more comprehensive studies have reported a significant decrease in HCC incidence.[500,501] Although multiple groups have reported higher crude incidence rates for HCC in patients receiving DAA versus IFN-based regimens, there is no significant association between treatment regimen and HCC risk after adjustment for important confounders, including severity of cirrhosis, age, diabetes, and low platelet counts and albumin levels.[500]

As with cirrhosis, several cofactors have been proposed in the development of HCV-associated HCC. HBV coinfection appears to increase the risk of HCC in HCV-infected persons.[387,502] Infection with genotype 3 virus also has been associated with HCC.[479,503,504] In addition, alcohol and tobacco use, older age, and male gender are associated with HCC among HCV-infected persons.[387,479]

Relatively little is known about how chronic HCV infection leads to cancer. The HCV genome is not reverse-transcribed to DNA and thus cannot integrate into host cell chromosomes. Transforming activities have been associated with the core and NS3 proteins.[59,505] Strong evidence in favor of a direct or indirect transforming action of the core protein comes from studies of transgenic mice that develop such tumors in the absence of an immune response.[264,506] In addition, it is possible that NS5A promotes the development of tumors by repressing the antitumor activity of PKR.[130] However, in humans, chronic HCV replication is not by itself sufficient to cause HCC, even when accompanied by inflammation, because overall cirrhosis is evident when HCC develops. Even in the transgenic mouse model, liver cancer probably occurs as a result of increased hepatocyte turnover, dysregulation of proapoptotic and antiapoptotic cellular signaling pathways, or the generation of free hydroxyl radicals that are capable of damaging cellular DNA.[385,507]

Extrahepatic Manifestations of Hepatitis C Virus Infection

Although the most common end-organ disease associated with chronic HCV infection occurs in the liver, there are numerous extrahepatic manifestations attributable to HCV infection that result in morbidity and mortality. Increased all-cause mortality, as reported in patients with HCV infection, is thought to be related in large part to the extrahepatic manifestations of the chronic infection. With IFN-based therapies, SVR was associated with a decrease in all-cause mortality—evidence of the extrahepatic health impacts of chronic HCV infection.[194,508,509] Similarly, SVR achieved with DAA therapy is associated with significant decreases in all-cause mortality, and this is true in patients with and without severe liver disease at the time of treatment.[510,511] The range of extrahepatic manifestations of chronic HCV infection is broad and includes vasculitis, glomerulonephritis, nonhepatic malignancies, neurologic disease, and metabolic diseases such as diabetes, insulin resistance, and cardiovascular disease.[512–515]

HCV infection is associated with essential mixed cryoglobulinemia, membranoproliferative glomerulonephritis, and porphyria cutanea tarda. Up to half of HCV-infected persons have circulating cryoglobulins. However, only a small percentage develop the vasculitic syndrome of essential cryoglobulinemia (type II or type III, with circulating polyclonal immunoglobulin G [IgG] and IgM immune complexes).[516,517] HCV infection is also associated with B-cell lymphoproliferative disorders.[518–520] Clonal expansion of B cells in response to viral antigens and production of immune complexes is believed to be the primary pathogenesis of these extrahepatic diseases.[521] These clonal expansions are found in patients with HCV and non-Hodgkin lymphoma (NHL), suggesting a strong shared pathogenesis among these immune-mediated conditions.[522] HCV is also associated with aggressive diffuse large B-cell NHL and follicular lymphoma; however, the pathogenesis is likely different, with

a greater association with direct viral infection of B cells.[523] Membranoproliferative glomerulonephritis may also occur in association with HCV-related cryoglobulinemia, generally in the absence of vasculitis.[228,517] There is accumulating evidence that treatment of HCV infection stabilizes or reverses renal disease progression, including in the DAA era.[524,525] Chronic HCV infection has also been found in 60% to 80% of persons with sporadic (but not familial) porphyria cutanea tarda.[526,527] To a lesser extent, HCV infection has been associated with Mooren corneal ulcers, Sjögren syndrome, lichen planus, and idiopathic pulmonary fibrosis.[528] The pathogenesis of such conditions remains unknown. However, sialadenitis resembling that occurring in Sjögren syndrome has been observed in transgenic mice expressing HCV envelope proteins.[529] Thyroid autoantibodies, Hashimoto thyroiditis, and hypothyroidism have been associated with chronic hepatitis C in women.[530]

Chronic HCV infection is also associated with metabolic disorders such as insulin resistance, type 2 diabetes mellitus, and atherosclerosis.[384,531–533] As previously mentioned, there is strong evidence of a direct viral link between steatosis and genotype 3 chronic HCV infection (see also "Disease Progression"). The prevalence of steatosis is increased in patients with genotype 3 HCV infection, and steatosis improves with successful HCV treatment.[384,534] In patients with the more common genotype 1 HCV infection, there is also a correlation with insulin resistance and steatosis.[532,533] Several studies have shown that patients with chronic HCV infection have an increased risk of diabetes mellitus compared with both uninfected and HBV-infected controls.[134,535–538] The elevated risk is likely to be due to the association of HCV infection and insulin resistance. HCV infection causes both hepatic and extrahepatic insulin resistance. Patients with HCV infection have been found to have an increased homeostasis model for assessment of insulin resistance (HOMA-IR) as compared with both uninfected and HBV-infected controls.[539–541] Furthermore, HCV clearance with IFN-based and DAA antiviral therapies results in reduced levels of HOMA-IR, suggesting that clearance restores insulin sensitivity.[542,543] It can be difficult to tease out the impact of HCV on these metabolic diseases. One large observational study found that HCV infection was associated with diabetes mellitus until liver enzyme levels were included as confounders, attenuating the association.[544] It is possible that the liver enzyme elevation represented steatosis and is the true mediator of diabetes risk in patients with HCV infection. It is also possible that liver inflammation is the driver of insulin resistance and diabetes risk, and that the virus itself has less role to play in these metabolic disease complications.

Although the relationship of HCV infection and insulin resistance, steatosis, and diabetes is generally well accepted, whether HCV is an independent risk factor for cardiovascular disease is less clear. Studies focusing on markers of atherosclerosis have generally suggested a greater risk among patients with HCV infection; however, the outcomes of these studies have been highly variable.[545–548] Furthermore, studies assessing myocardial infarction and cardiovascular-related mortality have conflicted.[549–552] One difference in outcomes that may affect the results of such studies is differentiating type I (e.g., thrombotic or plaque rupture) from type II (supply-demand mismatch) myocardial events. Similar to the situation with other metabolic diseases, there is increasing evidence that HCV eradication with antivirals decreases cardiovascular events.[553–555]

DIAGNOSIS LABORATORY ASSESSMENT OF HEPATITIS C VIRUS INFECTION

Screening Serology

The diagnosis of HCV infection is based principally on detection of antibodies to recombinant HCV polypeptides and by assays for HCV RNA. There have been several "generations" of enzyme immunoassays (EIAs) that measure antibodies directed against NS4, core, NS3, and NS5 sequences.[556–558] The US Food and Drug Administration (FDA) has approved at least six assays for detection of HCV antibodies by commercial laboratories.[559] The sensitivity of the third-generation assay is estimated to be 97%, and it can detect HCV antibody within 6 to 8 weeks of exposure.[560,561] These assays are measures of either past or current HCV infection, not immunity. Direct testing for HCV RNA is necessary to distinguish between ongoing or prior infection in persons with HCV antibodies. Surrogate neutralizing tests based on pseudotyped lentivirus particles were described earlier ("Humoral Immunity"). Assays for IgM HCV antibodies are not clinically useful.

Point-of-care, "rapid" HCV tests may also have sensitivity and specificity for detection of HCV antibodies in a fingerstick-derived capillary blood specimen, similar to what is obtained with venous blood assayed in a commercial laboratory (>98%), and have the added value of providing the information quickly to the patient and provider.[562] The OraQuick HCV Rapid Antibody Test (OraSure Technologies) has been given a waiver by the FDA for use to detect HCV infection in nontraditional settings.

In the past, supplemental serologic testing such as with the recombinant immunoblot assay (RIBA) has been used to improve the specificity of the screening tests by demonstrating to which HCV antigen there was reactivity.[563,564] For this purpose, the FDA licensed the Chiron RIBA HCV 3.0 SIA (Chiron Corporation, Emeryville, CA). However, supplemental antibody assays are no longer commercially available in the United States. Accordingly, use of high signal/cutoff ratios has largely replaced RIBA testing to improve the specificity of EIA results.[559] In clinical laboratories, EIA-reactive samples with high optical density (e.g., >3.8 for the Ortho and Abbott EIA tests) are nearly always RIBA positive or contain HCV RNA.[565] Thus, HCV testing recommendations generally call for screening for HCV antibodies followed by RNA testing, as detailed later.[566,567]

According to US Public Health Service Guidelines, all HIV-infected persons should be screened for HCV infection at entry into health care.[568] HCV screening should be done as in persons without HIV infection, as described earlier. In HIV-infected persons, HCV antibody titers may decline below the level of detection, especially in those with advanced immunodeficiency (CD4 cell count <100/mm^3).[569–572] There are case reports of HCV *seroreversion* occurring in association with immunosuppression, and *seroconversion* associated with antiretroviral therapy.[457,573] Thus, although uncommon in other settings,[574] HCV antibody–negative persons with HIV infection who have unexplained liver disease should be tested for HCV RNA to exclude the possibility of seronegative infection.[575,576] In addition, patients with HIV infection and ongoing risk of HCV exposure (men who have sex with men [MSM], people who inject drugs [PWID]) should be tested at least annually.[577]

RNA Detection

HCV RNA can be detected in plasma and serum by means of a variety of molecular techniques, including reverse-transcriptase polymerase chain reaction (RT-PCR), real-time PCR, transcription-mediated amplification (TMA), and branched DNA (bDNA) methods. Initially, tests used to quantify the amount of virus were less sensitive than those used to detect HCV RNA and also did not precisely characterize HCV RNA in persons with levels estimated at more than 6 $\log_{10}$IU/mL. Accordingly, some tests are FDA approved specifically for detection, and others for quantification. More recently developed real-time PCR and TMA assays quantify virus across a wide range and have comparable sensitivity (10–50 IU/mL) to qualitative assays.[578–580]

A number of factors may affect the estimate of HCV quantity, including the assay used, time to serum separation, storage temperature, collection tube, and testing laboratory.[579,581] To improve the comparability of HCV RNA results, clinicians should use the same assay and laboratory to monitor a patient on treatment, and laboratories should report quantitative results in international units (IU), which correspond to a standardized amount of HCV RNA rather than viral particles.[582] Algorithms have been published for the conversion of proprietary unit values provided by commercially available assays to international units.[583] In persistently infected persons not receiving treatment, HCV RNA levels tend to remain stable (within one-half $\log_{10}$) over years.[584,585] In the absence of treatment, there is little need to repeat HCV RNA tests frequently.

Core Antigen Detection

HCV core antigen (p21) is a highly conserved protein that is a component of the viral nucleocapsid and present in plasma throughout infection. HCV core antigen detection represents another means of confirming active viral replication and offers several potential advantages to RNA detection in the current therapeutic era in which RNA quantitation is generally not required in order to optimize therapy. Current advantages

include shorter assay time with automated sample processing and lower costs compared with HCV RNA detection; however, a theoretical benefit is improved potential for low-cost, point-of-care assay development—a key component of HCV treatment rollout in resource-limited settings.[586,587]

Several commercial EIAs are available, with most clinical data limited to two assays (Abbott ARCHITECT HCV Ag [Abbott] and Ortho HCV Ag ELISA [Ortho Clinical Diagnostics]).[583,588] Sensitivity and specificity of the assays are high (90%–94% and >97%, respectively), although sensitivity falls off at HCV RNA levels below 3000 IU/mL.[587] Emerging clinical data indicate that core Ag assays are highly accurate in determining HCV treatment outcomes with DAA-based therapies when compared with quantitative HCV RNA detection.[586,589,590]

Genotype

Determination of the viral genotype played a large role in predicting the response to IFN-based therapy (see "Genetic Diversity" earlier). With the currently available DAA regimens, responses are now uniformly high, with several regimens providing roughly equivalent response rates regardless of genotype.[591,592] However, depending on the DAA regimen used, genotype and (in some cases) subtype determination (1a vs. 1b) remains critical for appropriate treatment, including the need for further genotypic resistance testing in select situations (see "Treatment of Chronic Hepatitis C," sections on "Initial Treatment of Chronic Hepatitis C Virus Infection," "Genotype 1," and "Antiviral Resistance").[593,594] The reference method for determination of HCV genotype and subtype is based on sequencing of the core/E1 and NS5B regions; however, this approach is rarely used in the clinic.[185,595]

Several assays are commercially available to determine HCV genotypes on the basis of direct evaluation of 5′ noncoding sequence (5′UTR; Trugene 5′NC HCV Genotyping kit; Bayer Healthcare Diagnostics Division, Tarrytown, NY) and hybridization to genotype-specific 5′ noncoding and core region oligonucleotide probes (INNO-LiPA HCV II [Innogenetics, Ghent, Belgium] and Versant HCV Genotyping Assay 2.0 [Bayer Healthcare Diagnostics Division].[596] The first-generation line probe assays that use only the highly conserved 5′UTR accurately identify the genotype but have limited ability to accurately determine subtype (1a vs. 1b) and may not be able to distinguish subtype 1b from certain genotype 6 subtypes.[597–599] Second-generation line probe assays that also include core sequences have been shown to have improved ability to discriminate between genotype 1 subtypes.[599] Genotyping assays that use oligonucleotide probes to the 5′UTR for genotype assignment and NS5B for subtype determination also have improved ability to discriminate between genotype 1a and 1b subtypes (RealTime HCV Genotype II; Abbott Molecular, Des Plaines, IL).[599,600] In settings in which subtype determination is critical for treatment decision making, a second-generation assay (either line probe or oligonucleotide probe) that targets two regions of the HCV genome should be used.

Other Laboratory Testing

Some clinicians routinely test persons with confirmed HCV infection for related conditions. Typical evaluations include testing for HIV, hepatitis B surface antigen, and susceptibility to HAV and HBV infections, and screening for other underlying causes of liver disease; for example, autoimmune liver disease, hemochromatosis, Wilson disease, and α_1-antitrypsin deficiency.[575] With the recent recognition of HBV flares during DAA-based HCV treatment in patients with prior HBV infection, including a few patients with isolated HBV core IgG antibody positivity, expanded baseline testing for prior HBV exposure is now recommended.[598–602] All patients with a negative hepatitis B surface antibody test result (<10 mIU/mL) should be screened for prior HBV infection with HBV core total or IgG antibody testing. Treatment of HCV in patients who have evidence of prior HBV exposure yet lack of serologic evidence of protective immunity (HBV surface antibody titer <10 IU/mL) should be monitored more closely for evidence of HBV reactivation.

Clinical Application of Tests for Hepatitis C Virus

Detection of HCV infection begins with testing. In 1998, the CDC recommended testing persons with various HCV risk factors such as injection drug use or receipt of blood transfusion before 1992 (Table 154.2). That recommendation had little impact on HCV testing because most studies suggested that no more than half of HCV-infected persons were aware of their status (see "Epidemiology of Hepatitis C"). Thus, both the CDC and the US Preventive Services Task Force (USPSTF; grade B recommendation) recommended that all persons born from 1945 to 1965 undergo at least one HCV test.[472,603] Because roughly 75% of all those with HCV infection in the United States are in that birth cohort, this practice could lead to recognition of nearly 1 million new cases. Efforts to integrate this recommendation into clinical practice have met with mixed results. For example, a nationwide survey of Medicare data demonstrated a 91% increase in testing from 2011 to 2014 after the USPSTF recommendations were made; however, the absolute screening rate remained at a very low level (3.26% in 2014).[604] In isolated settings, approaches such as electronic medical record prompts and even laws requiring HCV screening have been effective.[605–609] Given the inconsistent nature of screening practices and the recognition of an increasing HCV incidence and burden in those outside the birth cohort, universal screening for HCV in adults should be considered and has been modeled to be cost-effective and to increase the number of people diagnosed and treated.[610]

Chronic HCV infection is usually diagnosed by testing for HCV antibodies, then by testing reactive sera for HCV RNA to assess if the infection is ongoing. As HCV treatments have evolved, the importance of accurately quantitating baseline HCV RNA levels and determining changes during therapy have been dramatically diminished.[611,612] Still, in most settings a quantitative HCV RNA test with a sensitivity limit of less than 15 to 25 IU/mL and a wide linear range is used. A negative RNA test result (<15 or <25 IU/mL, depending on the specific assay) in a person found to have HCV antibodies with EIA most likely indicates that HCV infection has resolved. Other interpretations include that the EIA result is falsely positive, that the HCV RNA test result is falsely negative, or rarely that a person has intermittent or low-level viremia. The latter condition may occur transiently in the initial 2 years of infection but is rare with chronic hepatitis C.

In acute HCV infection or in immunosuppressed states, a negative EIA does not exclude HCV infection. HCV RNA testing is helpful if seronegative acute HCV infection is suspected because HCV RNA can be detected within 2 to 3 days after an exposure, whereas antibodies to HCV are detectable an average of 7 to 8 weeks later.[273,274] HCV RNA testing can also be used to screen for HCV infection in persons with negative HCV EIA results who are known to have conditions associated with diminished antibody production, such as HIV infection and hemodialysis.

Liver Fibrosis Staging

A number of approaches are available for the staging of liver fibrosis in patients with chronic HCV infection. As the toxicity and efficacy of

TABLE 154.2 Persons Who Should Be Screened for Hepatitis C Virus (HCV) Infection

HIGH PREVALENCE	POSTEXPOSURE TESTING
Persons who ever injected illegal drugs Persons born from 1945–1965 Persons with elevated aminotransferase levels Persons on hemodialysis Persons who received transfusions or organ transplants, including clotting factor concentrates produced before 1987 or either a transfusion or organ transplant before July 1992 Persons in settings with demonstrated high HCV prevalence and where risk factor ascertainment may be poor (e.g., inmates, patients attending inner-city clinics for sexually transmitted diseases, and some university emergency departments)	Persons with percutaneous or heavy mucosal exposure to HCV-positive blood Children born to HCV-infected women Sexual partners of HCV-infected persons should be considered for HCV screening, although the risk of transmission during sexual intercourse is low

[a]See 1998 US Public Health Service Guidelines for full recommendations regarding HCV screening.[739]

hepatitis C treatment have dramatically improved, preferred approaches for staging have shifted away from liver biopsy to serum fibrosis markers and radiographic modalities (e.g., elastography; see later), often in combination. Despite therapeutic advances, proper fibrosis staging remains critical to proper treatment regimen selection and optimal patient management (e.g., esophageal varices and HCC screening).

Liver Biopsy

Although liver biopsy remains the most definitive method for assessing the stage of liver injury associated with HCV infection, it is infrequently used in the current HCV treatment era due to high treatment efficacy rates regardless of fibrosis stage and the lack of frequent treatment-associated side effects or toxicity. Biopsy may identify other causes of liver disease, such as alcohol use or hemochromatosis, and also provides information on distinct HCV-related processes: periportal necrosis (piecemeal necrosis), parenchymal injury, portal inflammation, and fibrosis.[413,475,613] Various systems have been proposed for grading inflammation and staging fibrosis (Table 154.3).[613–615]

There are important limitations to the liver biopsy. Although interobserver variance has been described, the main concern is with the insensitivity of the test (failure to detect significant fibrosis), which is a function of the size of the sample taken.[616–618] Tissue samples shorter than 15 mm are especially likely to miss significant disease. However, even in carefully controlled clinical trials, the average size of a liver biopsy specimen is often less than 20 mm.[619] Complications such as serious bleeding may also occur, although infrequently (<1%).[616,617] The histologic "snapshot" of the current state of inflammation and fibrosis also does not predict the future course of disease with the level of certainty expected for an invasive screening test. Contraindications to liver biopsy include uncorrectable coagulopathy and clinical evidence of decompensated cirrhosis.

Noninvasive Blood Markers of Hepatic Fibrosis

A large number of serum fibrosis markers have been considered as surrogates for the liver biopsy.[620] Studies have considered liver-related enzymes such as ALT, AST, and γ-glutamyl transferase (GGT); direct and indirect measurements of molecules made or processed by the liver, such as the prothrombin time, platelet count, and levels of serum albumin, bilirubin, γ-globulin, and apolipoprotein A_1; and markers of inflammation, fibrinolysis, fibrogenesis, or stellate cell activation (YKL-40, hyaluronic acid, procollagen III N-peptide, TGF-β, α_2-macroglobulin, α_2-globulin). However, these individual test results are not sufficiently sensitive or specific to play a major role in clinical decision making.

The validity of blood tests for liver fibrosis has been improved through use of algorithms that combine multiple individual results.[620] The most accessible of these algorithms are those that use common laboratory parameters, with or without patient age, and include the fibrosis 4 index (FIB-4) and AST-to-platelet ratio index (APRI).[621,622] The APRI uses platelet count and AST to estimate the likelihood of advanced fibrosis or cirrhosis. Use of a cutoff of ≤0.5 provides a high negative predictive value (NPV) (86%–90%) for advanced fibrosis or cirrhosis; in contrast, the positive predictive value (PPV) for cirrhosis with use of a cutoff of 2.0 is not as robust (57%–65%).[621] As an example, in 192 patients with chronic hepatitis C, significant fibrosis could be predicted accurately in 51% of patients by using the APRI, which is calculated by dividing the AST level (divided by the upper level of normal) by the platelet count (10^9/L) and multiplying by 100.[623]

Similarly, the FIB-4 uses AST, ALT, and platelet count combined with patient age to estimate the likelihood of advanced fibrosis or cirrhosis. Typical cutoffs used include <1.45, which has a high NPV for advanced fibrosis (95%), and >3.25, with a PPV of 82% for advanced fibrosis or cirrhosis.[622] The FIB-4 has also been validated in HIV coinfected population and is predictive of clinical outcomes.[624] Other groups have reported variable success with these "multitest" fibrosis algorithms designed for detection of significant fibrosis (METAVIR 2–4) or cirrhosis (METAVIR 4) among persons with chronic hepatitis C, with or without HIV coinfection.[623,625–627] It is important to recognize that both of these indices are relatively specific, provided there is no other process independently affecting the markers being used; however, they possess only moderate sensitivity for advanced fibrosis or cirrhosis, and therefore patients with clinically significant fibrosis may be missed.[628]

The European MULTIVIRC group found that a combination of markers (α_2-macroglobulin, haptoglobin, apolipoprotein A_1, GGT, ALT, and total bilirubin) had relatively high NPVs and PPVs for significant liver fibrosis.[629] However, the high NPVs and PPVs required scores that pertained only to 12% and 34% of patients, respectively. In addition, the test does not appear to predict future fibrosis progression as well as it does contemporaneous disease.[377] The related assay is commercially available in the United States (FibroSURE; LabCorp, Burlington, NC).

Diagnostic accuracies of the various marker panels vary across studies. In one systematic review, Chou and coworkers reported average areas under the receiver-operating curves of 0.82 to 0.9 for detection of cirrhosis compared with biopsy when these algorithms were used.[620] The consistency of the apparent ceiling of the accuracy of these tests may reflect error in the biopsy reference standard as much as inaccuracy in the surrogate tests themselves.[630]

Radiographic Tests

Liver stiffness measurement (LSM) with transient elastography has been introduced as a noninvasive method of staging liver disease in persons with chronic hepatitis C.[631–633] Based on the premise that liver fibrosis can be predicted through measurement of liver stiffness, this ultrasonography-like device provides a series of measures (shots) of liver stiffness ranging from 2.5 to 75 kilopascals (kPa). Typically, 10 individual measurements are obtained and averaged. Results less than

TABLE 154.3 Comparison of Systems Used to Grade Liver Fibrosis in Hepatitis C Virus–Infected Persons

	LIVER BIOPSY			NONINVASIVE		
HISTOLOGIC FINDING	MODIFIED HAI[615]	METAVIR[614]	KNODELL[613]	FIBROSCAN[a,631]	FIBROTEST[629]	APRI[623]
No fibrosis	0	0	0		<0.21	
Expansion of some portal zones	1	1	1			
Expansion of most portal zones	2	1	1	<7.1	0.27–0.31	≤0.5
Expansion of most portal zones and occasional bridging	3	2[b]	3	7.1–9.4	0.48–0.58	
Expansion of most portal zones and marked bridging	4	3[c]		9.5–12.4	0.58–0.72	>1.5
Marked bridging and occasional nodules	5	3				
Cirrhosis	6	4	4	12.5	>0.74	

[a]FibroScan is a radiographic technique. FibroTest is marketed as FibroSURE in the United States. Other thresholds have been used for some of these noninvasive tests. See text, "Noninvasive Blood Markers of Hepatic Fibrosis."
[b]More than one septum.
[c]Portal-central septae.
APRI, Aspartate aminotransferase–to–platelet ratio index; *HAI,* histologic activity index.

7.5 and more than 12.5 kPa generally correlate strongly with no or minimal fibrosis or cirrhosis, respectively.[378,634] One limitation of the test is that approximately 20% of persons do not have a valid result, which can occur if the machine does not receive 10 valid shots, the proportion of valid shots is less than 60%, or the interquartile range of the shot results divided by the median is greater than median value.[634] Obesity, female sex, operator inexperience, and age older than 52 were associated with invalid results. Falsely high estimates of liver fibrosis have also been reported with acute inflammation and recent food intake.[635] A liver stiffness test (FibroScan) was approved for use in the United States in 2013 but has been used in Europe since 2003. An interesting aspect of elastography to diagnose cirrhosis is that additional information can be provided as the LSM increases above 12.5 to the limit of 75 kPa. Data suggest that an LSM ≥21 kPa corresponds closely to a hepatic venous pressure gradient (HVPG) of ≥10 mm Hg—the accepted gold standard for the diagnosis of portal hypertension.[636,637] These data have been adapted by the American Association for the Study of Liver Diseases (AASLD) into recommendations that patients with compensated cirrhosis, platelet count >150,000/mm^3, and an LSM <20 kPa may have screening for esophageal varices deferred.[637]

Significant liver disease can also be detected by hepatic imaging with ultrasound, computed tomography, and magnetic resonance imaging. These modalities may depict a small nodular liver, ascites, an enlarged spleen, intraabdominal varices, and HCC. However, although potentially useful in the management of persons known to have cirrhosis (e.g., in screening for HCC), such imaging methods are not sensitive for fibrosis detection and should not be used for initial staging of HCV infection. Modification of MRI with the addition of LSMs, magnetic resonance elastography (MRE), yields a test that performs as well as or better than ultrasound-based elastography.[638] The major limitations of this modality are cost and limited availability, given the requirement for specialized equipment.

Approach to Staging Liver Disease

There are two major clinical applications for liver disease staging. One is to ascertain the appropriate length of treatment, and the other is to determine if there is cirrhosis because there will be an indication for routine screening for portal hypertensive complications and HCC. In the current HCV treatment era, the primary application of staging is detection of cirrhosis. The relative sensitivity of LSMs (FibroScan) for detection of cirrhosis and high tolerability make that method particularly attractive where it is available in clinical practice. Some combine liver stiffness evaluation with fibrosis blood tests to achieve greater confidence.[631] In one study, the predictive value of LSMs was better than that of liver biopsy for predicting liver-related outcomes in HIV- and HCV-coinfected persons.[639] Accordingly, liver biopsy now has a diminishing role in liver disease staging, being reserved for instances in which cirrhosis cannot confidently be excluded by other methods or when other disease processes need to be excluded by direct tissue examination.

Persons with chronic hepatitis C and bridging fibrosis or cirrhosis (METAVIR 3 or 4) should be screened for HCC.[640] Elevations in serum α-fetoprotein (AFP) may indicate the development of HCC in persons with HCV-related cirrhosis. But AFP is neither sensitive nor specific, and liver ultrasound testing every 6 months is the preferred method to screen for hepatocellular cancer in patients with bridging fibrosis or cirrhosis from chronic hepatitis C.[640] Endoscopy is also recommended for most HCV-infected persons with cirrhosis to assess for esophageal varices and provide appropriate treatment and follow-up.[637]

EPIDEMIOLOGY OF HEPATITIS C

HCV is most often transmitted through percutaneous exposure to blood. However, the predominant modes of transmission may change over time and differ between, and even within, countries. In economically developed countries, most new HCV infections are related to illicit injection drug use, although blood transfusions were once important sources of infection. HCV may also be transmitted between sexual partners and from a mother to her infant, although this is relatively uncommon compared with HBV. HCV can also be transmitted by means of percutaneous medical procedures, when there is a breach in infection-control protocols. This transmission route is especially important in settings with insufficient resources to maintain infection-control standards. Worldwide, unsafe medical practices are the dominant transmission route.

Prevalence of Hepatitis C Virus

HCV infection has been reported in virtually every country where it has been carefully evaluated, suggesting that HCV, unlike HIV, has a long-standing global distribution. Estimates for the global prevalence of viremic HCV infection have been recently updated and now stand at 71.1 million persons (approximately 1.0% of the population); although infection is prevalent, this is a marked decrease from prior estimates of 185 million HCV antibody–positive persons in 2005 due to updated and lower than previously estimated prevalence in sub-Saharan Africa.[206,641] In developed nations, the HCV prevalence is typically 1% to 2% in the general population. Estimates of hepatitis C prevalence in the United States are available from serial National Health and Nutrition Examination Surveys (NHANES). A random sample of the households from 2003 to 2010 suggested that 3.6 million persons had HCV antibodies or 1.3% of the general population.[642] The peak age of infection spanned 2 decades, with 41% of infections in those 40 to 49 and 38% in those 50 to 59 years. Epidemiologic factors associated with HCV infections included male sex, non-Hispanic black race, less than high school education, and income less than two times the poverty level.[642] Estimates accounting for high-risk population, such as the incarcerated or homeless, suggest there are at least 5.2 million anti-HCV–positive persons in the United States.[643] In comparison to prior NHANES surveys, the overall prevalence of HCV was seen to be decreasing in the United States with the aging of the cohort with the highest HCV prevalence. Given the aging of the HCV epidemic in the United States, approximately 75% of all HCV-infected persons in the United States were born between 1945 and 1965.[472] This finding, the discovery that about half of HCV-infected persons are unaware of their status, and improved treatments led the CDC to call for all persons born between 1945 and 1965 to be tested for HCV antibodies, in addition to those individuals with apparent risk factors.[603] However, with the ongoing shift in HCV epidemiology in the United States, such screening approaches are no longer adequate to capture many new infections, and updated approaches (e.g., universal HCV screening) are needed.[610]

There is considerable heterogeneity in HCV infection prevalence around the world. In Egypt, for example, HCV infection occurs in 5% to 30% of the general population.[644,645] Similarly high rates of infection have been found in certain regions of Japan, Taiwan, and Italy. As in the United States, HCV infection is generally more prevalent among persons older than 40 years and uncommon in those younger than 20.[646–649] This cohort effect suggests that transmission occurred through a practice that has been discontinued, such as traditional folk remedies or reuse of needles for injection.[646,650,651] It is likely that a national campaign to treat schistosomiasis infections with frequent reuse of needles and syringes was responsible for many infections in Egypt, and decades before in Japan.[652–654] Very high HCV prevalence rates are also reported in Pakistan and linked to unsafe injections.[655,656] One study estimated a mean of 13 injections per person per year in Pakistan, the highest in the world.[656]

A high prevalence of HCV infection has also been reported in some urban areas of developed countries. In Baltimore, Maryland, HCV infection was found in 18% of patients attending an inner-city emergency department and in 15% of persons attending a nearby clinic for sexually transmitted diseases.[657,658] A high prevalence of HCV infection (20%–40%) also has been noted among incarcerated persons in California, Maryland, and Texas.[659] Undoubtedly, in these settings, prior illicit injection drug use is chiefly responsible.

Prevalence of Hepatitis C Virus in Those With HIV

Because of shared routes of transmission, HCV infection is found in persons with HIV much more frequently than in the general population. In the United States and Europe, 15% to 30% of HIV-infected persons are coinfected with HCV.[576,660] However, the prevalence of HIV and HCV coinfection varies markedly depending on the route of HIV infection, with 50% to 95% of HIV-infected injection drug users being

coinfected, compared with less than 10% of HIV-infected men who have sex with men.[661] Globally it is estimated that there are around 2 to 5 million persons living with HIV who are coinfected with HCV.[662,663]

Incidence and Transmission of Hepatitis C Virus

Starting around 2011 in the United States, a dramatic increase in acute HCV infections in young persons (≤30 years old) in nonurban settings was noted, with an 85% increase in incidence between 2010 and 2011.[664] Although over 50% of the states surveyed for the time period from 2006 to 2012 had an over 200% increase in HCV incidence, the epicenter of the outbreak appeared to be the Appalachian region of the United States, with injection drug use as the major risk factor.[664,665] Fueled by these changes, the number of estimated acute HCV infections in the United States has increased year after year since 2010, with an estimated 34,000 infections in 2015, which was likely an underestimate.[666,666a]

Biologic Basis of Transmission

HCV transmission requires that infectious virions contact susceptible cells that are permissive for replication. HCV RNA can be detected in blood (including serum and plasma), saliva, tears, seminal fluid, ascitic fluid, and cerebrospinal fluid.[230,231,667] HCV RNA-containing blood is infectious when inoculated intravenously (e.g., by transfusion). In addition, a chimpanzee has been infected by intravenous inoculation of saliva.[255] However, there is very little information available regarding the potential infectivity of HCV RNA–containing body fluids. Furthermore, it is not clear whether cells other than hepatocytes can be infected (and thus whether infection requires direct percutaneous inoculation into the bloodstream).

Transmission appears to be enhanced by the stability of virus in environmental conditions such as in syringes, gauze, or water bottles.[668,669] In one study, a high HCV inoculum remained infectious in cell culture after storage in bottled water for 3 weeks.[669]

HIV infection may also enhance the transmissibility of HCV. The most conclusive data derive from studies comparing the rate of perinatal transmission of HCV from HIV- and HCV-coinfected mothers with those infected with HCV only.[670,671] Whether the association of HIV infection with higher HCV RNA level is the reason for more frequent perinatal (and sexual) HCV transmission is unknown.[672,673] There are fewer studies investigating whether HIV- and HCV-coinfected persons are more likely to transmit HCV by means of sexual intercourse, but such persons appear to be much more likely to transmit HIV than HCV to their partners. In one study, HIV infection was detected in 13% of 162 female sexual partners of HIV- and HCV-coinfected hemophilia patients, whereas only 3% were HCV infected.[674] The greater transmissibility of HIV by sexual intercourse and related recommendations to prevent HIV transmission by using barrier precautions for every act of sexual intercourse are more than sufficient to prevent spread of HCV to sexual partners.

Percutaneous Transmission

Infection occurs in more than 90% of seronegative recipients who are transfused with blood from HCV-antibody positive donors.[675,676] Before the introduction of nonspecific surrogate tests (serum ALT and antibody to HBV core protein), and specific EIA assays for detection of HCV infection in blood donations, approximately 17% of HCV infections in the United States were caused by transfusion.[677] Use of HCV EIA test to screen donations reduced the risk of transfusion-transmitted hepatitis C substantially, and that risk is less than 1 : 1,000,000 in areas (such as the United States) where donations are also screened for HCV RNA.[678–680]

HCV also has been transmitted by intravenous administration of contaminated blood products including immune globulin.[681,682] In recent years, however, the risk of transmission by these products has been effectively eliminated by the introduction of solvent-detergent and other virus inactivation procedures. Transplantation of organs from HCV-infected donors almost always results in HCV infection in seronegative recipients,[683] and in seropositive recipients may lead to superinfection with a second distinct viral strain.[684]

Contaminated needles and perhaps other paraphernalia associated with illicit drug use account for the majority of HCV infections in most developed countries. Since 1992, at least two-thirds of new HCV infections in the United States have been associated with illicit drug use.[685] Worldwide, 50% to 95% of persons acknowledging drug use have HCV infection.[686–689] HCV infection generally occurs within months of initiating the illicit use of injected drugs. In one cohort, 80% of subjects acknowledging 2 or more years of injection use were infected with the virus, a prevalence that was higher than that of HIV or HBV infection.[686,690] Early acquisition of HCV is probably related to the practice of older (infected) intravenous drug users teaching new (uninfected) initiates by demonstrating on themselves and then on the new initiates.[691] In the United States, there appears to have been a significant reduction in the incidence of HCV associated with illicit drug use in the late 1980s.[685] However, incidences of 5% to 30% are still reported in many urban areas. There are also HCV outbreaks in persons 15 to 24 years of age in urban and rural areas, linked to transitions to injection drug use.[692,693]

HCV may be transmitted by other percutaneous exposures that are not associated with drug use but that occur too infrequently to be detected in many studies. For example, tattoos have been associated with HCV infection in some studies, with tattooing in prison appearing to be a particularly high-risk procedure.[694–696] Human bite and folk remedies such as acupuncture and scarification rituals may also be associated with HCV infection.[697]

Nosocomial Infection

Worldwide, unsafe medical practices are the predominant route of HCV transmission. In countries where there are adequate resources to observe universal precautions, nosocomial transmission is uncommon and associated with breaches in infection-control protocols.[698,699] Nonetheless, a report from Spain suggests that nosocomial spread is one of the more preventable forms of HCV transmission.[700] The investigators considered 109 cases of acute hepatitis diagnosed between 1998 and 2005. In the 6-month period preceding the diagnosis of acute hepatitis C, hospital admission was recognized in 73 (67%) of cases, whereas intravenous drug use was reported in only 9 (8%) and sexual contact in 6 (5%). Among the 73 patients in whom hospital admission was the only risk factor, 33 underwent surgery and 24 were admitted to a medical emergency unit or a medical ward; the remaining 16 patients underwent an invasive diagnostic or therapeutic procedure. In some instances, the transmission vector was identified (e.g., multidose saline containers, heparin flush vials, or colonoscope).[701–704] In one example, 16 persons were infected from a single source patient through preparation of radiopharmaceutical injections.[705] The nosocomial transmission of HCV within hemodialysis units is a particular concern related to contamination of surfaces and failure to adhere to hand hygiene and glove use in dialysis units.[706–708] Adherence to strict universal precautions can significantly lower the risk of transmission in dialysis units.[666,680,696,709]

In economically developing regions of the world, HCV transmission occurs from the widespread use (and reuse) of injection supplies and other percutaneous practices in both traditional and unconventional medical settings. For example, one analysis estimated that the annual ratio of injections per person ranged from 1.7 to 11.3 and that reuse of equipment without sterilization was as high as 75% in some areas such as in Southeast Asia.[710] As mentioned earlier, widespread use of injections for schistosomiasis appears to explain the approximately 20% population prevalence of HCV in Egypt.[652] Likewise, frequent unsafe injections explain most infections in Pakistan.[656]

Transmission of HCV to health care workers occurs after 1% to 2% of accidental needlestick exposures to HCV-infected patients.[711–713] Studies of such accidents indicate that the risk of HCV transmission is intermediate (approximately 3% per documented exposure to susceptible host) between that of HIV (approximately 0.3%) and HBV (approximately 30%).[711,713,714] Although hollow-bore needlestick exposures account for most documented instances of HCV transmission, HCV infection has also been reported from blood splashed on the conjunctiva, and from a solid-bore needlestick.[715] Despite these risks, the prevalence of HCV infection among dental and medical health care workers is less than or similar to that of the general population.[716–718]

HCV also may be transmitted from health care providers to patients.[719,720] Performance of procedures in which percutaneous injuries can occur (e.g., intrathoracic surgery) is a common feature. In

community-based studies in the United States, patients with acute HCV infection do not commonly report recent interaction with a health provider, and work restrictions are not routinely required for HCV-infected health care workers.[685] Thus, although nosocomial exposure is a leading cause of infection in developing countries, it is rare where resources permit adherence to universal precautions.

Sexual Transmission

Although transmission of HCV during sexual intercourse is difficult to prove, there is mounting circumstantial evidence that it occurs. HCV RNA has been detected in semen and saliva,[230,721] and persons with multiple sexual partners and commercial sex workers have a high HCV prevalence.[722,723] In multiple studies of families of HCV-infected patients carried out in Japan and in Europe, sexual partners generally have been the only household contacts at increased risk for infection with the same viral variant, and the odds increase with the duration of the relationship.[722,724–726]

On the other hand, there are data that indicate that sexual transmission of HCV is relatively infrequent in monogamous partnerships. Studies of long-term sexual partners of HCV-infected hemophiliacs and transfusion recipients generally show little or no evidence for HCV transmission, even if there was unprotected sexual intercourse.[727–730] Vandelli and coworkers studied 895 monogamous sexual partners of persons with chronic hepatitis C for more than 8000 person-years and found no convincing instances of HCV transmission, despite unprotected intercourse occurring an average of 1.8 times per week.[731] Likewise, Terrault and colleagues[732] studied 500 anti-HCV–positive subjects and their long-term heterosexual partners. On the basis of 8377 person-years of follow-up, the maximum incidence rate of HCV transmission by sex was 0.07% per year.[732] HCV prevalence among MSM is also generally lower than for other infections such as HIV, HBV, and syphilis, for which sexual transmission is well established; yet HCV prevalence in MSM has also been found to be higher than in the general population.[733,734] In one study, only 4.6% of a cohort of MSM were infected with HCV, whereas 81% had been infected with HBV.[735]

Transmission of HCV among HIV-infected MSM has also been reported, including several recent outbreaks.[360,736,737] In one recent outbreak, 60 HIV-infected men who acquired HCV had more high-risk sexual practices and were more likely to have shared drugs via a nasal or anal route in the preceding year in comparison with 130 controls who did not acquire HCV infection.[360,736,737] Similar reports have come from other European countries, Australia, and the United States. Although it is difficult to exclude the possibility of unacknowledged (or unrecalled) prior injection drug use in these instances, cumulatively the reports underscore that HCV infection is an important risk for MSM, especially those who engage in high-risk practices. In addition, there are multiple well-documented outbreaks of HCV among men who report no other risk factor than having sex with other men.[722,724,738] These outbreaks generally occur among HIV-infected persons and have been associated with high-risk practices such as fisting that might involve mucosal tears, and noninjection recreational drug use.

Thus, available data suggest that HCV may be transmitted during sexual intercourse, but it occurs rarely in most populations outside of HIV-positive MSM. One conjecture is that sexual transmission occurs more often from persons with acute HCV infection than from those with chronic hepatitis C. This might explain the high prevalence in settings in which exposure to someone with acute hepatitis C is likely (e.g., partner of an injection drug user) and the relatively low transmission incidence in long-term sexual partners of those with chronic hepatitis C. Nonetheless, individuals in long-term monogamous relationships should be informed of the low risk of future transmission and, according to US Public Health Service Guidelines, encouraged to discuss this risk and the use of barrier precautions with their sexual partners.[575,739]

Maternal-Infant Transmission

HCV is uncommonly transmitted from mother to infant. Estimates of the perinatal transmission frequency range from 0% to 4% in larger studies.[670,740–742] The timing of transmission is not known. However, HCV RNA has been detected within a month of birth in non-breastfed infants delivered by cesarean section, suggesting that transmission occurs in utero in at least some instances.[741] Because of the passive transfer of maternal HCV antibodies, the diagnosis of infant HCV infection must be based on detection of viral RNA or the persistence of antibody after 18 months of age. Cases of vertically transmitted HCV are poised to dramatically increase in the United States owing to the expanding HCV epidemic in young persons due to injection drug use. For instance, from 2011 to 2014, the rate of HCV detection in women of childbearing potential in Kentucky increased 213%; during the same time frame there was a 124% increase in infants born to mothers infected with HCV (1.6% of infants born in Kentucky).[743]

HCV RNA has been detected in breast milk.[744] However, in most studies the risk of HCV transmission is similar in breastfed and bottle-fed babies.[670,745,746] Neither the CDC nor the American Academy of Pediatrics recommends that HCV-infected mothers bottle-feed to prevent HCV transmission.[739,747] Likewise, although one study showed a reduction in perinatal HCV transmission in women who had elective cesarean sections, this measure is not routinely recommended for HCV-infected mothers.[671,739] A systematic review failed to identify any intervention that was clearly demonstrated to reduce the risk of mother-infant HCV transmission.[748]

Transmission Cofactors

Risk factors for HCV transmission chiefly relate to the probability of virus reaching recipient bloodstream. Donor HCV RNA level matters, especially at the extremes. In a review of 2022 parenteral, sexual, and perinatal HCV exposures, HCV was transmitted only by individuals with detectable viremia.[749] Moreover, nonparenteral (e.g., perinatal) transmission of HCV is rare when the level of viremia is low.[750] Some but not all studies indicate that infection with HIV may be an important cofactor for both sexual and maternal-infant transmission,[670,674,751] possibly because HIV infection is also associated with higher HCV RNA levels.[672,673,752]

TREATMENT OF CHRONIC HEPATITIS C

Treatment of chronic hepatitis C has undergone extensive evolution. More details of current recommendations are presented in the AASLD and Infectious Diseases Society of America (IDSA) online guidelines (www.hcvguidelines.org).

Over the past 5 years, the FDA has approved for the treatment of chronic HCV infection nine oral, IFN-free therapeutic regimens that are capable of inhibiting HCV replication, eradicating infection, and improving the natural history of the disease. These regimens have allowed expanded access to previous "difficult-to-treat" populations including patients with HIV coinfection, chronic kidney disease, or decompensated cirrhosis; liver and kidney transplant recipients; and adolescents. An improved understanding of HCV replication and atomic level resolution structures of several critical viral enzymes have provided targets for specific antiviral agents (see Fig. 154.3).

Treatment Responses

Virologic Responses

The primary aim of treatment is to prevent complications of chronic hepatitis C by eradication of infection. Treatment can permanently eradicate HCV infection such that HCV RNA is no longer detectable in blood or liver, titers of antibodies to HCV decline (although seldom to an undetectable level), and HCV-related liver pathology remits or improves.[753,754] Accordingly, treatment responses chiefly are characterized by the results of HCV RNA testing at various time points. In the setting of DAA therapy, the most relevant clinical time point test is termed *sustained virologic response,* which is defined as the absence of HCV RNA in serum 12 weeks after the end of therapy. Sometimes SVR is modified to indicate the number of weeks after treatment when HCV RNA is not detected (e.g., SVR_{12} means no HCV RNA detectable 12 weeks after stopping treatment). SVR_{12} has been accepted by the FDA as the marker of successful treatment. A relapse is defined when HCV RNA is detected after completion of therapy in persons who achieved an on-treatment undetectable viral load. Relapse typically occurs within the first 12 weeks after treatment completion. *Breakthrough* refers to a greater than 1 log increase in HCV RNA level in a patient who is still on treatment. Undetectable virus at the end of therapy is referred to as

an *end-of-treatment response* (ETR). Assessing virologic response at early time points (week 4 or 12) was essential in the IFN era. Given the potency of current DAA regimens, responses at early time points (week 4) are no longer useful to predict treatment outcome; despite this, an HCV RNA level at week 4 may be required by payers for completion of treatment.[611]

Histologic and Clinical Responses to Therapy

There is consistent evidence that liver histologic findings (chiefly inflammation but in some cases fibrosis) and long-term clinical outcomes are improved in patients treated with antivirals, including DAA therapies, particularly in those patients achieving an SVR.[755–763] One retrospective study considered outcomes of 479 persons with chronic hepatitis C a median of 2.1 years after they underwent IFN-α–based treatment. The risk of a clinical event (liver failure, liver cancer, or death) was substantially lower in the 30% who had achieved an SVR compared with the others.[760,764] Another study of 530 persons with advanced fibrosis or cirrhosis (Ishak fibrosis stages 4–6) followed over a median of 8.4 years after IFN-based HCV treatment reported a 10-year mortality rate among persons with SVR of 8.9% compared with 26% among those without SVR.[765] The 10-year liver-related mortality or transplant rate was 1.9% with SVR compared with 27.4% without SVR. Another study of more than 17,000 US veterans who started HCV treatment between January 2001 and June 2007 found that SVR was associated with a reduced hazard ratio for death of 0.51 to 0.70, depending on the HCV genotype.[766] New studies assessing the same benefit of SVR in the setting of DAA therapy show similarly impressive benefits with HCV eradication in patients with and without severe liver disease, including patients with decompensated liver disease.[501,510,572,767] Successful treatment of HCV infection can also improve various measures of quality of life and patient-reported outcomes, and the benefit is seen in patients with minimal or no hepatic fibrosis and those with severe liver disease.[768–772]

Medications

Interferon-α

Before the availability of DAAs, IFN-α was the mainstay of antiviral therapy for HCV. The type 1 IFNs (which include multiple types of IFN-α and IFN-β) comprise a heterogeneous group of cytokines that are expressed in response to viral infection. IFNs may alter the course of virus infections both directly and indirectly. With HCV, there is strong in vivo and in vitro evidence for a direct, IFN-mediated antiviral response, which in some cases the virus may be able to partially evade (see "Viral Persistence" earlier).[773,774] In 2018, neither AASLD nor IDSA recommended IFN-containing regimens in treatment of hepatitis C.

Ribavirin

Ribavirin is a guanosine analogue with high oral bioavailability and exceptionally broad, although not particularly potent, antiviral activity. At least four different mechanisms of action have been proposed to explain its efficacy in the treatment of HCV infection. First, ribavirin is a potent inhibitor of cellular inosine monophosphate dehydrogenase and thus may influence intracellular nucleoside pools; second, it has been suggested that it may weakly inhibit the NS5B-encoded RNA-dependent RNA polymerase; third, it has been shown to promote the mutagenicity of RNA viruses; and, fourth, ribavirin may possibly modulate the Th1/Th2 balance in the host immune response.[775–778] When administered alone, ribavirin therapy appears to reduce liver enzyme levels in 21% to 43% of patients.[779–781] However, HCV RNA levels were significantly reduced in only a few percent of patients receiving ribavirin monotherapy. Ribavirin has a multiple-dose half-life of 12 days and can be administered once or twice daily. Interesting to note, the clinical importance of ribavirin appears to be in preventing relapse and improving DAA efficacy in certain difficult-to-treat populations such as those with cirrhosis (including decompensated cirrhosis) and resistance-associated substitutions (RASs).[593,782–784]

Protease Inhibitors

The early direct-acting inhibitors of HCV replication targeted the NS3/4 serine protease.[785] In an initial phase I study, 31 patients with genotype 1 infection were treated with BILN 2061 given orally twice daily for 2 days, and reductions in HCV RNA level of 2 to 3 $\log_{10}$ copies/mL were noted.[785] Although later-phase testing of that compound was aborted owing to toxicity, additional compounds (boceprevir and telaprevir) were licensed in 2011 for use for genotype 1 HCV infection in combination with IFN and ribavirin.[786] These agents were limited by their frequent dosing, limited genotype scope, and relatively poor tolerability and efficacy and thus were rapidly replaced by IFN-free DAA combinations, which became available in 2013. Both are no longer manufactured in the United States. Additional first-generation protease inhibitors active against a broader range of viruses and administered once daily were approved as part of DAA combination therapies. For example, simeprevir was approved for treatment in genotype 1 infection in combination with an NS5B polymerase inhibitor (sofosbuvir; see later); grazoprevir was approved for the treatment of genotype 1 and 4 infection in combination with elbasvir (an NS5A inhibitor); and paritaprevir was approved for the treatment of genotype 1 infection in combination with ombitasvir (NS5A inhibitor) and dasabuvir (NS5B nonnucleoside inhibitor) with or without ribavirin, depending on the genotype, and for the treatment of genotype 4 infection in combination with ombitasvir and ribavirin.[251,787,788] The limitation of these first-wave protease inhibitors was primarily the lack of pangenotypic activity, low genetic barrier to resistance, and safety in patients with severe liver disease, particularly decompensated disease.

Second-generation NS3/4 protease inhibitors glecaprevir and voxilaprevir are both pangenotypic, have higher genetic barriers to resistance (particularly glecaprevir), and are safer in patients with cirrhosis, although both remain contraindicated in patients with decompensated cirrhosis. Glecaprevir is approved in combination with pibrentasvir (NS5A inhibitor) and is active versus RASs that may develop from exposure to first-generation protease inhibitors including substitutions at the 80, 155, and 168 positions.[789] Voxilaprevir is approved in combination with sofosbuvir (NS5B polymerase inhibitor) and velpatasvir (NS5A inhibitor) and similarly remains active versus the 155 and 168 substitutions.[790,791] Although the Q80K RAS does not confer an increase in 50% effective concentration (EC_{50}) to voxilaprevir, it was associated with lower response rates to 8 weeks of sofosbuvir-velpatasvir-voxilaprevir in genotype 1a infection.[790,792]

Nucleos(t)ide and Nonnucleoside Polymerase Inhibitors

The NS5B polymerase activity can be inhibited by nucleoside and nucleotide substrates that incorporate into the growing RNA chain and terminate elongation. For example, sofosbuvir is a chain terminator prodrug with broad pangenotypic activity and received initial FDA approval for once-daily administration in December 2013. It is now most commonly used as a component of several fixed-dose combination therapies.[793] Additional nonnucleoside compounds bind to allosteric sites in the palm or thumb regions of the enzyme and induce conformational changes that diminish enzymatic activity. Nonnucleoside inhibitors have a narrower genotypic activity range than the nucleoside or nucleotide inhibitors and a lower barrier to resistance (see "Antiviral Resistance"). The only approved nonnucleoside polymerase inhibitor is dasabuvir, a palm region substrate, which as described earlier is approved in combination with paritaprevir (NS3/4 protease inhibitor) and ombitasvir (NS5A inhibitor) for the treatment of genotype 1 infection.

NS5A Protein Inhibitors

As described earlier, NS5A is a nonenzymatic phosphoprotein required for RNA replication and assembly of infectious particles. The protein has no human analogue, and disruption of its function results in potent suppression of replication. As a class, NS5A inhibitors are pangenotypic but have a low genetic barrier to resistance. First-generation NS5A inhibitors were approved as part of DAA combination therapies in 2014, 2015, and 2016. Ombitasvir and ledipasvir were both approved in 2014 as fixed-dose combination regimens. Ombitasvir is approved as part of a triple–mechanism-of-action regimen with an NS3/4 protease inhibitor (paritaprevir) and a nonnucleoside NS5B palm region polymerase inhibitor for the treatment of genotype 1 infection and as part of a dual–mechanism-of-action regimen with paritaprevir for the treatment of genotype 4 infection.[794,795] In the case of genotype 1a and

genotype 4 infection, ribavirin is also required as part of the treatment regimen. Ledipasvir is approved as part of a fixed-dose combination regimen with sofosbuvir (NS5B nucleoside polymerase inhibitor) in genotypes 1, 4, 5, or 6 infection.[796] Daclatasvir was approved in 2015 as part of a combination therapy with sofosbuvir in the treatment of genotype 1 or 3 infection.[797] Although daclatasvir has activity versus genotypes 1 to 6, there was insufficient data for the FDA to provide a broader pangenotypic indication. Elbasvir was approved in 2016 in combination with grazoprevir (NS3/4 protease inhibitor) for the treatment of genotype 1 or 4 infection.[798]

In genotype 1a infection, substitutions at amino-acid positions 28, 30, 31, and 93 confer high-grade resistance to elbasvir; thus baseline resistance testing is recommended by the FDA before use of this combination regimen for the treatment of genotype 1a infection.

Velpatasvir is not a true next-generation NS5A inhibitor because the Y93H substitution still confers high-fold resistance, but it does retain activity versus other first-generation RASs.[799] Velpatasvir, in a fixed-dose combination with sofosbuvir, was the first FDA-approved pangenotypic regimen.[800] Velpatasvir is also approved as part of a pangenotypic, triple-mechanistic, fixed-dose combination therapy with sofosbuvir and voxilaprevir (NS3/4 protease inhibitor) for the salvage of patients in whom prior DAA therapies have failed.[791] Pibrentasvir, a second-generation NS5A inhibitor, is approved as a pangenotypic, fixed-dose combination formulation with glecaprevir (NS3/4 protease inhibitor). In vitro pibrentasvir has the highest barrier to resistance of the NS5A inhibitors and retains activity against all common single-position NS5A RASs in all HCV genotypes (<10-fold shift in EC_{50}).[801]

Treatment of Chronic Hepatitis C

History and Progress (Fig. 154.9)

The first large-scale clinical trials of IFN-α compared 3 million units of IFN-α2b given subcutaneously three times a week for 6 months versus placebo and achieved SVR rates less than 15%.[802,803] Extension of the length of therapy to 12 to 18 months improved the SVR rates to 20% to 30%.[804] Higher SVR rates have been achieved with the pegylated (PEG) IFN-α products compared with standard IFN-α, and even higher rates when PEG IFN-α was combined with oral ribavirin given twice daily.[805–808] In 2011, boceprevir and telaprevir were approved for use together with PEG IFN-α and ribavirin for genotype 1 chronic HCV infection and provided a significant increase in SVR rates to 80% to 90% when response-guided treatment based on on-treatment viral kinetics was incorporated into the medical decision making for futility versus continuation of therapy (see "Virologic Responses" earlier).[786] However, these regimens continued to carry significant adverse event profiles and prolonged courses of therapy. Owing to the adverse event profiles and the need for IFN and ribavirin, these regimens remained inaccessible for many special patient populations including patients with psychiatric disease, decompensated liver disease, chronic kidney disease and end-stage renal disease, children and adolescents, and patients with organ transplants.

The first all-oral DAA regimen, sofosbuvir in combination with ribavirin, was approved for use in genotype 2 and 3 infections.[797,798,809] Although this regimen included only a single DAA, it increased SVR rates to 97% with 12 weeks of therapy in genotype 2 infection and 84% with 24 weeks of therapy in genotype 3 infection.[810,811] Sofosbuvir was also approved in patients with HIV infection and in patients with HCC awaiting liver transplant. Owing to the lack of IFN, this regimen was also explored for the first time in patients with decompensated cirrhosis and in those with liver transplants.[812] In addition, phase II studies of sofosbuvir with a next-in-class, first-generation NS3/4 protease inhibitor, simeprevir, or with a first-in-class NS5A inhibitor, daclatasvir, provided early insights into the true potential of potent DAA combinations.[813,814] The regimens would later be approved by the FDA for the treatment of genotype 1 infection and genotype 3 infection, respectively.[797,815] From 2014 through 2017, a total of six fixed-dose combination DAA regimens were approved by the FDA. These approvals included the first approved therapies for patients with decompensated liver disease, with liver and kidney transplants, or with chronic kidney disease and end-stage renal disease and for adolescents, and would shepherd in pan-genotypic regimens and next-generation regimens that could be used to salvage patients in whom other DAA regimens had failed.[791,796,798,800,816] In 2018, we reached the pinnacle of HCV therapeutics; with approved regimens offering >95% SVR for the vast majority of patients and options for salvage, there is unlikely to be a significant change in the approach to or recommended regimens for chronic HCV infection.

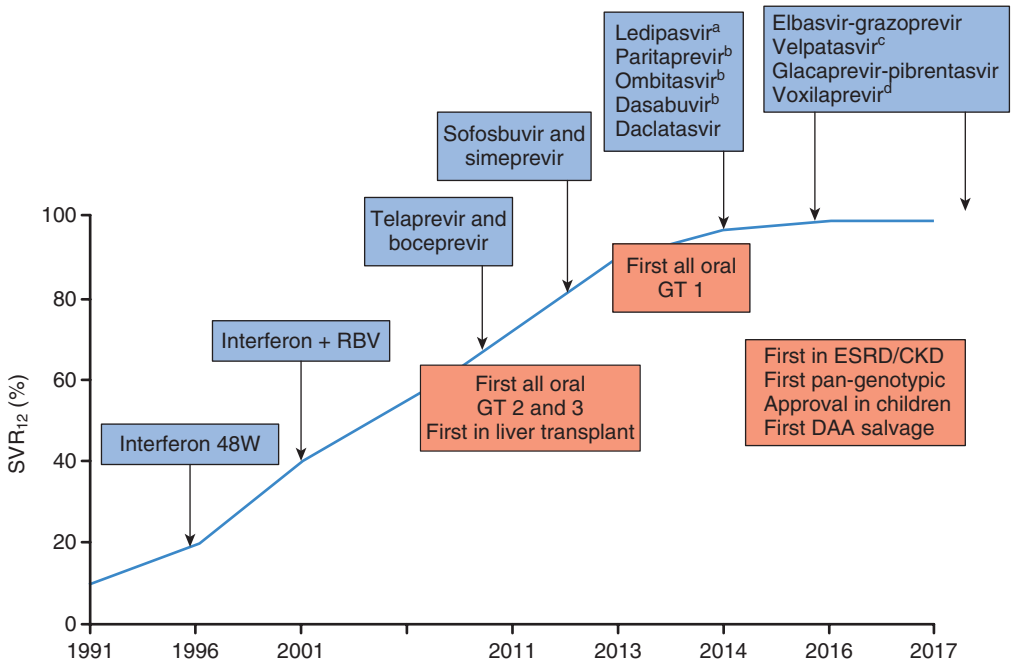

FIG. 154.9 Timeline of hepatitis C virus *(HCV)* therapeutic approaches indicating approval of new agents and approximate sustained virologic response (SVR_{12}). [a] Ledipasvir administered as fixed-dose combination with sofosbuvir. [b] Administered as a fixed-dose combination of paritaprevir/ritonavir/ombitasvir dosed with dasabuvir. [c] Velpatasvir administered as fixed-dose combination with sofosbuvir. [d] Voxilaprevir administered as fixed-dose combination with velpatasvir and sofosbuvir. From 2014 to 2017, multiple new HCV antiviral agents and fixed-dose combination treatment regimens were approved, revolutionizing the treatment of hepatitis C virus infection. *(Figure provided by Dr. Naggie.)*

Initial Treatment of Chronic Hepatitis C Virus Infection

Initial treatment of chronic HCV infection includes patients who have not been previously treated with any HCV antiviral regimen, including prior IFN-containing regimens. All recommended treatments for HCV infection include fixed-dose combinations of DAAs, and neither the AASLD/IDSA nor the European Association for the Study of the Liver (EASL) HCV treatment guidelines recommend IFN-containing regimens.[817–819] There are few recommended regimens that include ribavirin, and these are for use primarily in select, very difficult-to-treat populations (see "Response Indicators").

Choosing the most appropriate initial treatment DAA regimen includes considering the genotype and subtype (although there are examples where this may not be necessary), baseline HCV RNA, presence of cirrhosis, and, if the patient has cirrhosis, the Child-Turcotte-Pugh score, presence of renal dysfunction, and concomitant medications in order to manage drug interactions. All of these baseline characteristics will aid in selecting the safest DAA regimen and the appropriate length of therapy. In addition, owing to rapid realization that IFN-free therapies were significantly more efficacious and safe, the FDA allowed registration trials to include single-arm intervention studies with efficacy analyses in which a historical control was used. This resulted in a limited number of head-to-head comparisons of the currently available regimens.

Genotype 1

There are currently four recommended DAA regimens for the initial treatment of genotype 1 infection. There are minimal differences in approach to initial therapy for subtype 1a versus 1b, with the primary difference relevant only to the use of the elbasvir-grazoprevir regimen, a single-tablet fixed-dose combination regimen of NS5A and NS3/4 inhibitors. The phase III C-EDGE trial enrolled treatment-naïve patients, 91% with genotype 1 infection and 22% with cirrhosis, and randomized them to immediate or deferred 12-week treatment arms.[820] The SVR^{12} for patients with genotype 1a infection was 92% and with genotype 1b infection was 99%. Virologic failure was associated with baseline NS5A substitutions and emergent NS3 and/or NS5A variants (see also "Antiviral Resistance"). Based on these data, the FDA recommended baseline NS5A genotype testing before use of elbasvir-grazoprevir in patients with genotype 1a infection, and when NS5A substitutions associated with high-fold resistance are identified, recommendations include extension of therapy to 16 weeks plus the addition of ribavirin. The AASLD/IDSA HCV guidance similarly recommends baseline RAS testing for patients with genotype 1a infection, but owing to the longer course of therapy and the need for ribavirin, the 16-week regimen is listed as an alternative treatment option.

Otherwise, the treatment approach for the other three recommended regimens is the same across subtypes. Glecaprevir-pibrentasvir, a three-tablet fixed-dose combination of NS3/4 and NS5A inhibitors, is approved as an 8-week regimen in patients without cirrhosis and a 12-week regimen in patients with cirrhosis. The phase III ENDURANCE-1 trial randomized patients without cirrhosis and with genotype 1 infection to 8 or 12 weeks of therapy; the SVR_{12} in both arms was 99%.[592] The phase III EXPEDITION-1 single-arm, open-label trial investigated 12 weeks of glecaprevir-pibrentasvir in patients with chronic HCV infection (60% genotype 1) and cirrhosis; the SVR_{12} was 99%.[821] Ledipasvir-sofosbuvir, a single-tablet fixed-dose combination of NS5A and NS5B inhibitors, is approved as a 12-week regimen for initial treatment in patients with or without cirrhosis.[795,796] The phase III ION-1 trial randomized treatment-naïve patients with genotype 1 infection (16% with cirrhosis) to four different treatment arms: ledipasvir-sofosbuvir for 12 weeks, ledipasvir-sofosbuvir plus ribavirin for 12 weeks, ledipasvir-sofosbuvir for 24 weeks, and ledipasvir-sofosbuvir plus ribavirin for 24 weeks.[822] The SVR_{12} was 99%, 97%, 98%, and 99%, respectively, confirming the efficacy of the 12-week regimen. The phase III ION-3 study randomized treatment-naïve patients with genotype 1 infection and without cirrhosis to 8 versus 12 weeks of therapy.[823] Although the 8-week duration of therapy was noninferior to the 12-week duration, the 8-week arm with ledipasvir-sofosbuvir had a higher relapse rate (5%) versus the 12-week arm with ledipasvir-sofosbuvir (1%). Based on a post hoc analysis, a baseline HCV RNA of 6 million IU/mL was identified as a threshold under which the relapse rate in the 8-week arm was the same as in the 12-week arm (2%), whereas above this threshold it was much higher (10% versus 1%). Thus, shortening to 8 weeks of ledipasvir-sofosbuvir is an option for treatment-naïve patients without cirrhosis and with a baseline HCV RNA <6 million IU/mL.

Sofosbuvir-velpatasvir, a single-tablet fixed-dose combination of NS5B and NS5A inhibitors, is approved as a 12-week regimen for the treatment of chronic genotype 1 to 6 HCV infection with or without cirrhosis.[800] The phase III ASTRAL-1 double-blind randomized trial enrolled patients (19% cirrhosis) with genotype 1, 2, 4, or 6 infection to 12 weeks of sofosbuvir-velpatasvir versus placebo and enrolled patients with genotype 5 infection to 12 weeks of sofosbuvir-velpatasvir.[591] The overall SVR_{12} was 99%, with two relapses in patients with genotype 1.

Although there are other FDA-approved regimens for genotype 1, including sofosbuvir plus simeprevir and the fixed-dose combination ombitasvir-paritaprevir-ribavirin with dasabuvir (a nonnucleoside NS5B polymerase inhibitor), these are no longer recommended regimens owing to pill burden, side-effect profile, drug interaction risk, or some combination of these.

Genotype 2

There are two recommended regimens for the treatment of genotype 2 infection: glecaprevir-pibrentasvir and sofosbuvir-velpatasvir.[819] Both are pangenotypic regimens; the efficacy of either is ≥99%. The phase III ENDURANCE-2 double-blind, placebo-controlled trial randomized patients without cirrhosis and with genotype 2 infection to 12 weeks of therapy versus placebo and found a 99% SVR_{12}.[824] The phase II SURVEYOR-II, part 4 study was a single-arm study that investigated a shortened 8-week course of therapy among patients without cirrhosis and with genotype 2, 4, 5, or 6 infection. Among the treatment-naïve patients with genotype 2 infection, the SVR_{12} was 99%. For patients with genotype 2 infection, glecaprevir-pibrentasvir is recommended for 8 weeks in the absence of cirrhosis and 12 weeks when cirrhosis is present.[824] The phase III ASTRAL-2 study randomized patients with genotype 2 infection with or without cirrhosis to 12 weeks of sofosbuvir-velpatasvir or 12 weeks of sofosbuvir plus ribavirin, which was one of the previously recommended regimens.[825] The study showed superior efficacy of sofosbuvir-velpatasvir versus the comparator (SVR_{12}, 99% vs. 94%, respectively). The phase III ASTRAL-1 study also enrolled patients with genotype 2 infection with and without cirrhosis to receive 12 weeks of sofosbuvir-velpatasvir and found a 100% SVR_{12}.[591] For patients with genotype 2 infection, sofosbuvir-velpatasvir is recommended for 12 weeks regardless of the presence of cirrhosis.

Genotype 3

Two regimens are recommended for the treatment of genotype 3 infection: glecaprevir-pibrentasvir and sofosbuvir-velpatasvir. For treatment-naïve patients with genotype 3 infection and cirrhosis, there is a recommendation to check for NS5A RAS at baseline before starting therapy, when considering sofosbuvir-velpatasvir (discussed in more detail later). Phase III ENDURANCE-3 was a randomized trial comparing 12 weeks of glecaprevir-pibrentasvir versus 12 weeks of sofosbuvir plus daclatasvir (NS5A inhibitor), the prior recommended regimen, in treatment of patients with genotype 3 infection and without cirrhosis.[592] An 8-week arm of glecaprevir-pibrentasvir was later added as an amendment to the study. Both glecaprevir-pibrentasvir arms met noninferiority as compared with the comparator standard-of-care arm, with SVR_{12} of 95% for both versus 97%, respectively. For patients with genotype 3 infection, glecaprevir-pibrentasvir is recommended for 8 weeks in the absence of cirrhosis and for 12 weeks when cirrhosis is present. The phase III ASTRAL-3 study randomized patients with genotype 3 infection with or without cirrhosis to 12 weeks of sofosbuvir-velpatasvir or 24 weeks of sofosbuvir plus ribavirin, which was one of the previously recommended regimens.[825] The study showed superior efficacy of sofosbuvir-velpatasvir versus the comparator (SVR_{12} 95% vs. 80%). Of the 250 patients in the ASTRAL-3 study with cirrhosis, 16% had baseline NS5A RASs. Patients with baseline RASs had a lower SVR_{12} (88%) than did those without RASs (97%). This lower SVR_{12} was primarily driven by patients with the Y93H RAS at baseline. Currently, the guidelines recommend use of the

glecaprevir-pibrentasvir regimen or an alternative regimen, sofosbuvir-velpatasvir-voxilaprevir, if the Y93H RAS is present and the patient has cirrhosis. Sofosbuvir-velpatasvir-voxilaprevir is a triple-mechanism targeted, single-tablet, fixed-dose combination regimen that is approved by the FDA for the treatment of prior DAA failures.[791] Although it is approved by the FDA only for treatment of DAA failures, the phase III POLARIS-3 study randomized treatment-naïve patients with genotype 3 infection and cirrhosis to 8 weeks of sofosbuvir-velpatasvir-voxilaprevir versus sofosbuvir-velpatasvir for 12 weeks.[792] The SVR_{12} was 96% in both arms. Accordingly, this regimen is recommended as an alternative regimen to 12 weeks of sofosbuvir-velpatasvir when the Y93H RAS is present in patients with cirrhosis, because the Y93H RAS does not appear to affect the treatment response with the triple-drug regimen.

Genotype 4

The same four regimens recommended for genotype 1 infection are also recommended for genotype 4 infection, with only minor differences. One difference is that NS5A RASs do not affect the treatment response for elbasvir-grazoprevir in genotype 4 infection, and RAS testing at baseline is not recommended; therefore, 12 weeks of elbasvir-grazoprevir is recommended for genotype 4 infection regardless of presence of cirrhosis. Also, with genotype 1 infection there is the option to shorten ledipasvir-sofosbuvir to 8 weeks in select patients, but this is not an option for genotype 4 infection; therefore, 12 weeks of ledipasvir-sofosbuvir is recommended regardless of presence of cirrhosis. For the glecaprevir-pibrentasvir regimen, 8 weeks of therapy is recommended for patients without cirrhosis and 12 weeks is recommended for those with cirrhosis. Last, for sofosbuvir-velpatasvir, 12 weeks of therapy is recommended regardless of presence of cirrhosis.

The studies supporting the efficacy of these regimens in genotype 4 infection are primarily the same as those supporting the efficacy in genotype 1 because most studies included both genotypes. For sofosbuvir-velpatasvir, the ASTRAL-1 study included 64 patients with genotype 4 infection, with and without cirrhosis, and the SVR_{12} was 100%.[591] Another 57 patients with genotype 4 infection were enrolled as part of the sofosbuvir-velpatasvir comparator arm in the POLARIS-2 study, and SVR_{12} was 98%.[792] Both studies support the use of sofosbuvir-velpatasvir for 12 weeks in genotype 4 infection regardless of presence of cirrhosis. ENDURANCE-4 enrolled 76 patients with genotype 4 infection without cirrhosis to 12 weeks of glecaprevir-pibrentasvir and achieved 99% SVR_{12}.[824] In addition, SURVEYOR-2 part 4 investigated 8 weeks of glecaprevir-pibrentasvir in patients without cirrhosis, and 46 patients with genotype 4 infection were included; the SVR_{12} was 93%.[824] EXPEDITION-1 included 16 patients with genotype 4 infection and cirrhosis; all received 12 weeks of therapy, and all achieved SVR_{12}. These three studies support the recommendation of 8 weeks of glecaprevir-pibrentasvir in patients without cirrhosis and 12 weeks in patients with cirrhosis. Meanwhile, a pooled analysis of the phase II/III registration program found that a total of 101 patients with genotype 4 infection were treated with 12 weeks of elbasvir-grazoprevir and that the SVR_{12} was 96%.[826] Two small pilot studies support the use of ledipasvir-sofosbuvir for 12 weeks in patients with genotype 4 infection; the SYNERGY trial was an open-label study of 21 patients, and a second single-arm study enrolled 22 patients with genotype 4 infection.[827,828]

Genotypes 5 and 6

Three DAA regimens are recommended for treatment of patients with genotype 5 or 6 infection: glecaprevir-pibrentasvir, sofosbuvir-velpatasvir, and sofosbuvir-ledipasvir.[819] Because of the lower prevalence of these genotypes in North America and Western Europe, where many registration trials take place, the numbers of patients enrolled are limited. The studies supporting these regimens overlap with those supporting genotype 1 and 4 infection, as often the studies have enrolled across multiple genotypes. The approach to treatment is also similar to that for genotype 4, with glecaprevir-pibrentasvir recommended for 8 weeks in patients without cirrhosis and 12 weeks in patients with cirrhosis, and both sofosbuvir-velpatasvir and ledipasvir-sofosbuvir recommended for 12 weeks regardless of presence of cirrhosis. Across the registration program for glecaprevir-pibrentasvir, 30 patients with genotype 5 infection were treated, including 2 without cirrhosis who received therapy for 8 weeks and 2 with cirrhosis for 12 weeks; all patients achieved SVR_{12}.[824] Similarly, 36 patients with genotype 6 were treated across the same studies, including 10 without cirrhosis who received therapy for 8 weeks and 7 with cirrhosis for 12 weeks; SVR_{12} was 97% and there were no virologic failures.[824] Across clinical trials that included sofosbuvir-velpatasvir (ASTRAL-1 and POLARIS-2), 24 patients with genotype 5 infection received 12 weeks of the regimen and 96% achieved SVR_{12}. Similarly, 47 patients with genotype 6 infection received 12 weeks of the regimen and all patients achieved SVR_{12}.[591,792] For ledipasvir-sofosbuvir, several small pilot studies have supported the efficacy of 12 weeks of this regimen in patients with genotype 5 or 6.[829,830]

Response Indicators

Historically, the likelihood of SVR could be predicted according to viral and host factors before treatment. Aggregate response rates for almost all populations and genotypes are sufficiently high with current DAA regimens that response indicators are of significantly less value. Even in the presence of multiple negative predictors, response rates are sufficiently high that in no population would therapy be withheld; however, in the increasingly rare situation in which several of the most predictive factors are present, there may still be room for treatment optimization. In the DAA era, baseline predictors that can play a role in treatment response when present in combination with others include genotype, presence of cirrhosis (particularly decompensated), prior treatment experience (primarily although not entirely DAA experience), and presence of RASs (primarily but not entirely NS5A).

One of the most important predictors of the response to IFN-α–based HCV treatment was the viral genotype (Fig. 154.10). The lowest SVR rates were consistently reported in patients who had genotype 1 or 4 HCV infections; genotypes 3 and 5 were intermediate, and genotypes 2 and 6 were most responsive.[831–835] DAAs have now mostly leveled the playing field, although genotype 3 infection, particularly in patients in whom HCV therapy previously failed and/or who have cirrhosis, has emerged as a standout. This may at least in part be due to the higher rates of steatosis in genotype 3 infection, which was known to affect

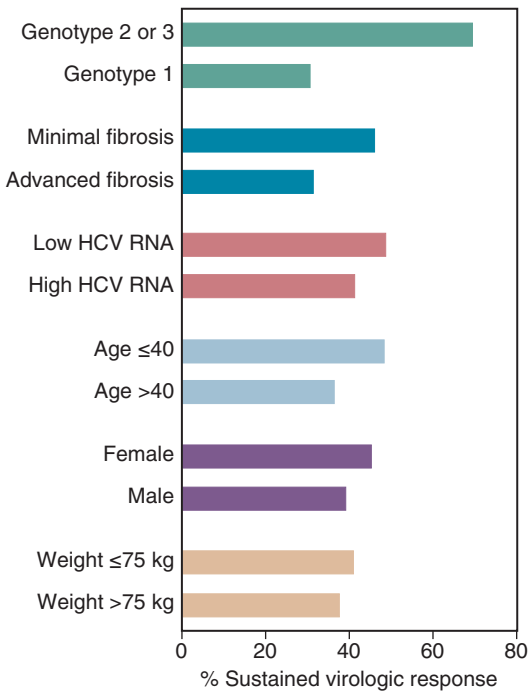

FIG. 154.10 Sustained virologic response rates in persons taking peginterferon and ribavirin according to duration of treatment and ribavirin dose, stratified by hepatitis C virus *(HCV)* genotype and HCV RNA level. *(From Hadziyannis SJ, Sette H Jr, Morgan TR, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose.* Ann Intern Med. *2004;140:346–355.)*

treatment response in the IFN era, and the higher prevalence of naturally occurring NS5A RAS.[592,825]

The amount of HCV detected in the blood before treatment, the baseline HCV viral load, was also predictive of responsiveness to PEG IFN and ribavirin.[831,832] As previously discussed (see "Initial Treatment," "Genotype 1"), baseline HCV RNA may still be predictive of treatment response with some DAA regimens, although this primarily occurs with attempts to shorten therapy.

In addition to viral factors, there are several important host determinants. Interesting to note, as with spontaneous resolution of infection (see "Viral Persistence" earlier), the strongest host determinant of response to PEG IFN and ribavirin is the patient's DNA sequence near *IL28B,* the gene for λ-IFN 3. With use of a commercially available test (the C vs. T allele for rs12979860), response rates for persons with genotype 1 chronic HCV infection who were treated with PEG IFN and ribavirin were 69%, 33%, and 27% in Caucasians who had the CC, CT, and TT genotypes, respectively; among black patients, SVR rates were 48%, 15%, and 13% for CC, CT, and TT genotypes, respectively.[836] However, the predictive value of *IL28B* genotype diminished significantly with combination DAA therapy and is no longer used clinically in the setting of chronic HCV infection. Yet, there are examples that highlight the potential role, albeit small, of the host immune response in DAA-mediated clearance. Both examples include studies of DAA therapies that attempted to shorten the course of therapy from 12 weeks, a duration in which high efficacy was noted, to 8 weeks. The ION-3 study, as discussed previously (see "Initial Treatment," "Genotype 1"), randomized treatment-naïve patients with genotype 1 infection and without cirrhosis to 8 versus 12 weeks of therapy.[823] Although the 8-week duration of therapy was noninferior to the 12-week duration, the 8-week arm with ledipasvir-sofosbuvir had a higher relapse rate (5%). A post hoc multivariable analysis assessed predictors of SVR and identified female sex and *IL28B* favorable CC genotype as associated with treatment response. In addition, from pooled data of the ledipasvir-sofosbuvir program, baseline NS5A resistance to ledipasvir is also predictive of relapse in patients receiving 8 weeks of therapy.[837,838]

Cirrhosis remains a host predictor that increases the risk for treatment failure; hence there are differences in the treatment approach with some regimens in patients with cirrhosis. Although DAAs have significantly improved the SVR rates in patients with compensated cirrhosis, patients with decompensated cirrhosis have a lower SVR.[782,783] Because of the lower SVR in patients with decompensated cirrhosis, studies investigated the benefit of adding ribavirin to the DAA regimen and consistently showed higher SVR; therefore regimens for decompensated cirrhosis include ribavirin. Cirrhosis appears to be especially important with genotype 3 infection.[825,832,839,840] The reason for the higher relapse rate in patients with cirrhosis, especially decompensated cirrhosis, is unclear but may be related to penetration of fibrotic nodules, which may increase the risk of RAS development. In addition, historically, HIV infection was associated with a lower treatment response rate, but this is no longer true. With combination DAA therapies, multiple phase III trials have found a similar efficacy and safety of DAA regimens in patients with HIV infection.[841–844] Thus, guidelines recommend treating patients with HIV infection the same as those without HIV infection, with attention to the potential for drug interactions, which now is the only clinically relevant difference when treating patients with HIV infection.

Antiviral Resistance

Antiviral resistance is a new clinical consideration directly related to the use of DAA agents. From a virologic perspective, the potential for development of resistance in HCV is greater than in HIV, and mathematical modeling studies accounting for daily virion production and the error-prone nature of the HCV RNA-dependent RNA polymerase (RdRP) suggested that all possible single- and double-position resistant variants are generated daily in chronically infected individuals.[845] Clinical data with the first-generation NS3 protease inhibitors seemed to validate these concerns and demonstrated that high-level resistance was selected within 1 to 2 weeks with monotherapy, and most patients (60%–80%) in whom IFN-based therapy with boceprevir or telaprevir failed were found to have genotypic portease inhibitor (PI) resistance.[283,846–850] However, several phenomena have converged to transform the major clinical impact of antiviral resistance to only a modest effect in select groups of patients (see treatment section). Those factors are (1) the rapid pace of drug development, with approval of multiple highly potent DAAs with increasingly high barriers to resistance; (2) the widespread use of combination therapy, including the availability of regimens in which three drug classes are used, and (3) in stark contrast to HIV and HBV, the lack of a long-lived cellular reservoir or integrated form.

The key populations or clinical scenarios in which antiviral resistance appears to have a detrimental impact on responses to DAA therapy include genotype 1a infection (treatment-naïve and treatment-experienced patients) with elbasvir-grazoprevir and genotype 1a infection in treatment-experienced patients with ledipasvir-sofosbuvir; genotype 3 infection in treatment-experienced patients and/or in the presence of cirrhosis with sofosbuvir-velpatasvir and possibly with shortened glecaprevir-pibrentasvir therapy (8 weeks); and previously DAA-treated patients, particularly those exposed to NS5A inhibitors with genotype 1a or 3. Important to note, in many of these scenarios the occurrence is sufficiently rare or the absolute decrease in SVR is small enough (combined with lack of statistical significance) that routine testing or changes in clinical management may not be recommended (see treatment section).

Testing for genotypic resistance is widely available in the United States, with several commercial assays available for the NS3, NS5A, and NS5B regions. Testing approaches vary in terms of how the sequencing is carried out and include Sanger sequencing (also known as population sequencing) and ultra-deep sequencing with a detection threshold set at 10% for reporting variants. Correlative clinical data suggest that detection levels in the range of those expected for population sequencing (15%–25%) enable identification of the vast majority of clinically significant RASs and avoid "overdetection" of RASs present at low levels (1%–5% of the virus quasispecies) that do not appear to affect clinical outcomes.

The NS3 protease inhibitors were the first class of DAAs used in the clinic; however, currently available agents are quite different with regard to structure and resistance profile. Telaprevir and boceprevir are linear ketoamide inhibitors of the HCV protease and readily selected for resistance if therapy was unsuccessful. The major clinical resistance mutation seen was the R155K variant in genotype 1a.[849,851] This variant was almost exclusively seen in genotype 1a owing to a lower barrier to resistance resulting from different codon usage in 1a versus 1b, which required only a single nucleotide transition in 1a (AGG to AAG) versus a two-nucleotide change in 1b (CGG to AAG) including a transversion change.[851,852] Although of negligible clinical impact currently, efforts to develop protease inhibitors active in vitro against R155K spurred the development of modern, highly potent protease inhibitors (grazoprevir, glecaprevir, and voxilaprevir) that retain wild-type activity against this variant in vitro.[789,790,853] The resistance barrier of early protease inhibitors was significantly higher in genotype 1b, although variants at positions V36, T54, V55, A156, and V170 were selected.[849,851]

The enhanced activity of modern HCV protease inhibitors has significantly lessened the impact of resistance for this class of agents. First, there are no commonly occurring baseline polymorphisms (variants found in the absence of drug selective pressure) that have been demonstrated to have a clinically significant impact on responses with current DAA protease inhibitor–containing regimens. The most prevalent NS3 polymorphism is the Q80K variant in genotype 1a, which is found in around 40% of clade I genotype 1a isolates.[854,855] In vitro, it results in low-level resistance to simeprevir (7–10×) and paritaprevir (3×); clinically, it adversely affects responses to simeprevir plus IFN and simeprevir plus sofosbuvir when used for short durations (8 weeks) or in those with cirrhosis treated for 12 weeks.[856–860] The Q80K variant does not affect the antiviral activity of grazoprevir, glecaprevir, or voxilaprevir in vitro.[789,790,853]

On virologic failure with grazoprevir- or glecaprevir-containing regimens, the most commonly selected NS3 RAVs include Y56H/N, Q80R, A156G/T/V, and D168A/E (and other D168 variants) in genotype 1 and Y56H and Q168R in genotype 3.[861–863] Resistance selection during a 3-day monotherapy trial with voxilaprevir suggested a similar resistance profile.[790] In vitro high-level resistance to all current NS3 PIs is expected for A156G/T/V variants, although testing is difficult given the very poor replicative fitness of these variants.[789] D168 variants are more variable in their in vitro impact, with most conferring ≤20× shifts in EC_{50} to glecaprevir and voxilaprevir.[789,790] Despite selection with failure of therapy and demonstrable impact in vitro on antiviral activity, there

is no clear role for NS3 resistance testing after DAA failure with or without a PI. In the setting in which a two-drug regimen is attempted for re-treatment after DAA failure, resistance in NS3 along with NS5A does predict a poor response to re-treatment with glecaprevir plus pibrentasvir and is not recommended.[863,864] However, this determination is typically made on clinical grounds rather than viral resistance genotype sequencing. In the case of three-drug re-treatment regimens, NS3 PI resistance does not appear to impair responses.[865]

NS5A inhibitor resistance, both preexisting and selected, has the greatest impact on DAA treatment outcomes and is the area where clinical resistance testing is advocated for select populations. Naturally occurring NS5A RASs of potential clinical significance are found with relative frequency in both genotype 1 and genotype 3. In a survey of over 2000 patients infected with genotype 1, baseline NS5A class RASs (conferring >2.5× shift in EC_{50} to any NS5A inhibitor) were found in 15%.[837] A second study refined these estimates, looking at RAS prevalence based on GT 1 subtype and detection threshold.[838] Although NS5A RASs are more prevalent in genotype 1b (18%) as compared with genotype 1a (13%), they do not have clinical significance for genotype 1b in the non–DAA-exposed patient. Specific RASs of interested in genotype 1a include the Y93H and Q30H variants, which were each found in 1% to 2% of sequences.[837] The Y93H variant in genotype 1a confers high-level resistance to all NS5A inhibitors except pibrentasvir.[801,866] The Q30H variant confers moderate to high-level resistance to all NS5A inhibitors except velpatasvir and pribrentasvir.[801,866] Interesting to note, the Y93H variant is found in 9% of genotype 1b sequences yet does not appear to have a large clinical impact on treatment outcomes.[837,838] Excluding the M28V variant, which is frequently found (5%–6% of genotype 1a sequences) but does not confer resistance in vitro to ledipasvir (LDV), elbasvir (EBR), velpatasvir (VEL), or pibrentasvir (PIB), shifts the baseline prevalence of NS5A RASs in genotype 1a to <10%.[801,866–868] This is of particular importance for elbasvir, for which baseline testing is recommended in genotype 1a before treatment; although the label does not distinguish based on the specific M28 RAS detected, guidelines do not recommend extending therapy or adding RBV if the M28V variant is identified.[797,798,819]

In post hoc analyses of registrational clinical trial programs, baseline NS5A RASs have a demonstrable negative impact on treatment outcomes with LDV-SOF and EBR-GZR. In a pooled analysis of genotype 1 patients treated in phase II/III trials with LDV-SOF for 6 to 24 weeks with or without RBV, baseline RASs were associated with a significantly lower SVR_{12} of 93.5% (316 of 338) compared with 98.4% (1741 of 1770) without NS5A RASs ($P < .001$).[837] The effect of RASs was most pronounced in treatment-experienced patients treated for 12 weeks who harbored LDV RASs that conferred high-level resistance (>100×; SVR 64.7%, 11 of 17). Addition of RBV resulted in a numeric but not statistically significant increase in SVR_{12} in those treated for 12 weeks. A second global analysis of LDV-SOF–treated patients expanded on these findings by looking at the impact of RASs by genotype subtype only in those treated according to guideline recommendations.[867] Consistent with the prior study, the only group in which baseline RAS significantly affected SVR was treatment-experienced patients with genotype 1a infection (88% vs. 98% SVR). High-level resistance (>100×) in this group portended worse outcomes, with only 70% SVR (16 of 23). Despite these data, firm management recommendation cannot be made for the use of LDV-SOF in the setting of NS5A RASs, given the relatively small number of patients,the contribution of other factors (e.g., stage of liver disease), and lack of randomized data to guide alternative treatment approaches. Guidelines do advocate for NS5A resistance testing in genotype 1a treatment-experienced patients with cirrhosis in whom LDV-SOF is being considered.[819] If NS5A RASs, particularly those conferring high-level LDV resistance, are identified, alternative treatment regimens are recommended.

Baseline NS5A RASs also affect outcomes for EBR-GZR in genotype 1a infection. An integrated analysis across phase II/III EBR-GZR studies demonstrated lower SVR rates in genotype 1a patients with baseline EBR-specific RASs: 52% (16 of 31) with RASs versus 98% (464 of 475) without EBR RASs.[593] Numerically, the impact was larger in treatment-experienced patients. Two important considerations for these data are that population sequencing was used and M28V was not included because it does not confer resistance to EBR in vitro.[868] Given these considerations, EBR-specific RASs were present in 6% of the population studied. Based on a very limited number of patients ($n = 6$), extension of therapy to 16 to 18 weeks with RBV appeared to abolish the adverse impact of NS5A RASs on response (100% SVR).[797,798] Finally, a multivariate analysis of response factors found only baseline viral load above 800,000 IU/mL and the presence of EBR-RASs to be significant predictors of response in genotype 1a patients treated with EBR-GZR.[594] Based on these data, baseline testing is recommended (see "Initial Treatment of Chronic Hepatitis C" section and Chapter 117).

The NS5A RASs of clinical significance in GT3 are A30K and Y93H. In non–NS5A-exposed patients, each RAS is found in 5% to 10% of GT3 sequences.[592,869,870] In vitro, the A30K confers moderate resistance to DCV (40×) and VEL (50×), whereas little impact is seen with PIB (1.1×).[798,799,801,869] The Y93H RAS confers high-level resistance in vitro (>100×) to both DCV and VEL, but no appreciable shift is seen with PIB (<3.0×).[798,799,801,869] In other genotypes—for example, GT2—polymorphisms such as L31M are found with regularity (approximately 40%), yet do not have clinical impact. Furthermore, sequence-based resistance testing is not commercially available outside genotypes 1 and 3.

The impact of NS5A RASs in patients infected with GT3 not previously exposed to NS5A inhibitors is more nuanced. The presence of baseline NS5A RASs (A30K, L31M, Y93H) resulted in a 93% SVR rate with SOF-VEL (98% without RASs).[870] In addition, response rates were lower in those with the Y93H variant, at 86% (19 of 22). However, response rate is also modified by prior treatment experience and/or the presence of cirrhosis.[824,825] The regimen of SOF-VEL-VOX does not appear to be affected by baseline NS5A resistance in GT3 infection not previously treated with an NS5A inhibitor (100% SVR, 32 of 32).[871,872] For the regimen of GLE-PIB, baseline presence of the A30K RAS has been associated with numerically lower response rates to 8 weeks of treatment in patients without cirrhosis and GT3 infection. In the phase III ENDURANCE-3 study, SVR rate with 8 weeks of GLE-PIB was 75% in those with baseline A30K (12 of 16).[592] An integrated analysis of phase II and III studies did not demonstrate an impact of NS5A RASs on responses with 8 weeks of GLE/PIB in GT3.[873] Notably, all GT3 virologic failures in the integrated analysis came from the phase III ENDURANCE-3 study. Finally, an integrated GT3 analysis that specifically assessed the impact of the A30K variant found a numerically lower SVR rate of 84% (16 of 19) with 8 weeks versus 94% (17 of 18) with 12 weeks of GLE-PIB.[874] Given the small numbers, this difference was not statistically significant. Mechanistically, despite the fact that neither the A30K nor the Y93H variant results in a significant shift in in vitro potency for PIB, the A30K variant lowers the barrier to development of the dual A30K + Y93H variant to a single nucleotide change, and this combination results in a 69-fold decrease in PIB potency in vitro.[801] Conversely, the baseline presence of the Y93H variant in GT3 still requires two nucleotide changes to result in the double variant (A30K + Y93H), and clinically a baseline Y93H variant alone does not appear to affect GLE-PIB in GT3.[592,873,874]

After unsuccessful therapy containing an NS5A inhibitor, genotypic resistance is frequently selected. For instance, after 12 weeks of LDV-SOF therapy, NS5A resistance is selected in 95%.[866] A number of factors contribute to rates of resistance selection including duration and regimen composition. Treatment with highly potent triple-drug regimens containing a nucleoside appear to infrequently select for NS5A resistance (<10%).[871] Long-term follow-up data on persistence of NS5A RASs indicate that the majority (>80%) continue to have detectable resistance mutations for over 3 years despite the lack of a true archiving mechanism in HCV.[875]

The impact of selected NS5A resistance on re-treatment has been significantly mitigated with the arrival of potent double- and triple-drug regimens, some of which are now approved for re-treatment of HCV in the setting of prior DAA exposure.[791,800,816] A clear impact of resistance was seen when a longer duration of the same regimen was used for re-treatment. In a small study of LDV-SOF failures ($N = 41$) re-treatment outcome with LDV-SOF for 24 weeks was determined by baseline resistance. Sustained response rate was 100% in those without resistance and 60% in those with NS5A resistance.[876] In contrast, there is no impact of NS5A resistance on re-treatment efficacy when the triple combination of SOF-VEL-VOX is used. In the phase III POLARIS-1 study, SVR rate

was 100% in genotype 1-infected patients with baseline NS5A RASs (n = 59) and 97% in those with both NS5A and NS3 RASs (n = 71).[865,877]

In the current treatment era, resistance to NS5B polymerase inhibitors, either naturally occurring or selected, does not appear to have a clinically meaningful impact. Dasabuvir is the lone nonnucleoside HCV RNA polymerase inhibitor approved for clinical use. On failure with a dasabuvir-containing regimen, resistance is selected in approximately 60% of patients, with variants S556G/R being most prevalent.[878] Sofosbuvir, the lone NS5B nucleotide inhibitor currently approved for treatment of HCV, is unique among all HCV DAAs in that it has an extremely high barrier to resistance and infrequently selects for resistance after DAA failure. The signature resistance mutation for sofosbuvir is the S282T in NS5B, which in vitro results in a 5-fold to 20-fold increase in EC_{50} depending on additional mutations present.[879,880] Replicative fitness of replicons or viruses harboring the S282T mutation in vitro are significantly diminished (approximately 10% of wild-type). Combined, the modest impact on potency and significant decrease in viral fitness likely explain why resistance is rarely seen in the clinical setting. Multiple baseline sequencing studies have not detected the S282T variant down to a 1% level.[866,880] A combined analysis from phase II and III studies of SOF plus ribavirin with or without IFN found the S282T variant in <1% (1 of 300) of virologic failures.[880] After virologic failure with LDV-SOF, the S282T variant was found in a single patient (2% of sequenced patients).[866] Consistent with its poor replicative fitness, the S282T variant quickly becomes undetectable via sequencing after withdrawal of drug selective pressure.[866,880,881] This appears to occur via reversion to wild-type, as opposed to outgrowth of persistent wild-type populations, as evidenced by altered codon usage for serine at the 282 position after reversion.[866,881] A handful of other mutations have been identified after unsuccessful sofosbuvir-containing therapy, with the two most frequently selected being L159F and V321A. In vitro, these mutations do not result in a significant fold-shift in sofosbuvir activity (1.2-fold to 1.3-fold), and their impact, if any, on responses to SOF-based therapies is unclear. At this time there is no defined role for NS5B genotypic resistance testing either at baseline or after failure of DAA treatment including an NS5B nucleotide.

Re-treatment of Chronic Hepatitis C Virus Infection

Re-treatment of patients in whom prior HCV antiviral therapy has failed falls into several subgroups, including failure of IFN and ribavirin with or without a single DAA (first-generation NS3/4 protease inhibitor), DAA failure without NS5A, and DAA failure with NS5A. Patients in whom failure of IFN and ribavirin with or without a single DAA has occurred are treated in the same manner as treatment-naïve patients, with few exceptions. Patients in whom an all-oral DAA regimen (especially NS5A-inclusive regimens) has failed represent a new and challenging re-treatment population and are potentially difficult to re-treat owing to increased likelihood of resistance (see section on DAA resistance) and enrichment of multiple other traditional negative predictors (male, high BMI, cirrhosis, *IL28B* unfavorable genotype).

Failure of Interferon and Ribavirin With or Without NS3/4 Protease Inhibitor

The re-treatment of patients with genotype 1, 4, 5, or 6 infection who experienced failure of previous treatment with IFN and ribavirin with or without NS3/4 protease inhibitors (NS3/4 protease inhibitors were approved only for genotype 1 infection) is the same as the initial treatment of patients with these genotypes with a few exceptions. The 8-week option for ledipasvir-sofosbuvir is not applicable, because this applies only to patients who have never received therapy for their HCV infection. Furthermore, a meta-analysis of a phase II/III study of ledipasvir-sofosbuvir found that patients with genotype 1 infection who had experienced previous failure of IFN and ribavirin with or without an NS3/4 protease inhibitor and had cirrhosis had a lower SVR when treated for 12 weeks than those who received 12 weeks of therapy with ribavirin or 24 weeks of therapy.[a] The phase II SIRIUS trial randomized patients to ledipasvir-sofosbuvir with ribavirin for 12 weeks versus 24 weeks without ribavirin and reported similar SVRs (96% vs. 97%, respectively).[784] With multiple ribavirin-free, 12-week options available for this subgroup of patients, this regimen is listed as an alternative, not a recommended, treatment option. This same approach is applied in patients with genotype 4 infection, but for genotype 5 and 6 infections, 12 weeks of ledipasvir-sofosbuvir is recommended regardless of the presence or absence of cirrhosis. This is based on very limited data. In addition, the elbasvir-grazoprevir regimen was studied (and approved) with ribavirin in patients with prior failure of an NS3/4- and IFN-containing regimen; thus, this regimen is an alternative, not a recommended, regimen in this re-treatment population.

The re-treatment of patients with genotype 2 infection in whom previous treatment with IFN and ribavirin failed is the same as initial treatment. Both glecaprevir-pibrentasvir and sofosbuvir-velpatasvir offer high efficacy for this patient population. However, the re-treatment of patients with genotype 3 infection who experienced previous failure of IFN and ribavirin has unique recommendations. As previously mentioned in the "Initial Treatment of Chronic Hepatitis C Virus Infection" section, there are subpopulations of patients with genotype 3 infection that present a therapeutic challenge. Consistently, across multiple DAA regimens, prior treatment failure and/or presence of cirrhosis increases the risk of treatment failure. It is also in the setting of these multiple baseline negative predictors that the presence of NS5A RASs has more impact on the risk of treatment failure. The ASTRAL-3 study, which investigated 12 weeks of sofosbuvir-velpatasvir in patients with genotype 3 infection, included 71 patients in whom therapy had previously failed and 80 patients with compensated cirrhosis. Although the overall SVR_{12} was 95%, lower SVR was reported for patients with previous failure (90%) and with cirrhosis (91%).[825] As previously described, when a sofosbuvir plus NS5A (velpatasvir or daclatasvir) regimen is used, baseline NS5A RASs, especially when other negative predictors are present, affect DAA treatment response for genotype 3 infection, with the Y93H variant having the greatest impact. Meanwhile, glecaprevir-pibrentasvir, which is approved as an 8-week regimen for initial treatment of genotype 3 infection, regardless of presence of cirrhosis, had a lower SVR rate when studied for 12 versus 16 weeks among 44 patients without cirrhosis in whom prior therapy had failed (previously treated with IFN and ribavirin with or without sofosbuvir; those previously treated with NS5A or NS3/4A protease inhibitors were excluded).[883] Owing to small numbers, the role of baseline NS5A RASs is less clear, but all patients who experienced treatment failure had RASs at baseline. In particular, two subjects (one in each arm) had the A30K substitution at baseline, and treatment failure occurred with the A30K plus emergence of the Y93H variant, a double RAS that confers 69-fold resistance to GLE-PIB. The phase III POLARIS-3 study randomized treatment-naïve patients with genotype 3 infection and cirrhosis to 8 weeks of sofosbuvir-velpatasvir-voxilaprevir versus sofosbuvir-velpatasvir for 12 weeks.[792] The SVR_{12} was 96% in both arms.

Thus, for patients with genotype 3 infection who have previously experienced failure of treatment with IFN and ribavirin and who do not have cirrhosis, the recommendation per the AASLD/IDSA HCV treatment guidance is to check for baseline NS5A RASs. If the Y93H RAS is not present, the recommendation is to use sofosbuvir-velpatasvir for 12 weeks. If the Y93H RAS is present, the recommendation is to use sofosbuvir-velpatasvir-voxilaprevir for 12 weeks. If neither of these is possible, alternative regimens are glecaprevir-pibrentasvir for 16 weeks or daclatasvir and sofosbuvir for 12 weeks (without Y93H RAS only).[819] The re-treatment approach for genotype 3 infection in patients in whom treatment with IFN and ribavirin previously failed and who have cirrhosis is more challenging. Because of the addition of the negative baseline predictor in cirrhosis, there is a need to further optimize therapy, and both recommended regimens are off-label recommendations. As previously mentioned, although sofosbuvir-velpatasvir-voxilaprevir is not approved for this indication by the FDA, POLARIS-3 supports the efficacy of this combination for 8 weeks in patients with genotype 3 infection and cirrhosis.[792] Owing to the small number of treatment-experienced patients, the guidelines recommend 12 weeks of therapy, because of the number of negative baseline predictors in this difficult-to-treat patient population and the need to optimize treatment response. The other recommended regimen is the combination of sofosbuvir with elbasvir-grazoprevir. Although elbasvir-grazoprevir is not FDA approved for genotype 3 infection, the regimen does have activity versus this genotype. The C-ISLE

[a]References 852–854, 856, 861, 864–867, 869, 871, 873–879, 881, 882.

study investigated this triple-drug regimen for 12 weeks with or without ribavirin or for 16 weeks without ribavirin, in patients experienced with IFN plus ribavirin ($n = 53$) with genotype 3 infection and cirrhosis.[884] The overall SVR_{12} in this subgroup was 100% in a per protocol analysis. Because of the unmet need in this very difficult-to-treat subgroup, 12 weeks of treatment with either sofosbuvir with elbasvir-grazoprevir or sofosbuvir-velpatasvir-voxilaprevir is recommended.[819]

Failure of Non-NS5A Inhibitor, Sofosbuvir-Containing Regimens

In patients who have previously experienced treatment failure with a non-NS5A inhibitor, sofosbuvir-containing regimen, failure primarily occurred with sofosbuvir and ribavirin (primarily genotypes 2 and 3), sofosbuvir plus IFN and ribavirin (primarily genotypes 1 and 3), or sofosbuvir and simeprevir (primarily genotype 1). For genotype 1 infection, regardless of the presence of cirrhosis, there are three recommended regimens: sofosbuvir-velpatasvir-voxilaprevir for 12 weeks for genotype 1a, sofosbuvir-velpatasvir for 12 weeks for genotype 1b, and glecaprevir-pibrentasvir for 12 weeks, regardless of subtype.[819] The phase III POLARIS-4 randomized trial compared sofosbuvir-velpatasvir-voxilaprevir for 12 weeks versus 12 weeks of sofosbuvir-velpatasvir.[877] Cirrhosis was common—46% in both study arms. SVR_{12} rates for patients with genotype 1 infection were 97% and 90%, respectively. Overall, there was only 1 relapse in the sofosbuvir-velpatasvir-voxilaprevir arm, and 14 relapses in the sofosbuvir-velpatasvir arm. Eight of the 14 relapses occurred in patients with genotype 3 infection and 5 had genotype 1a infection; accordingly, sofosbuvir-velpatasvir is not recommended for these two genotypes or subtypes, and sofosbuvir-velpatasvir-voxilaprevir is recommended. However, only one relapse occurred in a patient with genotype 1b infection and none occurred for genotype 2 infection, suggesting that sofosbuvir-velpatasvir for 12 weeks is an efficacious regimen with these genotypes or subtypes and that the triple salvage regimen is not required. It is also notable that the single patient with relapse in the sofosbuvir-velpatasvir-voxilaprevir arm did not experience treatment-emergent RAS, whereas 9 of the 14 relapses in the sofosbuvir-velpatasvir arm occurred in patients who developed treatment-emergent NS5A RASs. The data to support the use of glecaprevir-pibrentasvir in this re-treatment population for genotype 1 or 2 are limited.

The ENDURANCE-1 and EXPEDITION-1 studies included a total of 14 patients with genotype 1 infection in whom a sofosbuvir-containing regimen had failed and who had received glecaprevir-pibrentasvir for 12 weeks.[592,821] For genotype 2 infection, the ENDURANCE-2 study, which investigated 12 weeks of glecaprevir-pibrentasvir, included six patients who had previously experienced failure of sofosbuvir plus ribavirin with or without IFN.[824] All six patients achieved SVR_{12}. Thus, glecaprevir-pibrentasvir for 12 weeks is recommended in the case of genotype 1 or 2, non-NS5A, prior sofosbuvir-containing regimen failures, regardless of the presence of cirrhosis. This regimen is not recommended for similar patients with genotype 3 to 6 infections (see "Failure of Direct-Acting Antiviral Regimens Containing NS5A Inhibitors" section).

Failure of Direct-Acting Antiviral Regimens Containing NS5A Inhibitors

There are only two regimens approved by the FDA for patients in whom an NS5A-containing regimen has previously failed: sofosbuvir-velpatasvir-voxilaprevir for 12 weeks for all genotypes, and glecaprevir-pibrentasvir for 16 weeks for genotype 1. Owing to either poor performance of other regimens or limited data, sofosbuvir-velpatasvir-voxilaprevir for 12 weeks is recommended for genotypes 3 to 6 in situations in which any DAA regimen, including NS5A-containing regimens, has failed. POLARIS-1, a placebo-controlled phase III trial, supports the use of 12 weeks of sofosbuvir-velpatasvir-voxilaprevir in patients with all genotypes in whom an NS5A-containing regimen has failed, regardless of the presence of cirrhosis.[877] In the majority of patients a regimen containing NS5A plus sofosbuvir had failed, and the overall SVR_{12} was 97%. There were seven virologic failures, six of which were relapses, and all occurred in patients with cirrhosis. Four of the relapses occurred in patients with genotype 3 infection (and cirrhosis). Because of the difficulty in re-treating patients with genotype 3 infection in whom a previous DAA therapy has failed and who have cirrhosis, the AASLD/IDSA HCV treatment guidance recommends adding weight-based ribavirin to the 12-week sofosbuvir-velpatasvir-voxilaprevir regimen in order to optimize treatment outcomes. The phase II, open-label, MAGELLAN-1 and MAGELLAN-2 studies investigated the efficacy of glecaprevir-pibrentasvir for 12 to 16 weeks with or without ribavirin in patients primarily with genotype 1 infection in whom a prior DAA-containing regimen, including NS5A-inhibitors, had failed.[864,885] Although limited by small samples sizes, these studies reported that patients who had been previously exposed to DAA regimens with NS3/4 protease inhibitors and NS5A inhibitors had the highest relapse rates, regardless of the length of regimen; 12 weeks appeared sufficient for patients who had experienced failure of a prior NS3/4-only regimen, and 16 weeks appeared necessary for patients who experienced failure of an NS5A-only regimen. Accordingly, this regimen was FDA approved for DAA salvage only in genotype 1 infection and for the aforementioned durations and subpopulations. Owing to the need for more NS5A-failure salvage regimens in these patients, who may not be able to take the sofosbuvir-velpatasvir-voxilaprevir regimen, the AASLD/IDSA guidelines identify glecaprevir-pibrentasvir as an alternative regimen in genotype 1 infection. It is not recommended for DAA salvage for other genotypes owing to limited data.

Adverse Reactions

Adverse reactions were extremely common and often treatment limiting with IFN-α-based regimens. Flulike symptoms were experienced by most persons, and fatigue, depression, and cognitive changes were also common. In addition, IFN-α commonly caused mild-to-moderate transient bone marrow suppression, manifesting with anemia, thrombocytopenia, and especially neutropenia. Ribavirin also added to the side-effect profile, with psychiatric-associated symptoms and anemia being the most common manifestations. The side-effect profile of these medications and the lower chance of response significantly limited the uptake and the access for patients with concomitant medical conditions including psychiatric disease, cardiovascular disease, and HIV infection. Although the first DAAs, telaprevir and boceprevir, significantly improved the treatment response rates for genotype 1 infection, they were still used in combination with PEG IFN–ribavirin, and they had their own adverse event profiles, including severe anemia and, in the case of telaprevir, rash and severe anal burning and pruritus.

With DAA combination therapies and, increasingly, the limited need for ribavirin as part of most regimens, the safety and adverse event profile has dramatically improved, and therefore discontinuation of therapy due to adverse events is extremely uncommon. Several DAA regimen registration trials have randomized participants to receive placebo, providing an opportunity to assess the safety of these regimens. In POLARIS-1, a phase III placebo-controlled trial of sofosbuvir-velpatasvir-voxilaprevir, patients receiving the intervention arm reported headache (25%), fatigue (21%), diarrhea (18%), and nausea (14%), as compared with 17%, 20%, 12%, and 8% in the placebo arm, respectively.[877] There were no differences in laboratory abnormalities, with only 1% reporting hemoglobin <10 g/dL in both arms and only two participants in the intervention arm reporting elevated liver enzymes. Liver enzyme elevation was more common in the placebo arm owing to active HCV infection. Rates of discontinuation of therapy due to adverse events were 2% in the placebo arm and <1% (single subject) in the intervention arm. The C-EDGE study randomized patients to 12 weeks of elbasvir-grazoprevir versus a delayed-start arm.[820] In the first 12 weeks of follow-up, reports of adverse events (>10% incidence) were similar between the two arms: headache (17% vs. 18%, respectively) and fatigue (16% vs. 17%, respectively). Thus, whereas with IFN-containing regimens the management of adverse events was an important part of treatment, this is no longer the case. The need for growth factors and blood transfusions, hospitalization, and symptom management are almost nonexistent.

Drug interactions are important complications of therapy, especially with NS3/4 protease inhibitors. Because this field is rapidly changing, clinicians are strongly urged to check updated information (e.g., www.hep-druginteractions.org) before prescribing any DAA regimen to patients who are taking other medications. There are several key drug interactions that are worth mentioning, including sofosbuvir and amiodarone; ledipasvir or velpatasvir and proton pump inhibitors; and multiple DAA regimens with lipid-lowering drugs and antiepileptics.

Recommended Treatments for Chronic Hepatitis C as of 2018

The AASLD and IDSA recommendations for treatment of hepatitis C are presented in Table 154.4. AASLD and IDSA provide regularly updated treatment recommendations for hepatitis C. (See the online guidance for recommendations for re-treatment and for treatment of special populations, including patients who are HIV infected—available at https://www.hcvguidelines.org.)

Selection of Patients for Treatment Initial Treatment and Re-treatment of Patients With Chronic Hepatitis C, Including Those With HIV Infection

Historically, the risk of treatment with IFN-containing regimens, which, as noted earlier, carried significant adverse event profiles, had to be weighed against the benefit of SVR. Owing to low SVR rates in the IFN era and high rates of adverse events, only select patients were considered appropriate for therapy. Now, however, with DAA therapy that is very well tolerated and quite safe when the appropriate DAA is used in the correct patient population, the risk of therapy is low. Furthermore, the potential benefit of cure, which is afforded the vast majority of patients who take therapy, is high.[495,508,886] Therefore the AASLD/IDSA HCV guidance recommends treatment "for all patients with chronic HCV infection, except those with a short life expectancy that cannot be remediated by HCV therapy, liver transplantation, or another directed therapy."[819] In addition, patient subgroups previously considered poor candidates for therapy, including patients who inject drugs, patients who abuse alcohol, or patients with psychiatric disease, can do well with DAA therapies and should not be excluded based on their comorbidities.[887–889] Finally, patients with HIV infection should not be considered any differently for DAA therapy than a patient without HIV. Although rates of adverse events in the IFN era were greater in studies of patients with HIV infection, this is no longer true. Multiple phase III trials have supported not only the same efficacy of DAAs in patients with HIV infection, but the same degrees of safety and tolerance as well, with similar treatment discontinuation rates across both patient populations.[842,844,890] With few exceptions, owing to the benefit of ARV therapy, patients with HIV infection should be started on ARVs first, and then HCV therapy should be started once HIV suppression has been attained.[891] Attention to the potential for DAA drug-drug interactions is critical in choosing the ARV regimen, and in patients on stable ARV therapy, the choice of the DAA regimen should include considerations of drug-drug interactions.

TABLE 154.4 Current AASLD and IDSA Recommendations for Initial Treatment (September 2017)

Genotype 1a

- Elbasvir-grazoprevir for 12 weeks if no NS5A RAS for elbasvir is detected (no cirrhosis or compensated cirrhosis)
- Glecaprevir-pibrentasvir for 8 weeks if no cirrhosis and 12 weeks if compensated cirrhosis
- Ledipasvir-sofosbuvir for 12 weeks (no cirrhosis or compensated cirrhosis)
  - 8 weeks for patients without cirrhosis and baseline HCV RNA <6 million IU/mL
- Sofosbuvir-velpatasvir for 12 weeks (no cirrhosis or compensated cirrhosis)

Genotype 1b

- Elbasvir-grazoprevir for 12 weeks (no cirrhosis or compensated cirrhosis)
- Glecaprevir-pibrentasvir for 8 weeks if no cirrhosis and 12 weeks if compensated cirrhosis
- Ledipasvir-sofosbuvir for 12 weeks (no cirrhosis or compensated cirrhosis)
  - 8 weeks for patients without cirrhosis and baseline HCV RNA <6 million IU/mL
- Sofosbuvir-velpatasvir for 12 weeks (no cirrhosis or compensated cirrhosis)

Genotype 2

- Glecaprevir-pibrentasvir for 8 weeks if no cirrhosis and 12 weeks if compensated cirrhosis
- Sofosbuvir-velpatasvir for 12 weeks (no cirrhosis or compensated cirrhosis)

Genotype 3

- Glecaprevir-pibrentasvir for 8 weeks if no cirrhosis and 12 weeks if compensated cirrhosis
- Sofosbuvir-velpatasvir for 12 weeks if no cirrhosis or if compensated cirrhosis
  - Cirrhosis: testing for NS5A RASs for velpatasvir is recommended
    - Consider alternative treatments if Y93H present

Genotype 4

- Elbasvir-grazoprevir for 12 weeks (no cirrhosis or compensated cirrhosis)
- Glecaprevir-pibrentasvir for 8 weeks if no cirrhosis and 12 weeks if compensated cirrhosis
- Ledipasvir-sofosbuvir for 12 weeks (no cirrhosis or compensated cirrhosis)
- Sofosbuvir-velpatasvir for 12 weeks (no cirrhosis or compensated cirrhosis)

Genotype 5 or 6

- Glecaprevir-pibrentasvir for 8 weeks if no cirrhosis and 12 weeks if compensated cirrhosis
- Ledipasvir-sofosbuvir for 12 weeks (no cirrhosis or compensated cirrhosis)
- Sofosbuvir-velpatasvir for 12 weeks (no cirrhosis or compensated cirrhosis)

Clinicians are urged to consult online guidelines for the latest recommendations on HCV treatment (www.hcvguidelines.org).
[a]Regimens are listed in groups by level of evidence, then alphabetically. See www.hcvguidelines.org for alternative treatments, treatments in experienced patients, and special populations.
AASLD, American Association for the Study of Liver Diseases; *FDA,* US Food and Drug Administration; *IDSA,* Infectious Diseases Society of America; *RAS,* resistance-associated substitution.

Treatment of Acute Hepatitis C

In 2015, 1.75 million new HCV infections were reported by the World Health Organization (WHO), an epidemic that is widespread.[665,892,893] Because prevention of future infection is a mainstay of the elimination plan, early identification and treatment of incident HCV infections are critical. Currently, national guidelines for the management of HCV infection provide different recommendations for the treatment of acute HCV infection. EASL recommends an 8-week DAA regimen of an NS5A inhibitor–sofosbuvir combination.[818] Furthermore, EASL recommends that patients with HIV infection and/or those with HCV RNA >1,000,000 IU/mL should have therapy extended to 12 weeks. The AASLD/IDSA Guidance Panel recommends that acute infection be treated in the same manner as chronic infection, in which case, as with EASL, use of a shortened course of therapy to 8 weeks for persons with HIV infection is not recommended.[819]

Historically, with IFN-based regimens, treatment response was improved when patients were treated in the acute infection period, which allowed for shortening of the course of therapy from 12 to 6 months for genotype 1 infection.[894] Over the past 2 years, several pilot clinical trials of all-oral, DAA therapies have been reported in the setting of acute or early HCV infection.[895–899] These studies, which have investigated shortened courses of DAA therapy, show significant variability in study population, definitions of acute or early infection, presence or absence of symptoms attributable to HCV infection, frequency of the *IL28B* favorable genotype, DAA therapy potency, and/or duration of DAA therapy. Although small and variable, these studies have identified challenges in the treatment of acute infection including the potential impact of high baseline HCV RNA on treatment response.[896] Viral load in the setting of acute infection may correlate with the host immune profile, with higher viral load correlated with a more chronic immune profile. Because of the potential for natural clearance of acute HCV infection, there are some concerns that immediate treatment results in overtreatment of a group of patients who would otherwise naturally clear the infection. Natural clearance of HCV infection has been reported in 30% to 50% of patients, although this is lower in patients with HIV infection.[900–902] The majority of patients (two-thirds) will clear the infection in the first 6 months after exposure.[902]

The WHO targets for HCV elimination include a 90% reduction in incident infections by 2030. The epidemic of new HCV infection is reported primarily in PWID and MSM with HIV infection. Modeling indicates that HCV elimination among these two groups is achievable with scale-up of access to DAA therapy and thus cure.[903] In the Netherlands, universal access to DAA therapy among MSM with HIV infection resulted in >70% HCV eradication and a 50% decline in incident HCV

infections.[904] These studies suggest that treatment is prevention, and that the earlier a patient with HCV can be treated, the less likely it is that he or she will transmit the infection to another patient. This is a rapidly evolving area of investigation, and we are likely to see sustentative changes over the next few years, with a predicted movement to treat earlier and potentially to use abbreviated courses of DAA therapies.

PREVENTION

Preexposure Prevention

The key to reducing the incidence of HCV infection is decreasing exposure to contaminated blood. The incidence of posttransfusion HCV infection has been reduced to very low levels by screening blood donations for HCV antibody and surrogate markers of HCV infection.[678,905] Although the impact is more difficult to measure, nosocomial HCV transmission in developing countries should decrease with worldwide adherence to universal precautions. Among people with illicit drug use, harm-reduction interventions such as opiate substitution therapy (OST) and needle and syringe programs (NSPs) have been the traditional backbone of HCV prevention. A meta-analysis found that OST reduces risk of HCV acquisition by 50%, and when combined with high coverage of NSPs results in a 71% reduction in the risk of HCV acquisition.[906] In fact, harm-reduction interventions are viewed as a critical component of the push toward HCV elimination.

Efforts to develop an HCV vaccine are complicated by the extensive antigenic diversity existing among different HCV genotypes, in addition to the absence of solid immunity following natural infections, as discussed earlier. Nonetheless, there is evidence that immunity can be acquired that protects against *persistent* HCV infection, both in chimpanzees and in humans. Chimpanzees that responded immunologically to an initial HCV infection or that were immunized with an experimental vaccine have been readily infected on subsequent experimental challenge with the virus.[907] Significantly, however, infection appears to be attenuated following prior infection or immunization and rarely becomes persistent.[272,344,908–911] Lanford and coworkers have shown that such protection can be achieved even across HCV genotypes.[912] Likewise, injection drug users who recovered from an initial HCV infection were shown to be less likely to become viremic.[286,338] When viremia did occur, it often was at a low level and resolved. These data suggest that a vaccine might be capable of preventing viral persistence and the significant pathologic consequences of HCV infection. Although recent studies have greatly expanded the number of well-characterized broadly neutralizing human monoclonal antibodies (bNAbs) against HCV, there remain a paucity of human prevention studies.[913] More work is needed to identify optimal vaccine antigens and adjuvants. It remains unclear that anti-HCV antibody responses induced in animals are predictive of responses that would be induced by the same vaccine in humans.

Postexposure Prevention

Early studies provided conflicting data about the extent to which HCV infection is modified by administration of pooled human immune globulin.[328,914,915] However, because HCV-seropositive donations are no longer included in the plasma pools from which immune globulin is manufactured, no benefit would be expected from products on the market today. Administration of immune globulin is not recommended after exposure to HCV in US Public Health Service guidelines.[739]

An individual who has a documented exposure (e.g., a health care worker sustaining a needlestick derived from a patient who is known to be infected) should be screened for HCV antibodies as soon as possible after exposure to exclude prior infection.[275,739] Serology and ALT testing should be repeated at least once 6 months later. Some authorities also test for HCV RNA 2 to 4 weeks after exposure. Currently, postexposure prophylaxis (PEP) is not recommended for HCV exposures. The rationale for postexposure chemoprophylaxis is based on several core principles: (1) the pathogenesis and time course of early infection; (2) the biologic plausibility that infection could be prevented with antiviral drugs; (3) evidence of antiviral efficacy of the drugs being used for PEP; and (4) the risk to the health care worker from exposure to PEP.[916] The impact of the failure to prevent the development of a chronic infection also drives the clinical need for exploring PEP for infectious pathogens. For example, in the case of chronic HBV infection and HIV, there is no cure for chronic infection, and the long-term impact of infection may be substantial; on the other hand, chronic HCV infection is curable in the vast majority of patients. Therefore the impact on the health care worker of the failure to prevent chronic infection is less critical for HCV compared with HIV or HBV infection. Cost-analysis modeling, assuming a seroconversion rate of 1.9% and PEP with DAA daily for 4 weeks versus no PEP and treatment of patients who develop active infection, concluded that there is unlikely to be a scenario in which PEP is cost-effective compared with early HCV treatment.[917]

HEPATITIS C VIRUS ELIMINATION

Thus far in human history, eradication or elimination of an infectious disease has been achieved only when a highly effective vaccine was available (e.g., smallpox, polio) or through environmental measures to eliminate transmission such as vector control (e.g., malaria in North America) or improved sanitation (e.g., dracunculiasis).[918–920] The availability of multiple highly efficacious and well-tolerated HCV treatment regimens that can eradicate infection within an individual and prevent spread to others has spurred a movement toward elimination of HCV. Although many hurdles remain across all facets of HCV care, from screening and diagnosis to access and treatment to harm reduction and reinfection, the timing is right, and without aggressive goals true progress will languish.

World Health Organization Goals

WHO commissioned a mathematical model, which suggested that HCV could be eliminated as a public health threat by 2030 if the response reached coverage targets for core interventions. Core interventions identified for HCV include prevention and cascade of care.[890–892,906,916,917] Prevention interventions for HCV include blood safety (donations screened with quality assurance), injection safety (proportion of unsafe injections), and harm-reduction strategies (syringes and needles distributed among PWID per year). The "cascade of care" is a continuum of services that persons living with HCV should receive as they go through the progressive stages, from diagnosis to treatment to chronic care and ultimately to cure. Testing and treatment are the identified coverage indicators for WHO (i.e., the percent of HCV-infected persons diagnosed and the percent diagnosed with HCV started on treatment). The WHO targets for the core interventions for 2020 and 2030 are 95% and 100% for blood safety, 90% for injection safety, 200 and 300 needles and syringes per person who injects drug per year for harm reduction, 30% and 90% for testing, and 80% treated for HCV by 2030.

Current State: Hepatitis C Virus Treatment Cascade

Although the WHO targets are aggressive, with commitment from local and national stakeholders they are achievable. As of 2015, the baseline goals were 97% for blood safety, 5% for injection safety, 27 needles and syringes per PWID/year for harm reduction, 20% for testing, and 7% for treatment. For the current global cascade of care, in 2015 the gap for testing was 70%; for treatment, 73%; and for cure, greater than 90%. These gaps are not uniform across the globe because there are disparities in access to testing and to treatment. Although access to DAAs, which offer the greatest chance of cure, is increasing globally, there are many regions of the world where DAAs are not available, significantly limiting the ability to achieve cure. In order to reach sufficient coverage levels, there must be universal provision of a safe blood supply and safe injection equipment to ensure that HCV is not spread through unsafe medical practices; scaling up of harm-reduction services; provision of testing services; and secure access to safe, effective treatment for those with identified infection. This summary is theoretical, but there are emerging real-world examples of how such interventions translate to decreased incidence of HCV infection.

From Modeling to Real-World Examples

Taking into account current prevalence and incidence rates and factoring in different treatment efficacies and penetration rates, a time horizon for changes in HCV epidemiology can be modeled. After the introduction of modern DAA-based HCV therapies wherein extremely high efficacy can be expected, multiple modeling studies in specific populations (PWID,

individuals with HIV coinfection, and prisoners) have come to the same conclusion: Substantial reduction in HCV prevalence (>50%) can be achieved within 1 to 2 decades with relatively modest scale-up of DAA treatment (approximately 10% treated per annum).[921–924] Furthermore, the addition of harm-reduction measures such as OST or NSPs for PWID or safer sex counseling for high-risk MSM can significantly enhance responses compared with antiviral treatment alone.[921,924]

Treatment of individuals with acute or recent HCV infection will be a key component of elimination strategies because, by definition, these are persons at high risk for onward transmission and, largely theoretically, they may be more infectious to others. Although the prospect for spontaneous clearance of HCV has given some pause about rapidly initiating therapy in this setting, a cost and decision analysis convincingly demonstrated cost-effectiveness, and even cost savings, of acute HCV treatment when there was risk of onward transmission.[925] Identification of acute HCV infection is problematic, but individuals in specific populations, such as those with HIV, are more likely to be diagnosed and should be a particular focus for treatment of acute HCV infection.

Despite the convincing data from modeling studies, there are as yet no large-scale demonstrations of the impact of DAA treatment on HCV incidence and prevalence. Several ongoing or localized programs with impressive early results are highlighted.

The country of Georgia has a high HCV prevalence (approximately 7%) and committed early to a program to eliminate HCV.[926] In cooperation with the CDC, the elimination program was launched in April 2015 with initial efforts focused on developing an updated assessment of disease prevalence, building laboratory support and treatment capacity, and targeting initial treatment to those with more advanced liver disease. During the first 20 months of the program, nearly 26,000 persons initiated treatment for HCV with sofosbuvir plus ribavirin (with or without IFN) or, later, LDV-SOF. Sustained response to treatment has been assessed in over 6000 persons with an overall SVR_{12} of 79.5%, and 98% in those treated with LDV-SOF.[927] Based on current data, Georgia is on target to achieve a 90% reduction in HCV prevalence by 2020—a full decade ahead of WHO goals.

A high HCV prevalence combined with the ability to provide monitored treatment makes prisons an attractive venue to launch "micro-elimination" programs.[928,929] Dramatic results from a comprehensive diagnosis, treatment, and re-infection program in Queensland, Australia demonstrated a decrease in HCV prevalence from 12% to 1% in just 22 months.[930] In the United States, the fragmented nature of health care and significant funding challenges for HCV treatment in prisons precludes the use of such an approach at present.

Elimination of HCV in those with HIV is perhaps a more readily attainable goal because this is a relatively small population with levels of engagement in care that result in more effective diagnosis of HCV.[931] In addition, HIV coinfection may improve access to HCV medications because this is seen as a priority population for treatment. Modeling studies specific to this population have indicated that increased rates of treatment combined with harm-reduction measures could have a significant positive impact. Based on the HIV- and HCV-coinfected population of MSM in the United Kingdom, the impact of multiple different treatment approaches on HCV incidence and prevalence was modeled.[924] With the conservative assumption of 90% SVR with DAA treatment, maintaining 2015 treatment levels (approximately 50% of recent infections and 20% of chronic infections treated), there was little impact of DAA therapy on chronic HCV prevalence (8% with DAAs vs. 9% with IFN) or incidence (1.3 per 100 person-years vs. 1.5 per 100 person-years) in HIV- and HCV-coinfected MSM by 2025. As increased rates of treatment of recent and chronic infections were scaled up to 80% and 20%, respectively, dramatic decreases in prevalence and incidence were predicted; the largest impact was realized when 20% risk reduction was also included in the models (2.4% prevalence and incidence of 0.4 per 100 person-years in 2025). An important corollary to these findings is that although the number of treatments increases transiently during the rollout, within a few years the annual treatment rates drop below the base-case scenario as a significant net impact on new infections is realized in later years.[924]

In support of modeling predictions, real-world cohorts demonstrating the power of intense screening campaigns coupled with universal access to therapy are beginning to emerge. For example, in the Netherlands, universal access to DAA therapy arrived in late 2015; after this, data from the ATHENA HIV cohort indicated that by 2017, of the 1471 HIV- and HCV-coinfected persons (69% MSM) in care, 76% had been treated.[932] Notably, the bulk of treatment uptake occurred after removal of treatment restrictions. During this same time period (2014–16) the incidence of acute HCV infection dropped dramatically in HIV-positive MSM, from 1.12 per 100 person-years to 0.5 per 100 person-years, translating to a 51% decrease.[904] Concomitant diagnoses of sexually transmitted infections (e.g., syphilis and gonorrhea) increased during the same time frame, which suggests that it was not a change in risk behaviors that led to the decrease in acute HCV infections. Despite these encouraging data, the impact may not be universal because data from the French HIV cohort have not yet realized a decrease in HCV incidence despite similar rates of DAA treatment uptake.[933]

In addition to concerns about generalizability, the high rates of reinfection seen in some HIV-positive MSM cohorts coupled with a lack of universal, contemporary rollout of aggressive screening and treatment programs have raised concerns over reintroduction of HCV.[934–936] This is particularly relevant given the demonstration of international HCV transmission networks in HIV-positive MSM.[893]

ACKNOWLEDGMENTS

We thank Stanley Lemon, Stuart Ray, and David Thomas for their extensive contributions to earlier versions of this chapter. We also thank Stacey Horner for her expert review of this chapter.

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The complete reference list is available online at Expert Consult.

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155 Coronaviruses, Including Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS)

Stanley Perlman and Kenneth McIntosh

SHORT VIEW SUMMARY

Definition
- The coronaviruses (CoVs) commonly cause mild but occasionally more severe community-acquired acute respiratory infections in humans. CoVs also infect a wide variety of animals, and several CoVs (e.g., severe acute respiratory syndrome [SARS], Middle East respiratory syndrome [MERS]) have crossed the species barrier, producing outbreaks of severe human respiratory disease. While SARS-CoV was eradicated, MERS-CoV continues to circulate in human and camel populations. As of March 10, 2019, 2374 cases of laboratory-confirmed MERS were reported to the World Health Organization, with 823 deaths.

Epidemiology
- Community-acquired CoV infections cause about 15% of common colds. They are typically epidemic in the winter months. MERS has occurred in patients in the Arabian Peninsula and those who recently traveled from this locale.

Microbiology
- CoVs are members of the Nidovirales order, single-stranded, positive-sense RNA viruses with a large genome. They mutate and also recombine frequently.

Diagnosis
- Laboratory diagnosis is best accomplished by finding viral RNA through polymerase chain reaction.

Therapy
- There are no accepted effective antiviral drugs for CoVs.

Prevention
- Prevention is through epidemiologic methods and the use of appropriate respiratory precautions in hospital settings. The SARS epidemic and MERS outbreaks were controlled through careful case identification, quarantine, and use of barrier precautions.

The family Coronaviridae, within the order Nidovirales, presently contains two subfamilies, the Coronavirinae and the Torovirinae. However, increased recognition of the genomic diversity of viruses within the Nidovirales order makes it likely that nidovirus, coronavirus, and torovirus taxonomy will require modification. Coronaviruses (CoVs) are a large group of viruses infecting mammals and birds and producing a wide variety of diseases. They have been divided into four genera, two of which contain viruses infecting humans (see later). All human coronaviruses (HCoVs) are primarily respiratory pathogens. During the winter of 2002–2003, an alarming new disease appeared: severe acute respiratory syndrome (SARS), which was quickly attributed to a new CoV, the SARS-CoV. The outbreak originated in southern People's Republic of China, with evidence that the virus was first derived from bats and was transmitted to humans through intermediate hosts, probably the palm civet *(Paguma larvata)* and raccoon dog *(Nyctereutes procyonoides)*.[1–3] The SARS epidemic was controlled through a massive effort at case identification and containment, and the last known case occurred in mid-2004. In retrospect, the emergence of SARS is consistent with what is known about CoVs as a group: They are important pathogens in animals causing a wide variety of diseases through a wide variety of pathogenic mechanisms, and they have been noted to mutate frequently and infect new species.[4,5]

More recently, a related but different CoV producing severe respiratory disease has emerged, the Middle East respiratory syndrome coronavirus (MERS-CoV). MERS-CoV was grown in June 2012 from a sputum sample obtained from a man in Saudi Arabia who died of overwhelming pneumonia. The virus was quickly identified as a new CoV most closely related to several bat CoVs.[6] This report was followed by a number of other reports identifying a total of 2374 infected individuals, most of whom had acute respiratory symptoms, severe in most and fatal in 823 (as of March 10, 2019).[7,8]

Human-to-human transmission has been documented but appears to be inefficient except in hospital settings.[9–11] The animal reservoir of MERS-CoV is believed to be camels, although evidence suggests that bats may be infected with related viruses.[12–14] Infection of camels on the Arabian peninsula and throughout Africa is widespread, and several cases of camel-to-human transmission have been reported, generally from juvenile camels.[12,15]

HISTORY

Community-Acquired Respiratory Coronaviruses, Severe Acute Respiratory Syndrome, and Middle East Respiratory Syndrome

In 1965, Tyrrell and Bynoe[16] cultured a virus obtained from the respiratory tract of a boy with a common cold by passage in human embryonic tracheal organ cultures. The media from these cultures consistently produced colds in volunteers. The agent was ether sensitive but not related to any known human virus. Subsequently, electron microscopy of fluids from infected organ cultures revealed particles that resembled infectious bronchitis virus of chickens.[17] At about the same time, Hamre and Procknow recovered a cytopathic agent in tissue culture from medical students with colds.[18] The prototype virus was named 229E and was found on electron microscopy to have a similar or identical morphology (Fig. 155.1).

Using techniques similar to those used by Tyrrell and Bynoe, McIntosh and colleagues[19] reported the recovery of several infectious bronchitis–like agents from the human respiratory tract, the prototype of

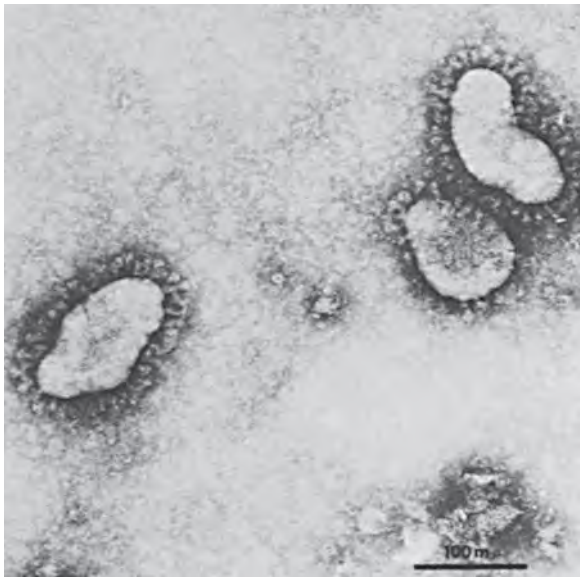

FIG. 155.1 Coronavirus strain HCoV-229E, harvested from infected WI-38 cells (phosphotungstic acid stain). *(From McIntosh K, Dees JH, Becker WB, et al. Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease.* Proc Natl Acad Sci U S A. *1967;57:933–940.)*

which was named OC43 (OC for organ culture). At much the same time, mouse hepatitis virus and transmissible gastroenteritis virus of swine were shown to have the same morphology on electron microscopy.[20] Shortly thereafter, the name *coronavirus* (the prefix *corona* denoting the crownlike appearance of the surface projections) was chosen to signify this new genus.

The number of animal CoVs quickly grew, including viruses causing diseases in rats, mice, chickens, turkeys, various other bird species, cattle, several wild ruminants, beluga whales, dogs, cats, rabbits, and pigs, with manifestations in the respiratory and gastrointestinal tracts, central nervous system, liver, reproductive tract, and other locations. Through sequencing and antigenicity studies, the animal CoVs and HCoVs initially were divided into three groups: group 1, which contained HCoV-229E, as well as numerous animal viruses; group 2, which contained HCoV-OC43 plus the closely related animal viruses, bovine CoV and mouse hepatitis virus; and group 3, which included only avian viruses related to infectious bronchitis virus (Fig. 155.2). Current taxonomy divides the subfamily Coronavirinae into four genera: *Alphacoronavirus* (which includes viruses previously in group 1); *Betacoronavirus* (which includes viruses previously in group 2, most notably SARS-CoV and MERS-CoV); *Gammacoronavirus* (which includes viruses previously in group 3); and *Deltacoronavirus* (which includes several newly described avian and swine viruses).[21]

SARS was first identified in Guangdong Province of the People's Republic of China in November 2002 and spread from there to Hong Kong and then throughout the world.[22] A CoV was independently and

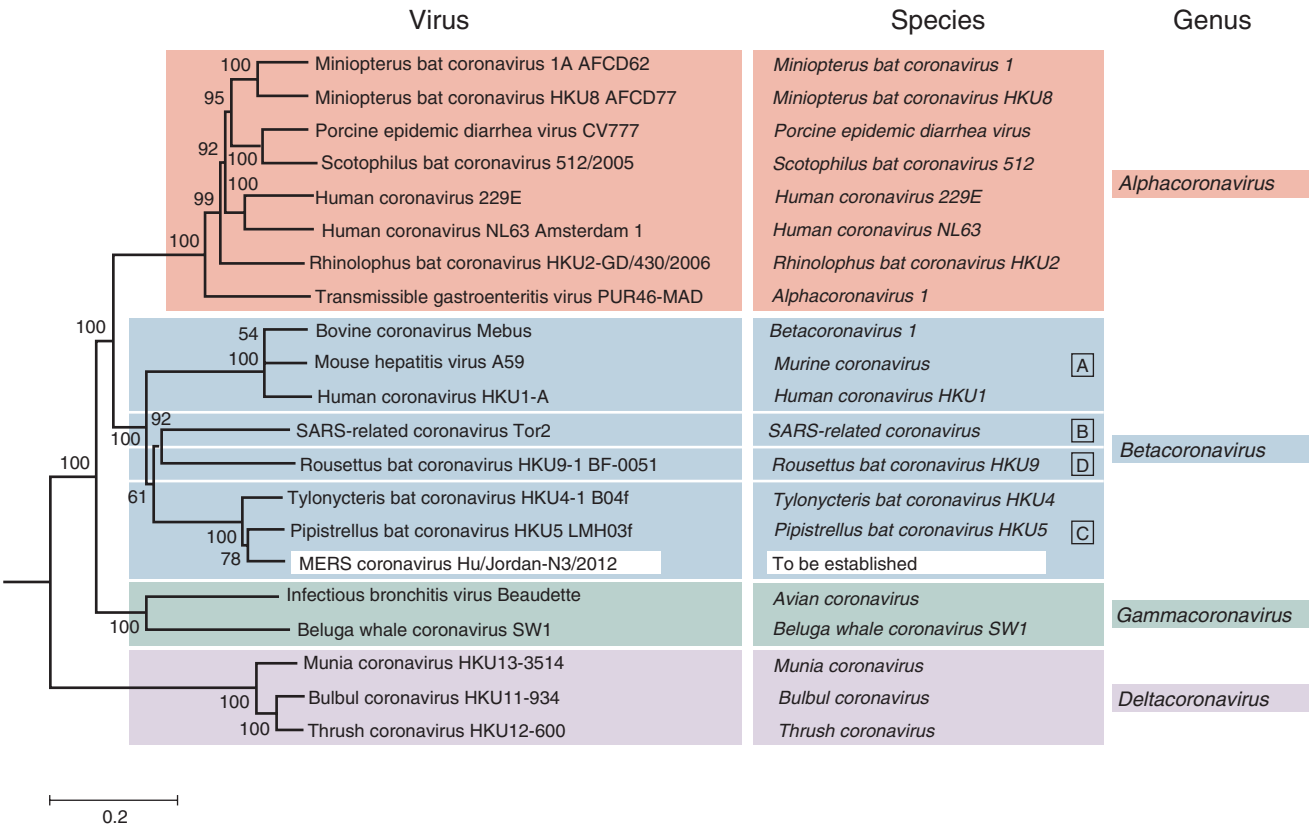

FIG. 155.2 Phylogenetic relationships among members of the subfamily Coronavirinae. A rooted neighbor-joining tree was generated from amino-acid sequence alignments of Coronaviridae-wide conserved domains in replicase polyprotein 1 (ADRP, nonstructural protein [nsp]3; Mpro, nsp5; RdRP, nsp12; Hel, nsp13; ExoN, nsp14; NendoU, nsp15; O-MT, nsp16) for 21 coronaviruses, each a representative of a currently recognized coronavirus species. Five of the six known human coronaviruses (HCoV-229E, HCoV-NL63, HCoV-HKU1, SARS-CoV, and MERS-CoV) are indicated. HCoV-OC43 is closely related to bovine coronavirus, which is shown in the figure. Equine torovirus Berne served as the outgroup. Virus names are given with strain specifications; species and genus names are in italics as per convention. The tree shows the four main monophyletic clusters, corresponding to genera *Alpha-, Beta-, Gamma-,* and *Deltacoronavirus* (color coded). Also indicated are betacoronavirus lineages A through D (corresponding to former CoV subgroups 2A through D). Bootstrap values (1000 replicates) are indicated at branch points. The tree is drawn to scale (scale bar, 0.2 amino-acid substitutions per site). *(From de Groot RJ, Baker SC, Baric RS, et al. Middle East respiratory syndrome coronavirus [MERS-CoV]: announcement of the Coronavirus Study Group.* J Virol. *2013;14:7790–7792.)*

almost simultaneously isolated from SARS patients by several laboratories and found by sequencing to be only distantly related to previously characterized CoVs.[23–26] The SARS outbreak stimulated a rapid and intense public health response coordinated by the World Health Organization (WHO), and by July 2003, transmission had ceased throughout the world. Despite this effort, however, 8096 probable cases had occurred in 29 countries, with 774 deaths.[22]

With the identification of the SARS-CoV, the HCoV field became much more active. Sensitive molecular methods were developed to detect RNA from viruses identical or closely related to HCoV-229E and HCoV-OC43 in the respiratory tract, and two new species were discovered: NL63, an alphacoronavirus, and HKU1, a betacoronavirus.[27–29] HCoV-NL63 was found independently by three groups, two in the Netherlands and, somewhat later, the third in New Haven, Connecticut.[30] In all three cases, positive samples were from infants and children with respiratory disease. Notably, HCoV-NL63 and HCoV-229E were estimated to have originated from a common bat precursor and diverged approximately 1000 years ago.[31,32] CoVs related to HCoV-229E have been isolated from camels.[33] All CoVs are susceptible to recombination, but HCoV-OC43 may be especially susceptible, with multiple recombinant strains identified in a confined geographical setting.[34] HCoV-HKU1 was found in Hong Kong in an adult with respiratory disease. These two new HCoV strains subsequently have been found worldwide and appear to have pathogenicity similar to that of HCoV-229E and HCoV-OC43, with the possible exception that NL63 is more frequently found in children with croup.[35,36]

The MERS-CoV was found when a man was admitted in June 2012 to a hospital in Jeddah, Saudi Arabia, with overwhelming acute pneumonia and renal failure. A sample of sputum grew a cytopathic virus that, on sequencing, proved to be a CoV, classified as a *Betacoronavirus* and most closely related to two bat CoVs, HKU4 and HKU5.[6] MERS-CoV continues to cause new infections, with all but a few of them sporadic or hospital-based and in individuals living or traveling in the Middle East.[37,38]

In the remainder of this chapter, the group of respiratory HCoVs first discovered in the 1960s and containing HCoVs 229E, OC43, NL63, and HKU1 are referred to as community-acquired respiratory (CAR) HCoVs to distinguish them from the SARS-CoV and the MERS-CoV.

Gastrointestinal Coronaviruses and Toroviruses

In view of the prominence of CoVs in animal enteric diseases, there have been extensive efforts to identify enteric HCoVs. There are numerous reports of CoV-like particles (CoVLPs) found by electron microscopy in human fecal matter, but these particles have been difficult to characterize further. Efforts to detect CoV RNA in feces using polymerase chain reaction (PCR) and primers for respiratory HCoVs have had limited success and have failed to associate CoVs with gastrointestinal disease.[39,40] In one instance, a human enteric CoV with high identity to bovine CoV was isolated from a child with diarrhea and shown to cause diarrhea when reintroduced into calves, demonstrating its pathogenic potential.[41]

Toroviruses were, like CoVs, first described in animals. They were first detected in the feces of cattle (Breda virus) and horses (Berne virus).[42,43] While previous publications suggested that particles resembling toroviruses could be detected in human fecal material using electron microscopy,[44] there are presently no reports definitively showing the existence of human toroviruses.

DESCRIPTION OF THE PATHOGENS

The CoV nucleic acid is RNA, approximately 30 kb in length, of positive sense, single stranded, polyadenylated, and infectious. The RNA, the largest known viral RNA (Fig. 155.3), codes for (in order from the 5′ end) a large polyprotein that is cleaved by virus-encoded proteases to form several nonstructural proteins, including an RNA-dependent RNA polymerase, methyltransferases, and a helicase, followed by either four or five structural proteins intermingled with a variable number of nonstructural and minor structural proteins.[4] The first of the major structural proteins is a surface hemagglutinin-esterase (HE) protein, present on HCoVs OC43 and HKU1 and some animal betacoronaviruses, that may play some role in the attachment or release of the particle, or both, at the cell surface. The gene for the HE protein contains sequences similar to the hemagglutinin of influenza C virus, likely evidence of an interfamily recombinational event that occurred many years ago. Notably, the HE receptor binding activity of HCoV-OC43 and probably also of HKU1 was progressively lost along with a decrease in HE-associated esterase activity during evolution in humans. These changes most likely reflected adaptation to the human sialic acid receptor after introduction into humans from zoonotic sources.[45] The next gene encodes the surface glycoprotein that forms the petal-shaped surface projections and is responsible for attachment and the stimulation of neutralizing antibody. This is followed by a small envelope (E) protein, a membrane glycoprotein, and a nucleocapsid protein that is complexed with the RNA. There are several other open reading frames, which are unique to each strain of CoV. While their coding functions are not clear, many of them are probably involved in immune evasion.[46,47] The strategy of replication of CoVs is similar to that of other nidoviruses, in that all messenger RNAs form a nested set with common polyadenylated 3′ ends, with only the unique portion of the 5′ end being translated.[4] As in other RNA viruses, mutations are common in nature, although the mutation rate is much lower, approximately 2×10^{-6} per site per replication cycle.[48] Unlike other RNA viruses, CoVs encode a 3′→5′ exonuclease that has proofreading activities, playing a critical role in maintaining replication fidelity in cell cultures and in animals.[49] CoVs are also capable of genetic recombination if two viruses infect the same cell at the same time.

All CoVs develop exclusively in the cytoplasm of infected cells (Fig. 155.4). They bud into cytoplasmic vesicles from membranes of the pre-Golgi endoplasmic reticulum. These virus-filled vesicles are then extruded by the exocytic secretory pathway with the small E protein critical for this process.[50] The resultant virus particles have a diameter of 70 to 80 nm on thin-section electron microscopy and 60 to 220 nm on negative staining. They are pleomorphic, with widely spaced, petal-shaped projections 20 nm long (see Fig. 155.1).

The cellular receptor for 229E and most other alphacoronaviruses is aminopeptidase N (APN).[51] Interestingly, NL63, the other known human alphacoronavirus, uses as its cellular receptor angiotensin-converting enzyme 2 (ACE2),[52] the same receptor as is used by the SARS-CoV.[53] Mouse hepatitis virus, a betacoronavirus related to strain OC43, uses as its receptor a member of the carcinoembryonic antigen family.[54] HCoV-OC43 and bovine CoV, which is closely related to HCoV-OC43, bind to 9-*O*-acetylated neuraminic acid as part of the entry process.[55] The host cell receptor for MERS-CoV is dipeptidyl peptidase 4, which, like ACE2 and APN, is an ectopeptidase that is abundantly expressed in the respiratory and enteric tracts.[56,57] This preferential usage of large host ectopeptidases for CoV entry is notable but not understood.

All the CAR HCoVs grow only with difficulty in tissue culture. Despite this, both 229E and NL63 were discovered because they produced a detectable cytopathic effect, the first in human embryonic kidney[18] and the second in LLC-MK2 cells.[27] Both the SARS-CoV and the MERS-CoV were initially isolated and grew readily in Vero cells.[6,25] HCoVs OC43 and HKU1 have been grown in tissue culture after laboratory adaptation or in primary ciliated human airway epithelial cells.[58,59] Detection of all these viruses in clinical specimens is most conveniently and sensitively achieved using PCR.

The enteric CoVs have been difficult to cultivate in vitro. All but a few strains have been detected only by electron microscopy of human fecal material. Two strains obtained from an outbreak of necrotizing enterocolitis in Texas were reported to contain four or five proteins with apparent molecular weights similar to those of other CoVs but not related antigenically to known human or animal strains.[60] The evidence favors the view that these isolates, as well as particles antigenically related to HCoV-OC43, are members of the family Coronaviridae, although their association with human disease is not proven.

EPIDEMIOLOGY

CAR Coronaviruses

Evidence of CAR CoV infections has been found wherever in the world it has been sought (e.g., Japan,[61] Ghana[62]). In temperate climates, CAR CoV infections occur more often in the winter and spring than in the summer and fall. The contribution of CAR CoV infections to the total

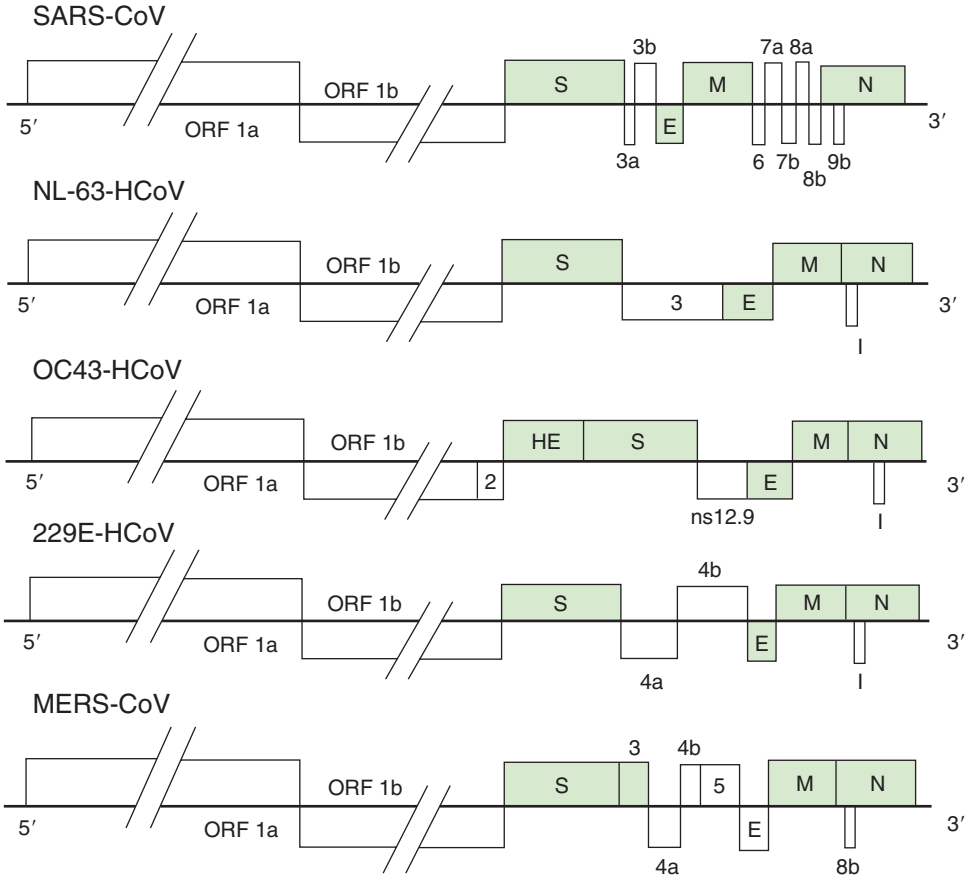

FIG. 155.3 Genome organization of representative human coronaviruses. All coronavirus genomes have the same basic structure and mechanism of replication.[4] The 5′ end of each genome encodes a leader sequence, which is attached to each virus-specific messenger RNA transcript by a novel mechanism of discontinuous replication. The first two-thirds of each genome encode replicase-associated genes. Gene 1 is translated as two large polyproteins, with the first expressed from ORF1a and the second from ORF1a/b following a −1 frameshift event. These polyproteins are then cleaved into individual proteins by two virus-encoded proteases. The major structural genes, the hemagglutinin-esterase *(HE),* surface *(S),* envelope *(E),* transmembrane *(M),* and nucleocapsid *(N)* proteins, are indicated in green. The nonreplicase, accessory genes located at the 3′ end of the genome are indicated with open boxes. The functions of these proteins are largely not known, and there is no sequence homology between accessory proteins of different coronaviruses. Some of these proteins are virion associated, but none is required for virus replication. The open reading frames (*ORFs*) encoding these proteins are numbered in order of appearance from the 5′ end of the genome, with the exception of ns12.9 of human coronavirus *(HCoV)*-OC43. I is an internal protein expressed from an alternative reading frame located within the N gene. It is equivalent to severe acute respiratory syndrome coronavirus *(SARS-CoV)*–specific protein 9b and the Middle East respiratory syndrome coronavirus *(MERS-CoV)*–specific protein 8b. *(Figure prepared by Rahul Vijay.)*

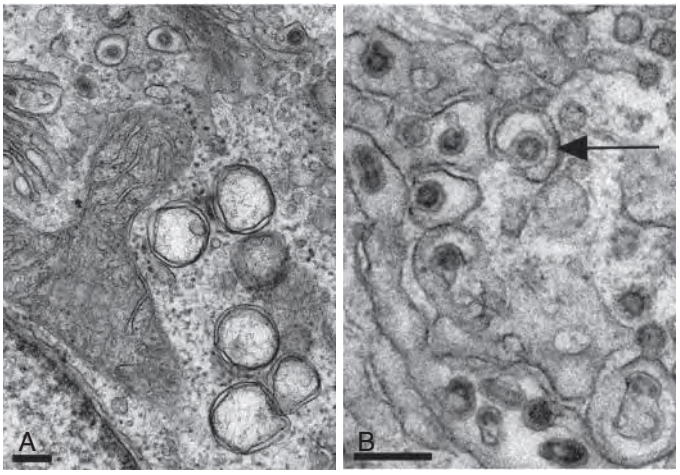

FIG. 155.4 Coronavirus strain 229E in Huh-7 cells. Huh-7 cells infected with HCoV-229E and fixed at 18 hours postinfection. (A) Double membrane vesicles, which are sites of virus replication, are shown. (B) Sites of virus assembly and budding *(arrow)* are shown. *(Images courtesy Drs. Eric Snijder and Montserrat Bárcena, Leiden University Medical Center, The Netherlands).*

number of upper respiratory illnesses may be as high as 35% during times of peak viral activity. Overall, the proportion of adult colds produced by CAR CoVs may be reasonably estimated at 15%,[63] with HCoV-NL63 and HCoV-OC43 being more common than HCoV-229E or HCoV-HKU1 in infants.[63,64]

Early studies of HCoV-OC43 and 229E in the United States demonstrated periodicity, with large epidemics occurring at 2- to 3-year intervals.[65] Similar studies of NL63 and HKU1 have not been done, but it seems from the available data that they also vary widely in incidence from year to year and place to place. Reinfection is common and may be due to the rapid diminution of antibody levels after infection.[66–68] Infection occurs at all ages but is most common in children. The ratio of symptomatic to total infections varies between 50% and 90%, depending on the age of the population studied, the method of virus detection, and the definition of "infection."[67,68] Among adult volunteers, 72% of those infected with HCoV-229E developed colds.[69]

MERS Coronavirus

Middle East respiratory syndrome (MERS) was first identified in 2012 in a man from Jeddah, Saudi Arabia, who developed pneumonia in June and died of respiratory and renal failure.[6] A virus was grown from a sputum sample that was subsequently sequenced and found to be a betacoronavirus most closely related to bat CoVs HKU4 and HKU5.

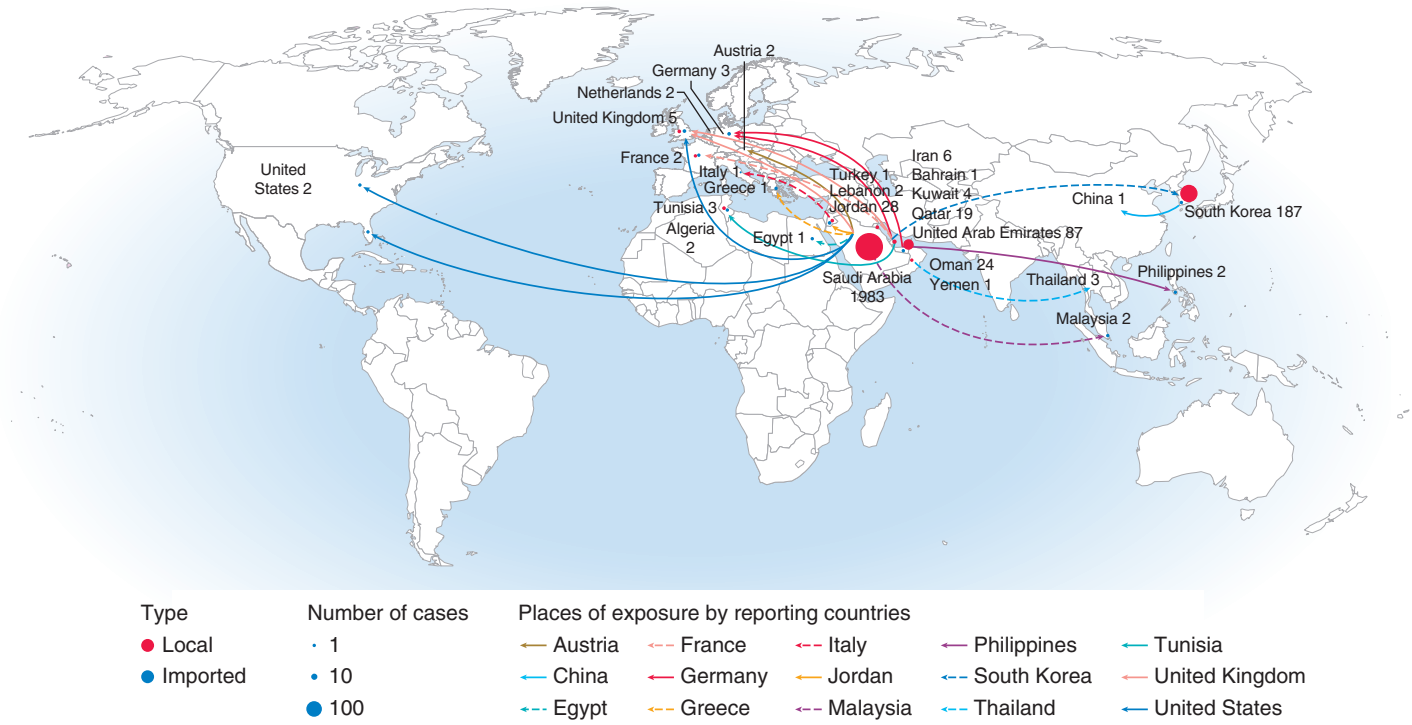

FIG. 155.5 Distribution of confirmed cases of Middle East respiratory syndrome coronavirus by reporting country, March 2012 to March 2019. This map is based on one published by the World Health Organization and shows the predominance of cases reported from the Kingdom of Saudi Arabia, with other cases arising in the Middle East, as well as the routes of travel during importation to other countries *(colored arrows). (Modified from World Health Organization. Middle East respiratory syndrome coronavirus [MERS-CoV].* http://www.who.int/emergencies/mers-cov/en/*. Accessed December 18, 2017.)*

Between then and March 10, 2019, a total of 2374 cases occurred, all infected by this virus, now termed the *Middle East respiratory syndrome coronavirus*.[70] The vast majority of these have been acquired and diagnosed in the Kingdom of Saudi Arabia, with most of the remainder in the United Arab Emirates, Qatar, Jordan, Oman, Lebanon, Iran, and Kuwait (Fig. 155.5). Cases originating in the Arabian peninsula have also occurred in travelers to Egypt, Tunisia, Germany, Italy, Great Britain, Greece, Malaysia, the Philippines, the United States, and the Republic of Korea, with secondary cases sometimes occurring in those locations through close family or hospital spread. In the United States, these include two unrelated MERS cases.[37,38,71]

WHO, the CDC, the Saudi Arabian Ministry of Health, and the Korean Centers for Disease Control and Prevention have published case definitions as well as surveillance instructions to aid in epidemiologic control of the MERS-CoV.[7,8,72,73] The majority of MERS-CoV transmission in the early years after virus identification reflected nosocomial spread.[74] Spread commonly occurred in emergency rooms and during aerosol-generating procedures. Environmental MERS-CoV contamination can be commonly detected in the vicinity of MERS patients, indicating that fomite and contact spread could occur and emphasizing the need for careful surface hygiene management.[75] However, as better infection control measures have been followed in hospitals, approximately 50% of cases are now believed to be primary cases, often acquired from camels.[76] (Of note, only a minority of presumptive primary cases describe camel contact.[11]) Virus may spread from camels through exposure to nasal or other body secretions, via the consumption of raw camel milk, or via environmental contamination[12] since virus can survive on hard surfaces for at least 48 hours.[77] Spread within family settings outside of the hospital is uncommon.[78]

In May and June 2015, a large outbreak of MERS occurred in South Korea; the index case was in a traveler returning from the Arabian Peninsula.[79] By the end of the outbreak, 186 cases, including 38 deaths, had been reported in Korea among household and hospital contacts. The large number and severity of cases resulted from several factors, including lack of timely diagnosis, "doctor-shopping," "super-spreading events," patterns of familial caregiving, and inadequate hospital infection control measures.[80,81]

Studies of MERS prevalence rely on virus detection at the time of acute infection or on measurements of anti–MERS-CoV antibodies. Prevalence studies may underestimate the numbers of exposed patients because antibody titers either do not develop (subclinical infection) or are transient (mild pneumonia).[78,82] Measurements of human MERS-CoV T-cell responses, although technically challenging, may provide better estimates of prevalence since they tend to decline less rapidly in both SARS and MERS survivors.[83,84]

Camels are almost certainly the major if not sole source for human infections. High percentages of dromedary camels that are currently or were previously infected have been detected throughout the Arabian peninsula, Africa, central Asia, and Pakistan.[12] Serologic studies demonstrated the presence of MERS-CoV antibodies in camels in Africa since at least 1983,[85] even though the first human infection was not diagnosed until 2012. MERS-CoVs have only been rarely detected in other animal species, with no evidence that they are involved in transmission to humans. Circulating MERS-CoVs with 98% to 99% identity were detected in camels and patients in the Kingdom of Saudi Arabia,[13] and in some cases virtually identical viruses were detected in patients and their contact camels.[15,86] While camels are the probable source for human MERS, bats may be the original source for the virus. Viruses related to MERS-CoV have been isolated from bats in Africa.[14,87]

SARS Coronavirus

The SARS epidemic began in Guangdong Province in the People's Republic of China in mid-November 2002.[22] It came to worldwide attention in March 2003 when cases of severe, acute pneumonia were reported to WHO from Hong Kong, Hanoi, and Singapore. Disease spread in hospitals to health care workers, visitors, and patients; among family members; and, on occasion, in hotels, apartment complexes, markets, and airplanes. Worldwide spread was rapid but focal. The

largest numbers of cases were reported from the People's Republic of China, Hong Kong, Taiwan, Singapore, and Toronto, Canada. The overall case-fatality rates in these locations ranged from 7% to 17%, but persons with underlying medical conditions and those older than 65 years of age had mortality rates as high as 50%. There was no mortality in children or in adults younger than the age of 24 years.[88]

In response to the global spread and associated severe disease, WHO coordinated a rapid and effective control program that included isolation of cases, careful attention to contact, droplet and airborne infection control procedures, quarantine of exposed persons in some settings, and efforts to control spread between countries through travel advisories and travel alerts. Presumably as a result of these efforts, global transmission ceased by July 2003. A few subsequent cases of SARS were detected, but all were either a result of laboratory spread or individual cases related to presumed contact with civet cats or other intermediate hosts. The last known case occurred in mid-2004.[89]

Spread of SARS to humans is thought to have occurred primarily through droplet or contact transmission, with a possible role for fomites. In most instances, an individual case transmitted to very few others, although super-spreading events were well documented,[90] likely involving small-particle airborne transmission.[91] Spread in hospital settings appeared to be surprisingly efficient, but it could be effectively suppressed with the enforcement of droplet and contact precautions and airborne precautions during aerosol-generating procedures.[92] Containment measures were efficacious, in part, because patients were most contagious only after lower respiratory disease developed.[93] The chain of spread was finally broken in the People's Republic of China, the last country to experience epidemic spread, in July 2003.

It now seems almost certain that the human epidemic began with the spread of a closely related bat virus first to palm civets or other animals sold in live wild game markets and then to humans in Guangdong Province in the People's Republic of China, and that the virus adapted itself through mutation and possibly recombination, until it transmitted readily among humans.[3,94–96] The virus that spread worldwide came largely from a single infected individual who traveled from Guangdong Province to Hong Kong and infected a large number of individuals before himself succumbing to the disease. In contrast, the virus that was epidemic in the People's Republic of China was more variable.

Gastrointestinal Coronaviruses

Although an etiologic role is not proven, enteric CoVLPs have been most frequently associated with gastrointestinal disease in neonates and infants younger than 12 months. Particles have been found in the stools of adults with the acquired immunodeficiency syndrome.[97,98] Asymptomatic shedding is common, particularly in tropical climates[99] and in populations living in poor hygienic conditions.[100] The particles can be detected for prolonged periods and without any apparent seasonal pattern.[101–103]

PATHOGENESIS

CAR Coronaviruses

CAR CoVs (HCoV-229E, OC43, NL63, HKU1) generally replicate in ciliated (HCoV-OC43, NL63, HKU1) and nonciliated (HCoV-229E) epithelial cells of the nasopharynx,[104] probably producing both direct cell degeneration[105] and an outpouring of chemokines and interleukins, with a resultant common-cold symptom complex similar to that produced by rhinovirus infection.[106] The incubation period is, on average, 2 days, and the peak of respiratory symptoms, as well as viral shedding, is reached at approximately 3 or 4 days after inoculation.[69]

The pattern of virus replication of CoVs is at least in part determined by virus-receptor interactions. The two best-defined receptors for the CAR CoVs are APN for strain HCoV-229E and ACE2 for NL63.[51,52]

MERS Coronavirus

Understanding of the pathogenesis and pathology of MERS has been hampered by a lack of surgical and autopsy specimens, largely for cultural and religious reasons. Autopsies from an immunocompetent patient in the United Arab Emirates and an immunocompromised patient in the Kingdom of Saudi Arabia revealed severe changes in the lungs, including hyaline membrane formation, alveolar fibrin deposition, and alveolar septal edema.[107,108] Hemorrhagic changes were observed in the lungs of the immunocompromised patient. Viral protein was detected by immunohistologic staining in the lungs of the patient from the United Arab Emirates. Particles resembling HCoVs were seen by electron microscopy in both lungs and kidney of the immunocompromised patient, but immunohistology was not recorded.

SARS Coronavirus

The pathogenicity of SARS includes systemic spread. Although the lung is the primary focus of the disease process, there are often signs of involvement in other organ systems, including diarrhea, leukopenia, thrombocytopenia, and, most notably, pan-lymphopenia.[109] Virus has been detected in respiratory secretions, blood, stool, and urine specimens and in lung, spleen and lymph nodes, brain, kidney, and intestine tissues when examined at autopsy.[110,111] On the basis of PCR testing, virus titer is highest during the second week of illness[112] and can often be detected in the stool into the third week of illness or longer.[26] Pulmonary symptoms may worsen late in the course of the illness, with the development of adult respiratory distress syndrome.[112] There may also be late evidence of liver and kidney involvement.

The pulmonary pathology of infection by the SARS-CoV has been described extensively,[25,111,113,114] but less has been published about the pathology in other organ systems.[110,111,115] The extrapulmonary pathologic changes found most consistently at autopsy are extensive necrosis of the white pulp of the spleen and a generalized small vessel arteritis. In the lung, there is hyaline membrane formation, interstitial infiltration with lymphocytes and mononuclear cells, and desquamation of pneumocytes in the alveolar spaces. Giant cells are a constant finding and usually have macrophage markers. In bronchoalveolar lavage, biopsy, and autopsy specimens, viral particles and viral RNA and protein have been noted in type I and II pneumocytes.[110,116]

CLINICAL MANIFESTATIONS

CAR Coronaviruses

Administration of antigenically distinct CAR CoV to volunteers produced illness with similar characteristics.[16,69,117] A summary of these characteristics is given in Table 155.1, in which a comparison is made with colds produced by rhinoviruses in similarly inoculated volunteers. The incubation period of CoV colds was longer and their duration somewhat shorter, but the symptoms were similar. Asymptomatic infection was sometimes seen and, indeed, has been a feature of both serologic surveys and PCR-based studies of natural infection of infants, children, and adults.[118,119]

More serious respiratory tract illness is probably also caused by all four strains of CAR HCoV. The evidence for this is not conclusive, but it seems likely that all strains can produce pneumonia and bronchiolitis in infants,[35,39,120,121] otitis and exacerbations of asthma in children and young adults,[122–124] pneumonia in healthy adults,[125] exacerbations of asthma and chronic bronchitis in adults,[126–128] influenza-like illness, serious bronchitis and pneumonia in the elderly,[63,67,129,130] and pneumonia in the immunocompromised host.[131,132] HCoVs are found in asymptomatic individuals of all ages, and, when accompanied by illness, are also sometimes accompanied by infections with other potential respiratory pathogens. Infection without disease and coinfection during disease are features of many respiratory pathogens, including rhinoviruses, adenoviruses, human metapneumovirus, human bocavirus, and parainfluenza viruses, but also (although less frequently) respiratory syncytial virus and influenza virus, making pathogenicity difficult to prove. Because infections with CAR HCoVs are so common, however, it is possible that they are responsible for a significant portion of these serious lower respiratory tract diseases, even though the basic pathogenicity of HCoVs (judging from volunteer studies) is similar to that of rhinoviruses, and clearly less than that of respiratory syncytial virus, influenza viruses, and certain adenovirus types. There is some evidence that HCoV-OC43 is more pathogenic in the elderly than HCoV-229E[133] and that NL63 differs from the other CAR HCoVs in preferentially causing childhood croup.[36]

MERS Coronavirus

MERS-CoV predominantly, if not solely, initiates infection via the respiratory tract. The median incubation period is 7 days, with a

TABLE 155.1 Clinical Features of Colds Produced by Experimental Infection With Four Viruses

	CORONAVIRUSES		RHINOVIRUSES	
FEATURE	**229E**	**B814**	**Type 2 (HGP or PK)**	**DC**
No. of volunteers inoculated	26	75	213	251
No. (%) getting colds	13 (50)	34 (45)	78 (37)	77 (31)
Incubation period (days)				
Mean	3.3	3.2	2.1	2.1
Range	2–4	2–5	1–5	1–4
Duration (days)				
Mean	7	6	9	10
Range	3–18	2–17	3–19	2–26
Maximum no. of handkerchiefs used daily				
Mean	23	21	14	18
Range	8–105	8–120	3–38	33–60
Malaise (%)	46	47	28	25
Headache (%)	85	53	56	56
Chill (%)	31	18	28	15
Pyrexia (%)	23	21	14	18
Mucopurulent nasal discharge (%)	0	62	83	80
Sore throat (%)	54	79	87	73
Cough (%)	31	44	68	56
No. (%) of volunteers with colds of indicated severity				
Mild	10 (77)	24 (71)	63 (80)	36 (47)
Moderate	2 (15)	7 (20)	12 (15)	28 (36)
Severe	1 (8)	3 (9)	4 (5)	13 (17)

From Bradburne AF, Bynoe ML, Tyrrell DAJ. Effects of a "new" human respiratory virus in volunteers. Br Med J. *1967;3:767–769.*

range of 2 to 17 days.[9,79] MERS-CoV causes a spectrum of illness ranging from subclinical disease to lethal pneumonia.[134–136] Although infections with severe respiratory involvement have occurred at all ages, the elderly and those with underlying conditions (diabetes, renal disease, immunosuppression) are the most often severely or fatally affected.[134,135,137] MERS requiring admission to the intensive care unit cannot be distinguished by clinical, radiologic, or standard laboratory criteria from other causes of severe pneumonia, so laboratory-based diagnosis is critical.[138,139] Outcomes are worse in critically ill MERS compared to non-MERS patients, with mortality rates of approximately 58% to 78% in patients admitted to the intensive care unit.[134,135,138,139] Health care workers typically develop mild disease, although MERS-CoV has also caused lethal disease in this group.[140] Patients usually present with nonspecific symptoms, including fever, shortness of breath, cough, fever, and diarrhea. Radiographic examination of MERS patients revealed a ground-glass appearance, initially located in the lung periphery. The presence of a pleural effusion was associated with a worse outcome.[141–143]

Acute renal injury occurs commonly in patients in the Arabian peninsula with severe disease, but less so in Korean patients, possibly reflecting differences in the extent of comorbidities such as diabetes and hypertension.[37,38,135,136,138] The MERS-CoV host cell receptor, dipeptidyl peptidase 4, is expressed at high levels in the kidney,[144] raising the possibility that direct infection of this organ contributes to renal disease. Particles resembling coronaviruses were seen by electron microscopy in the renal proximal tubular cells of a fatal case of MERS in a man who had recently received chemotherapy for T-cell lymphoma.[108] MERS patients often presented with hematologic abnormalities, including leukopenia, lymphopenia, and thrombocytopenia and elevated lactate dehydrogenase, alanine aminotransferase, and aspartate aminotransferase, although this was less common than observed in patients with SARS.[37,138,145] As in patients with SARS, expression of proinflammatory mediators such as interferon-α, interleukin-6, and chemokine (C-X-C motif) ligand 1 is prolonged in severe compared to mild cases. Virus-specific antibody responses developed more rapidly and robustly in MERS patients who survived the infection than in those who died.[146]

Pediatric patients with MERS generally develop mild or subclinical disease, although fatal disease in patients with underlying disease has been reported. MERS in pregnancy has been associated with fetal and/or maternal demise.[147–150]

SARS Coronavirus

SARS is generally initiated through the respiratory tract. After an incubation period that is usually 4 to 7 days, but could be as long as 10 to 14 days, the disease begins, usually with fever and other systemic (influenza-like) symptoms, with cough and dyspnea developing a few days to a week later.[22,151] Approximately 25% of patients have diarrhea. Interestingly, upper respiratory symptoms such as rhinorrhea and sore throat usually do not occur.[112,151–154] The chest radiograph is frequently abnormal, showing scattered airspace opacification, usually in the periphery and lower zones of the lung.[155] Spiral computed tomography demonstrates both ground-glass opacification and consolidation, often in a subpleural distribution.[155–158]

Lymphopenia is common,[112,113,159] with normal or somewhat depressed neutrophils. Paradoxically, neutrophilia was associated with poor outcomes.[109] Creatine kinase is often abnormal, as are lactate dehydrogenase and aspartate aminotransferase. Levels of proinflammatory cytokines were elevated at early times during infection in patients with severe clinical disease[160] and decreased in those patients who resolved the infection.[161]

Approximately 25% of patients develop severe pulmonary disease that progresses to adult respiratory distress syndrome, most commonly in patients older than 50 years or with underlying disease such as diabetes, cardiac disease, and chronic hepatitis.[112,154,159,162] The overall mortality rate is between 9% and 12%, with the highest rates in the elderly and adults with underlying liver disease. In some patients, clinical deterioration occurred during the second week of illness, as virus levels decreased, suggesting that disease was partly immune mediated.[112,154,159,162] Clinical improvement was associated with the onset of a virus-specific antibody response.[161]

Pediatric disease is significantly less severe than adult disease, although the features are similar.[163] Disease during pregnancy is severe, with high mortality in both the mother and fetus.[164] Congenital transmission has not been described.

Gastrointestinal Coronaviruses

The nature of the illness associated with enteric CoV infection is much less clear. One study found a significant association of gastroenteritis in infants 2 to 12 months of age with the presence of CoVLPs in the stool.[165] Another study, confined to infants in a neonatal intensive care unit, found highly significant associations between the presence of CoVLPs in the stool and the presence of water-loss stools, bloody stools, abdominal distention, and bilious gastric aspirates.[103] Finally, CoVLPs have been associated with at least three outbreaks of necrotizing enterocolitis in newborns.[60,103,166] Efforts to detect HCoV RNA by PCR using primers that would detect the known CAR HCoVs in stool have been disappointing, with most HCoV-positive samples also containing rotavirus or norovirus.[39,40,167,168] Of note, all of the studies that associate CoVLPs with gastrointestinal disease were done before the development of diagnosis by PCR.

Neurologic Syndromes

Like many other viruses, CoVs have been sought as possible etiologic agents in multiple sclerosis. The search has been stimulated by the capacity of JHM, a well-studied strain of mouse hepatitis virus, to produce in mice and rats an immune-mediated chronic demyelinating encephalitis histologically similar to multiple sclerosis.[169] HCoV-OC43[170,171] and HCoV-229E[172] have been detected in brain tissue from multiple sclerosis patients using virus isolation,[170] in situ hybridization, immunohistology,[171] and PCR.[172] The strongest support for CoV-mediated infection of the brain comes from a study in which HCoV-OC43 was identified in neurons in an 11-month-old patient with severe combined immunodeficiency and lethal encephalitis.[173] Except for this one report, evidence is lacking to establish an etiologic or pathogenic association of CoVs with human central nervous system disease.

LABORATORY DIAGNOSIS

CAR Coronaviruses

Although some human CAR CoVs grow in tissue culture directly from clinical samples and although antigen detection systems have been developed for both HCoV-OC43 and HCoV-229E,[174,175] laboratory diagnosis of CoV respiratory infections is best accomplished by molecular methods. Reverse-transcriptase PCR (RT-PCR) systems have been developed using many different primers and detectors.[176–182] From a clinical point of view, a single generic test for respiratory CoVs would be desirable, and such tests have been developed. However, when tested side by side with specific systems, the generic systems have somewhat lower sensitivity.[120]

MERS Coronavirus

MERS-CoV was originally isolated in Vero and LLC-MK2 cells, and standardized methods using RT-PCR–based examination of respiratory and other clinical samples have been published.[183,184] These methods can detect as little as 10 to 15 copies of viral RNA. Peak virus titers were detected at approximately 14 days after infection, and viral RNA could be detected for greater than 21 days in patients with severe disease.[185] MERS-CoV infection can be diagnosed beginning 2 to 3 weeks after the onset of illness using serologic methods, enzyme-linked immunosorbent assay (ELISA) initially, with confirmation by indirect immunofluorescence assays (IFA) and neutralization assays.[186] The transient nature of the antibody response in MERS patients with subclinical or mild disease has diminished the diagnostic utility of this approach.[78,82]

SARS Coronavirus

Although SARS-CoV was grown from respiratory tract specimens in Vero E6 and fetal rhesus monkey kidney cells, the more sensitive and rapid RT-PCR assays were most widely used to detect infection. Virus was detected by RT-PCR in upper and lower respiratory tract, blood, stool, and urine specimens. Early in the illness, specimens were found positive only in approximately one-third of patients.[112] Use of samples from multiple sources increased the yield. Virus was detected most frequently during the second week of illness.[112,187]

Antibody tests have been developed using tissue culture–grown virus and ELISA and IFA. Immunoglobulin M antibody can be detected in most patients for a limited period of time, and immunoglobulin G antibody appears first approximately 10 days after onset of fever in patients with good outcomes and becomes essentially universal after 4 weeks.[112,161]

THERAPY

Given the severity of SARS, clinicians throughout the world empirically treated most patients with corticosteroids and intravenous or oral ribavirin.[188] It is now known that ribavirin has little activity against SARS-CoV in vitro, and corticosteroid usage may have resulted in worse outcomes.[189,190] Lopinavir-ritonavir and intravenous immune globulin were also used in some patients, without conclusive evidence that they were helpful or harmful. There is anecdotal evidence of the benefit of either interferon-α or interferon-β treatment. However, no therapy has proven efficacy, and therapy continues to be largely supportive.[190]

Similarly, treatment of MERS-CoV infection at present depends entirely on supportive measures. No antiviral drugs are recommended,[191,192] although several studies have indicated that MERS-CoV is more sensitive to interferon-α or interferon-β than is SARS-CoV.[193,194] Combinations of interferon alfa-2b and ribavirin inhibit MERS-CoV in vitro, and administration of the combination improved outcomes in MERS patients at 14 but not 28 days.[195] A new broad-spectrum drug, GS-5734, has demonstrated efficacy against SARS-CoV and MERS-CoV in cells and experiment animals and may be a useful therapeutic option.[196] Standard droplet precautions should be used, with aerosol precautions during certain high-risk procedures.[197]

PREVENTION

Rigorous application of hospital infection control procedures, particularly those directed at contact and droplet spread, was shown to have a major beneficial effect on the spread of the SARS-CoV.[92] The containment of the global SARS outbreak is a testament to the power of the cooperation and collaboration engendered by WHO to address a major public health threat. Similarly, standard droplet precautions are recommended for patients with suspected or confirmed MERS-CoV infections, with aerosol precautions during certain high-risk procedures.[196,198,199] Suitable barrier precautions are especially critical in the case of MERS patients since a large fraction of infections result from nosocomial transmission.[11,74]

Vaccines for animal CoVs have been developed and widely used with variable efficacy. Vaccines are likely to be key in disrupting MERS-CoV camel-to-human and interhuman transmission. A variety of vaccination strategies, including inactivated, subunit, live-attenuated, DNA, and nanoparticle vaccines, are being pursued.[200,201] Vaccines will need to be carefully evaluated because an inactivated SARS-CoV vaccine and a feline infectious peritonitis surface glycoprotein vaccine caused immunopathologic disease after challenge.[202–204] Monoclonal antibodies that neutralize MERS-CoV have been identified and proposed for use therapeutically.[205,206] Given the small number of human cases and the high prevalence of virus in camels, vaccination of juvenile camels may be the preferred approach to protecting human populations.[207] Such vaccines are under development.

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