Viral Diagnostics

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Background and Introduction

Advances in diagnostic testing for transplant-related infections, particularly molecular viral diagnostic assays, constitute one of the most notable changes in transplant infectious disease over the last two decades [1, 2]. This chapter discusses recent developments in diagnostics for cytomegalovirus (CMV), Epstein-Barr virus (EBV), BK virus (BKV), community respiratory viruses (CRVs), parvovirus, hepatitis viruses, HIV, and other viral agents of importance in solid organ and hematopoietic stem cell transplantation. The recent debate regarding the extent of nucleic acid amplification (NAT) testing for HIV, HBV, and HCV in proposed transplant donors is reviewed [3]. Different uses for molecular viral tests in the transplant recipient are discussed, ranging from facilitation of antiviral preventive strategies to determination of length of therapy for active infections. The advantages and disadvantages of single vs. multiplex assays are explored [2]. Challenges in this field include interlaboratory variation [4], management of false-positive and discordant test results, and need for consensus on which patients should receive which testing, at what intervals, and for what period of time. Despite these challenges, molecular viral diagnostics have clearly contributed significantly to the reduction of infectious morbidity, by enabling early diagnosis and intervention, resulting in such notable examples as the reduction in severe CMV disease [5, 6] and in kidney allograft loss due to BKV [7]. Future clinical trials in the field of transplantation should incorporate accepted definitions of infection and practices of viral monitoring for transplant-associated viruses [8].

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General Considerations, Definitions, and Uses of Viral Diagnostic Tests

The term "serology" or "serologic test" refers in general to an assay which detects an antibody to a specific pathogen, usually IgG or IgM. A panel of serologic tests is performed on both donor and recipient prior to transplantation. The results may be used to disqualify a prospective donor or to restrict the use of the donor to a specific subgroup of recipients or more commonly may be used for risk stratification and posttransplant management for particular infections (e.g., the donor-seropositive, recipient-seronegative or D+/R- group which is the subgroup at the highest risk for both CMV and EBV, respectively, in solid organ transplantation) [9]. Serologies are of limited value in diagnosing active infections in the posttransplant patient, since immunosuppressed patients may not mount an IgM response even in the setting of an active infection and some recipients with de novo posttransplant hypogammaglobulinemia have globally low IgG levels [10]. IgG serology remains positive for life, and pathogen-specific IgG titers do not usually correlate with the activity of infection, so obtaining an IgG level (for CMV or EBV, among others) is not generally helpful in diagnosing an acute illness in a transplant recipient (an exception is when the clinician wants to know if a previously seronegative patient has seroconverted, which might have prognostic value, for example, in predicting ongoing risk for recurrences of CMV viremia) [11].

Antigen-based testing, such as the pp65 antigenemia test for CMV, does have a potential role in posttransplant recipients, as this is a direct detection of the virus and not a reflection of the patient's immune response to the virus [12, 13]. However, in most cases, antigen detection is semiquantitative and does not provide an exact viral load to follow over time. In addition, some antigen tests (such as the pp65 antigenemia test for CMV) decay with time, and thus lose sensitivity, if the sample is mailed into a central laboratory or if there is a delay between obtaining the blood sample and laboratory performance of the test.

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Table 49.1 Molecular tests for selected transplant-related viruses

Test Name	Method	Dynamic range at Copies/ml	Sensitivity	Specificity	Test Status
Qiagen Artus CMV RGQ	Real-time PCR	159 IU/ml–1.0 × 10^7 IU/ml	96.6%	100%	IVD
Abbott RealTime CMV	Real-time PCR	31.21 IU/ml–156 × 10^6 IU/ml	95–97%	99%	IVD
Roche CMV [19]	Real-time PCR; CAP/CTM	137 IU/ml–1 × 10^6 IU/ml	97.5–98.0%	100%	IVD
RealStar qCMV (Altona) CMV	Real-time PCR	150 IU/ml–1 × 10^6 IU/ml	100%	100%	ASR
Qiagen Artus EBV	Real-time PCR	500–5.0 × 10^6 IU/ml	95–97%	99%	ASR
RealStar EBV qPCR (Altona)___	Real-time PCR	500–10,000,000 copies/ml	94.5%	98.1%	ASR
Qiagen BKV	Real-time PCR	500–5.0 × 10^6 copies/ml	95–97%	99%	ASR
RealStar BKV (Altona)	Real-time PCR	300–100,000,000 copies/ml	100%	100%	ASR

ASR analyte-specific reagents, *IVD* in vitro diagnostic test (FDA-cleared)

Molecular testing has revolutionized viral diagnosis in transplantation [14–16]. Molecular diagnostic tests are generally highly sensitive assays that directly detect the virus' genetic material such as DNA or RNA (depending on the type of virus) and can be qualitative or quantitative. There are a variety of methodologies, including polymerase chain reaction (PCR) technologies, hybrid capture assay, nucleic acid sequence-based amplification (NASBA), transcription-mediated amplification (TMA), and others [1, 2, 17, 18]. Performance characteristics of some of these tests in comparative studies are shown in Table 49.1**.** Testing may be *monoplex* (a single pathogen tested at one time) or *multiplex* (which refers to several or many pathogens tested in one sample).

The advantages and disadvantages of each strategy are discussed below. The uses of molecular diagnostic assays are many. Most commonly these tests are performed on whole blood or plasma (and may be referred to as the blood "viral load") when quantifying the virus, although other relevant samples may be tested, such as urine in the case of BK virus or a nasopharyngeal swab or bronchoalveolar lavage fluid in the case of respiratory viruses. A list of potential uses for quantitative molecular diagnostic tests is shown in Table 49.2. The most common uses are in preemptive therapy or screening for viral infection prior to symptoms, in diagnosis of an acute infectious syndrome, and in monitoring for blood viral clearance to help determine the duration of therapy for an infection episode**.**

An even newer set of diagnostic tests is currently under development, namely, pathogen-specific assays of cellular immune function. Of these, the one in widest use so far is the interferon-gamma release assay (IGRA) for tuberculosis (QuantiFERON-TB Gold in-tube, Cellestis/Qiagen Inc., Germantown, Maryland) [20]. This assay is specific for *Mycobacterium tuberculosis* and avoids false-positive results due to BCG vaccination, as with the tuberculin skin test [20]. Similar assays for CMV and BKV have been an area of intense research interest. Recent results suggest that measurement of CMV-specific immune function is useful in risk stratification of high-risk organ transplant recipients [21] and in hematopoietic stem cell transplant recipients [22]. It is likely that these tests will be more commonly used in the future in transplant virology, with an eye to devising personalized prevention programs using assessments of individual patients' pathogen-specific immunity. However, testing for virus-specific cell-mediated immunity has not yet become widely performed at the time of this writing, so the current chapter will focus mainly on molecular diagnostic testing.

Table 49.2 Potential uses for quantitative molecular diagnostic tests

Screening of living or deceased prospective organ donors
Diagnosis of an acute infectious syndrome
Preemptive therapy or monitoring/screening for viral infection
Prediction of severity of disease (quantitative viral load)
Monitoring for resolution of infection and guidance for duration of therapy
Monitoring for recurrence of infection after completion of therapy
Determining the success of viral suppression or secondary prophylaxis
Clues to the presence of antiviral resistance (rise in viral load or failure to decrease on therapy)
Genotypic antiviral resistance testing (e.g., UL97 or UL54 mutations in CMV)

Cytomegalovirus

CMV remains one of the most common viruses to reactivate in the posttransplant patient. In the early years of transplantation, diagnosis of CMV infection relied on detection of viral growth in tissue culture, which was laborious and could take several weeks for a positive result to be obtained, particularly if the samples have low viral load. The advent of shell-vial centrifugation culture methodology shortened the turnaround time from 4–5 days to 48 h, but this test was less sensitive at lower viral loads and did not provide quantitative results [13]. The pp65 antigenemia test was then devised, and multiple studies have validated its utility in posttransplant monitoring and as a basis for preemptive therapy [12, 13, 23]. The pp65 antigenemia test detects CMV-infected white blood cells in peripheral blood using a

fluorescent assay which requires the laboratory technician to visually scan the slide and to report the number of positive (infected) cells per unit of area. It is thus a semiquantitative test. Although this does give some idea of the magnitude of the viral load, it is not as definitive in viral load measurement as quantitative molecular tests, which are usually expressed as DNA copies/ml or, most recently, in international units. The pp65 antigenemia assay is labor-intensive for the laboratory and thus may be problematic for transplant centers with very high volumes of tests. In addition, the results decay after obtaining the blood sample, so it is less suitable for mailing in to a central laboratory from patients who live a long distance away from the transplant center.

Molecular diagnostic tests for CMV have largely supplanted previous tests at many centers. Their quantification of the blood viral load, ease of handling high volumes of samples, and lack of decay with time if properly handled make the CMV DNA by PCR a useful choice for a transplant center with high volumes of samples and/or patients outside the immediate area. Quantitative viral loads often correspond to severity of disease, although interlaboratory variation has hampered the attempt to describe universal cutoff values for clinical categories and decision-making [4, 24, 25]. In solid organ transplant recipients, tissue-invasive CMV episodes generally have the highest viral loads (e.g., >50,000–100,000 copies/ml); asymptomatic viremia has the lowest viral loads (e.g., <5000 copies/ml); and the intermediate category of "CMV syndrome" has viral loads in between the other two categories, although exceptions may occur. However, widespread adoption of the WHO standard should allow for more reliable, shared correlations between viral loads and clinical manifestations, after the initial period of clinician adjustment to a new scale [25]. The quantification of the viral load also allows for following levels over time, so that treatment decisions, including when to initiate antiviral therapy, when to discontinue antiviral therapy, or when to switch from full-dose therapy to secondary suppressive dose prophylaxis, can be based on serial results of these quantitative tests (Table 49.2). A notable example of the use of sequential viral load measurements is the use of a risk-adapted, CMV viral load-based preemptive therapy program for CMV prevention in hematopoietic stem cell transplant recipients, utilized at the Fred Hutchinson Cancer Research Center [26].

Molecular tests are not without problems, however. PCR is a highly sensitive test, and false positives can occur, leading to unnecessary therapy or unnecessary concern on the part of patients and clinicians; such false-positive tests, however, are usually low level and may be subjected to repeat analysis or verified by obtaining a new sample. The risks of false-positive testing in disqualification of potential donors have been a topic of discussion regarding the revised solid organ transplant donor guidelines [3].

Other potential problems with molecular testing include logistics. A highly developed system must be in place, particularly for preemptive therapy, for the loss of even one sample or failure to act upon one sample result might lead to full-blown symptomatic infection. But perhaps the most problematic aspect is that of interlaboratory variation [4, 24, 25], depending on the use of whole blood versus plasma; commercial versus individually developed assays, different reagents, and primers; and a host of other factors. The American Society of Transplantation (AST)'s Infectious Disease Community of Practice, together with the Canadian Society of Transplantation, published an interlaboratory comparison of CMV PCR testing involving 33 laboratories, showing wide variation in results (between a 2- and 4-$\log_{10}$ copies/ml difference in some cases) and need for more standardization [4]. The World Health Organization (WHO)'s standardization initiative should help to ameliorate this situation and to improve the comparability of viral loads obtained in different laboratories. As of 2010, the WHO announced an international standard for CMV molecular testing, which enables laboratories to calibrate their assays and which involves reporting in international units per mL [25]. Another recent development, in 2012, was the first FDA approval of a quantitative CMV PCR test (the COBAS AmpliPrep/COBAS TaqMan CMV test or CAP/CTM CMV test) which is a fully automated test, and the one copy of CMV DNA (as defined by the COBAS® AmpliPrep/COBAS® TaqMan® CMV test) is equivalent to 0.91 international unit (IU) on the First WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (NIBSC 09/162) [19, 25]. An international multicenter comparison of the CAP/CTM CMV test in five laboratories at transplant centers compared the performance of this test with local assays, using blinded samples and clinical specimens [19]. This study showed high interlaboratory agreement of the CAP/CTM test and quantification differences using local assays [19]. It has been suggested that this test might serve as the basis for more widely accepted cutoffs for prediction of CMV disease and thresholds for preemptive therapy [25].

A final category of molecular diagnostic tests for CMV is those used to determine genotypic resistance, on analogy to HIV. CMV antiviral resistance commonly occurs in two sites, known as UL97 and UL54 [27, 28]. UL97 relates to the ability of a viral-encoded thymidine kinase to initiate triphosphorylation of ganciclovir to its active form, and thus UL97 mutations confer resistance to ganciclovir but not to foscarnet or cidofovir. UL54 mutations, on the other hand, affect the viral DNA polymerase and so may confer resistance to ganciclovir, foscarnet, or cidofovir or more than one of these [27, 28]. Phenotypic resistance testing is less commonly used now, as it is time-consuming and labor-intensive. Genotypic resistance testing should be obtained in any clinical situation where resistance is suspected, such as a

persistently high CMV viral load, failure of the viral load to decrease on therapy, or clinical lack of response to therapy after sufficient time has elapsed.

Epstein-Barr Virus

The utility of EBV serologic testing such as EBV VCA-IgG is principally in the pretransplant period, establishing whether or not the donor and the recipient have ever been infected with EBV. Most adults, 90% or greater, are EBV-seropositive, although pediatric transplant recipients are more likely to be seronegative [29]. As with CMV, the donor and recipient serogroups carry differential risks for the development of serious infections. In the case of EBV, the main issue is risk for EBV-related posttransplant lymphoproliferative disorder (PTLD). The highest-risk category in solid organ transplantation is the EBV D+/R- category; as similar to the case of CMV, there is no antecedent immunity in the recipient, but a viral load is acquired from the donor at the time of transplantation [29]. Knowledge of this D+/R- status may allow for closer surveillance, modulation of immunosuppression, and, at some centers, serial monitoring of the EBV DNA viral load [30, 31]. There are several different EBV serologies that are commonly performed: the Epstein-Barr nuclear antibody (EBNA), Epstein-Barr early antigen (EA), viral capsid antigen (VCA) IgG, and VCA-IgM. Of these, the VCA-IgG is the most reliable test for assessing whether or not the patient is seropositive (i.e., whether they have ever had EBV infection), while the VCA-IgM correlates better with current or recent disease although IgM response may be blunted in an immunocompromised host, so there is limited utility in ordering EBV serologies in patients following transplantation. For the diagnosis of active EBV infection and the assessment of PTLD risk, obtaining quantitative blood PCR testing is more helpful than serologies [29, 30]. EBV DNA viral loads may be performed on plasma or on whole blood. As in the case of CMV, interlaboratory variation also exists with respect to EBV DNAemia measurement [24]. EBV DNA viral loads may be followed over time in high-risk patients and may be useful as a gauge of the degree of success of interventions such as reduction of immunosuppression, which should be followed by a corresponding decrease in the EBV DNA blood viral load, unless the patient has active PTLD. Green et al. have demonstrated the predictive value of this monitoring [30], and McDiarmid has shown the utility of EBV DNA monitoring, coupled with reduction of immunosuppression and ganciclovir therapy, in the reduction of PTLD risk (from 10% to 5%) in a cohort of pediatric liver transplant recipients [31]. Successful therapy of PTLD with rituximab or rituximab plus combination chemotherapy is often associated with a rapid fall of the EBV DNA blood viral load to undetectable levels. However, later rebounds of EBV DNAemia may occur and do not necessarily portend recurrences of PTLD [30].

BK Virus

BK virus (BKV) is a member of the polyomavirus family, along with JC virus, SV40, and others. Acquisition of BKV is common in the general population and may occur early in life in asymptomatic form. BKV has a predilection for cells of the urinary tract including the bladder, ureters, and kidneys. BKV can cause hemorrhagic cystitis in HSCT recipients. In kidney recipients, its effects can be devastating [7]. After kidney transplant, BKV can silently reactivate and can cause a type of allograft nephropathy (BKVAN) that begins with interstitial nephritis and progresses to fibrosis and nonfunctioning allograft tissue. If no prevention program is in place, between 4% and 8% of all kidney allografts may be lost to BKV.

Screening and early intervention for BKV have been a major advance over the last 10 years and have led to an approximately eightfold reduction in kidney graft loss due to BKV. Most kidney transplant centers now employ BKV screening of asymptomatic patients using one of several available tests on blood or urine [7]. Serial screening for BKV allows for early reduction of immunosuppression, which is the most established therapy for BKV, and may reduce viral load by allowing for a more vigorous host immune response to BKV [7, 32, 33].

The tests available for BKV screening include urine cytology for the evidence of polyomavirus-related changes in the form of inclusion-containing "decoy cells," quantitative or qualitative BKV DNA performed on urine or blood, and BKV VP1 mRNA [34]. If urine is screened, a positive test might trigger testing of blood for the BKV DNA viral load. Blood BKV DNA viral loads correlate more with the presence of BKV in renal allograft tissue, as urine may frequently be positive for lower levels of BKV DNA without active involvement of renal tissue. Urine BKV DNA viral loads are typically several logs higher than blood viral loads. International consensus guidelines have established the blood viral load of 10,000 copies/ml as a common threshold for intervention in kidney recipients [35]. By contrast, BKV blood viral load has not traditionally been considered as predictive of symptomatic disease in HSCT recipients, although recent results by Gilis et al. suggest that BKV viremia is correlated with severity of disease in HSCT also [36].

BKV DNA testing is also useful for monitoring responses to interventions such as reduction of immunosuppression. If reduction of immunosuppression appears not to have produced the desired reduction in viral load, some centers employ off-label antiviral therapies for BKV [37] such as cidofovir, quinolones, intravenous immune globulin (IVIg),

and leflunomide. There are no randomized trials to date comparing these therapies, and reduction of immunosuppression remains the cornerstone of management. Thus, BKV DNA quantitative monitoring can serve as a guide to institution of interventions and as a guide to resumption of full-dose immunosuppression after an episode has resolved.

Routine serial monitoring of BKV DNA is not currently recommended in solid organ transplant recipients other than kidney or kidney-pancreas recipients. In liver, lung, and heart transplant recipients, reactivation of BKV may also occur, but the clinical significance is less certain. In hematopoietic stem cell transplant recipients, routine serial BKV viral load monitoring has not been standard in the past, but may emerge as a strategy in the future based on recent results [36].

Community-Acquired Respiratory Viruses

Community-acquired respiratory viruses (CRVs) pose a threat to transplant recipients in two ways: the risk of severe respiratory involvement during an infection episode and the late risk in lung transplant recipients for transient or permanent decreases in lung allograft function after a CRV infection has resolved [38]. Early diagnosis is crucial in allowing for rapid treatment; a multicenter study of pandemic H1N1 influenza in SOT recipients demonstrated that early treatment, within 2 days of onset of symptoms, was associated with lower risk of ICU admission and respiratory failure [39]. Early and rapid influenza diagnosis is particularly important, as antiviral medications effective against influenza are available. The CDC and Advisory Committee on Immunization Practices (ACIP) publish an annual guide to prevention and treatment of influenza which contains antiviral resistance information pertinent to the particular strains that are circulating in any given influenza season [40]. For other respiratory viruses, there is less agreement on treatment protocols, but many transplant centers use ribavirin (inhaled or oral) for treatment of respiratory syncytial virus (RSV) [41] and sometimes parainfluenza virus and human metapneumovirus (hMP) infections as well [42].

Diagnosis of CRV infections is also very important for infection control programs, as such viruses can spread rapidly through transplant wards and may have devastating effects particularly in patients with recent transplants or active rejection. Different respiratory viruses have different modes of transmission, so droplet precautions, contact precautions, or both may be appropriate. In any case, rapid application of appropriate precautions can prevent harm to other vulnerable hospitalized patients.

Diagnosis of CRVs has traditionally been performed on respiratory samples, most commonly nasopharyngeal (NP) swabs or washes or BAL fluid. Diagnosis may be accomplished by direct fluorescent antibody testing (DFA), by PCR, or by culture in tissue culture. Since culture-based diagnostics take at least several days, these are not suitable for rapid diagnosis and are now utilized primarily for determination of viral viability in a patient who is persistently PCR-positive, for example.

The choice of DFA or PCR for initial testing depends upon the virus(es) being detected. Some multiplex assays are wholly PCR-based and some are a combination of DFA and PCR tests. Since respiratory viruses have considerable overlap in their clinical presenting symptoms, and coinfections may occur, it makes sense to perform a multiplex assay incorporating the most likely agents, rather than testing for a single virus at a time. Common combinations of tests include influenza/RSV, influenza/RSV/parainfluenza/adenovirus/human metapneumovirus, and other more extensive combinations including rhinovirus and coronaviruses. Even rhinovirus infection (the "common cold") may have severe consequences in immunocompromised patients [43], so expanded multiplex testing is increasingly of interest.

In addition to initial diagnosis of an infection episode, repeat testing may be used for assessment of viral clearance in patients with ongoing symptoms or for infection control purposes in determination of the length of isolation precautions. It should be noted, however, that testing which does not rely on viral viability may be detecting residual fragments of nonviable virus.

There are special considerations for lung transplant recipients with regard to respiratory viruses, since long-lasting allograft dysfunction may result some months after resolution of the viral illness [38]. This may be true even for such common viruses as rhinoviruses and also for asymptomatic or minimally symptomatic infection episodes. At such times, viruses are not usually detectable, but progression to bronchiolitis obliterans syndrome (BOS), a chronic progressive form of lung allograft dysfunction, may occur due to cytokine release and injury and repair processes that are the subject of current research. It is thus of particular importance to test lung transplant recipients early, even if they are only minimally symptomatic, as viral detection might lead to therapy that can lessen the risk of this later allograft dysfunction. Obtaining a nasopharyngeal swab on all lung transplant recipients with new-onset respiratory symptoms is reasonable (Table 49.3).

Parvovirus

Parvovirus B19 is an under-recognized cause of anemia in transplant recipients [44]. While many centers test for parvovirus in patients who present with anemia without other

Table 49.3 Respiratory viruses (FDA cleared and commercially available)

Test name					Methods	Sample	Sensitivity/specificity	Status
xTAG Respiratory Viral Panel (12 viruses); Luminex NxTAG RPP (Luminex) ARIES FluA/B &RSV (Luminex)					PCR and Luminex; detection (9 h) MAGPIX RPP (12 viruses; 3 h) MultiCode PCR, WalkAway system	NP swabs NP swabs NP swabs	78.3–100%/91.3–100%; depends on which virus 97%/99% 97%/99%	IVD IVD IVD
ProFlu+ (FluA/FluB and RSV); Hologic					Real-time PCR; sample to results system – Panther	NP swabs/throat swabs	Sen/Spe 95–100%/ 92.6–98.6%	IVD
Simplexa FluA/B, RSV Focus (DiaSorin)					Real-time PCR (3.5 h)	NP swabs; tracheal aspirates	98–100%/93–99%	IVD
Verigene Respiratory Panel(FluA/B/RS) Luminex Nanosphere Luminex					Real-time PCR on Verigene SP system (3 h)	NP swabs/viral cultured samples	89.8–99.2%	IVD
ProFAST+ (Flu A/H1, A/H3); A/H1N1.2009; Hologic					Real-time PCR smart cycler (5.5 h)	NP swabs	95.4–100%/99.0–100%	IVD
RP FILMARRAY (22 viruses and bacteria), BioFire (BioMerieux)					Nested PCR; rapid test (70 min) WalkAway system	NP swabs, throat swab, and BAL	97% sensitivity 99.7% specificity	IVD
eSensor RVP (GenMark dx)	XT-8. Tat 5.5 hrs	NP swabs & throat swabs	99.2%/ 99.7%	IVD	XT-8 System Detection; TAT 6.0 hrs (12 viruses)	NP swabs and throat swabs	99.2%/99.7%	IVD

explanations, it has only recently been recognized that parvovirus can also cause milder degrees of anemia in a larger number of patients [45]. Diagnosis by serology is less useful, as the majority of adults are IgG-seropositive and transplant recipients may not mount an IgM response even during active infections. Bone marrow examination may reveal disordered erythroid progenitors including giant erythroblasts. Parvovirus PCR testing on blood is the most useful noninvasive test for diagnosing active parvovirus infection in a transplant recipient. This testing is important both as a basis for therapy with IVIg and reduction of immunosuppression and also for infection control and isolation purposes. Molecular testing has also led to the suggestion that viral loads of parvovirus from the donated organ, detected in graft preservation solution, may correlate with increased risk for posttransplant parvovirus infection in the recipient [46].

Agents of Viral Hepatitis

Viral hepatitis agents include hepatitis A, hepatitis B, hepatitis C, hepatitis D, and hepatitis E abbreviated as HAV, HBV, HCV, HDV, HEV, respectively. Of these, HBV and HCV are most commonly found in transplant recipients, although recently detection of hepatitis E has been on the rise [47]. HBV or HCV may be pre-existing in the recipient or may be acquired de novo posttransplant, either from the donor or from transfusions [48]. In the pre-existing category, HBV or HCV may be the reason for performing a liver transplant or may be a comorbidity in a patient receiving a non-liver transplant.

Hepatitis B

Serologic testing for HBV is complex and relies on an understanding of the timing of detection of several different HBV antigens and antibodies. Active infection is characterized by a rise in the hepatitis B surface antigen (HBsAg), which then falls to undetectable in about 90% of patients; then subsequently there is a rise in the anti-HBs (hepatitis B surface antibody) titer. Between the time that HBsAg becomes undetectable and anti-HBs appears, there is a "window period" during which time neither is detectable, but the HBV core IgM antibody (anti-HBc IgM) and HBV DNA are detectable. After natural infection, the anti-HBc IgM disappears and the anti-HBc IgG appears, so persons in whom HBV infection has been successfully controlled by the immune system generally are positive for both anti-HBc and anti-HBs, but negative for HBsAg. In about 10% of infected individuals, antibody seroconversion does not occur and the HBsAg is persistently positive in chronic infection. Such individuals may progress to cirrhosis and/or hepatocellular carcinoma and may require a liver transplant with the goal of eradicating HBV infection, utilizing posttransplant prophylaxis with hepatitis B immune globulin (HBIg) and an anti-HBV antiviral agent such as entecavir. In such individuals the blood HBV DNA is also commonly positive pretransplant and may be serially followed posttransplant to detect any recurrence early.

Pretransplant screening of the recipient commonly includes HBsAg, anti-HBc, and anti-HBs. A positive HBsAg is indicative of current infection which may represent either recent infection or chronic carriage. An isolated positive anti-HBs is usually the result of HBV vaccination, as the

vaccine is produced from recombinant HBsAg. A positive anti-HBc and anti-HBs are indicative of natural infection which has been controlled by the immune system. An isolated positive anti-HBc may either be a sign of recent or ongoing infection during the window period with positive IgM antibodies or past resolved infection if IgG antibodies are positive, where the anti-HBs titer has waned below the level of detectability. Alternatively, an isolated positive core antibody may be a false-positive test.

Occasional potential organ donors may be identified as "core-positive" donors, that is to say, the HBsAg is negative but anti-HBc is positive. Often only the total core antibody result is available and not whether it is IgM or IgG; also donor anti-HBs information may not be available. Although such donors may be in the "window period" between disappearance of HBsAg and appearance of anti-HBs, the risk of transmission of HBV to non-liver recipients is low ranging from 1:30 to 1:60 [49, 50] and can be further reduced by immunization of the recipient prior to transplantation; in some cases, antiviral prophylaxis is also given [48, 51]. The risk of transmission to a liver recipient from an HBV core-positive donor is higher, about 1:2 [49], but also can be minimized by use of pretransplant immunization and intensive posttransplant prophylaxis with hepatitis B immune globulin and an antiviral agent such as lamivudine or entecavir [48].

For any patient who is at risk for posttransplant HBV, either as a recurrence of their own previous infection or through donor-derived transmission, it is recommended to include serial posttransplant monitoring of the HBV DNA since posttransplant patients may not seroconvert but would still have viral DNA detectable if reactivation or transmission had occurred [48].

Hepatitis C

Hepatitis C is one of the most common indications for liver transplantation. In the past, HCV recurrence posttransplant was frequent and could be either early and aggressive or later and more slowly progressive [48]. Until recently there were no prophylactic antiviral protocols available for prevention of posttransplant HCV recurrence, although this has rapidly changed in the era of new and more effective HCV drugs.

In both liver and non-liver solid organ transplantation, donor-derived de novo HCV is a clinical concern because of poorer graft and patient outcomes in some settings in patients who are hepatitis C seronegative but experience HCV transmission from the donor [52], although a large study by Abbott et al. of kidney transplant candidates and recipients demonstrated improved survival with transplantation with HCV+ donors compared with the remaining on the waiting list [53]. The risk of transmission of HCV from a seropositive donor to a naïve recipient varies in different series, but has been reported to be as high as 75% in some studies [48]. An HCV-seropositive, HCV RNA-negative donor appears to be less likely to transmit HCV than a donor with detectable HCV RNA, but further data are awaited. Unlike the HBV core-positive donor, until recently the risk could be mitigated by prior immunization since there is no vaccine for HCV. Thus, transplantation from an HCV-seropositive donor to an HCV-seronegative recipient (HCV D+/R-) was usually reserved for situations where other donor offers were unlikely, with stringent informed consent [9]. However, the advent of effective HCV therapy is expected to change practice rapidly.

By contrast, the transplantation of a solid organ like a kidney from an HCV-positive donor to an HCV-positive recipient (HCV D+/R+) has been an accepted practice [53, 54]. Multiple studies have suggested that outcomes for transplantation are superior to those remaining on dialysis for an HCV+ transplant candidate [55], even if the donor is HCV+ [53, 54]. Since the waiting list is long and deceased-donor kidney transplants may not occur for years, it makes sense for the HCV-seropositive kidney transplant candidate to consider accepting an organ from an HCV-seropositive donor [53, 54].

In any of the above cases, where either the donor or the recipient (or both) is seropositive for, or at risk for, HCV, monitoring posttransplant for HCV reactivation in the recipient is important [48]. However, antibody seroconversion may be delayed or absent in the immunocompromised patient, even though HCV serology testing has undergone considerable evolution and improvement over time. Since HCV seroconversion may be delayed or absent in posttransplant patients experiencing transmission of HCV from the donor, molecular testing of HCV RNA is important in serial monitoring of the posttransplant patient at risk of HCV acquisition.

HIV

In the early years of transplantation, HIV seropositivity in the donor or the recipient was held to be an absolute contraindication. However, in recent years, a multicenter study of outcomes of solid organ transplants in selected HIV-positive recipients has been found to be comparable to those of HIV-negative recipients for kidney and liver transplantation [56, 57]; although the incidence of acute rejection in 150 HIV-positive kidney recipients was higher than expected, patient and graft survival were high [56]. These recipients are chosen because their kidney or liver disease is more clinically significant than their HIV-related illness, they have not had certain HIV-related opportunistic infections, and their HIV viral loads are well controlled except in the case of some liver candidates who could not tolerate antiviral therapy in

the setting of end-stage liver disease. Careful monitoring of the drug interactions between calcineurin inhibitors and protease inhibitors by an experienced pharmacist is necessary, but excellent outcomes can be achieved in certain patients in this category.

Until recently, HIV-seropositive donors were not accepted for donation in the United States, but data from South Africa suggested that HIV-seropositive donors can be associated with acceptable outcomes in selected HIV-seropositive recipients [58]. This is an evolving field, spurred by the shortage of deceased donors and restricted availability of dialysis in resource-limited settings, and further data are awaited. In the United States, the HIV Organ Policy Equity (HOPE) Act was passed in 2015 [59], which allows for research into transplantation from HIV-positive donors to HIV-positive recipients; and the first such transplants in the United States were performed at Johns Hopkins in 2016 [60].

As with HBV and HCV, HIV antibody seroconversion may be delayed or absent in the transplant recipient, and serial monitoring with HIV molecular testing is suggested for any patient at risk for HIV acquisition or reactivation posttransplant [61]. Patients who are HIV-seropositive pretransplant should have HIV RNA viral loads and CD4 counts serially monitored in addition to drug levels and posttransplant lab testing.

NAT of Donors and CDC/PHS High-Risk Donors

For many years, until the development of rapid molecular tests that could be performed in the deceased donor testing time frame, testing of prospective deceased donors relied on antibody serologies for HIV, HBV, and HCV, which are performed as part of a serologic panel by the organ procurement organization (OPO). However, the window period prior to seroconversion that can occur for each of these viruses resulted in infection transmissions from apparently seronegative donors, yielding for a search for more accurate laboratory tests. For example, a donor transmitted HIV and HCV to multiple organ transplant recipients after testing negative for antibody serology for both of these viruses [62]. In addition, a case was reported of a living donor that transmitted HIV after initially testing negative but then continuing risky behavior between the time of initial donor evaluation and the time the transplant was performed [63].

Nucleic acid amplification testing (NAT) is a technology for rapid molecular testing that is highly sensitive and has been used in blood banking. In recent years it has become possible to perform this testing in the rapid time frame needed for making decisions about whether or not to accept a deceased donor, including nights and weekends. The availability of NAT has spurred a national debate in the United States regarding whether all potential deceased donors should be subjected to NAT for HIV/HBV/HCV or just those in the CDC-specified high-risk categories including sexual promiscuity, injection drug use, incarceration, and other categories of behavioral risk. A survey of OPOs revealed a heterogeneity of practices in this regard, with some OPOs performing NAT on all donors, some on a subset of donors, and some not at all [64]. In 2010, an expert consensus panel recommended restricting NAT to donors in the above risk categories, citing concerns about false-positive testing that could lead to discarding otherwise potentially acceptable donors and thus leading to increased deaths on the waiting list for transplantation [3]. Then in the fall of 2011, the Centers for Disease Control and Prevention (CDC) and the US Public Health Service (USPHS) published a comprehensive guideline which recommended NAT of all deceased donors and also retesting of potential living donors shortly before intended donation. After discussion within the transplant community, these recommendations were revised, and the current guidelines call for HCV NAT testing of all donors, with HIV NAT testing only of PHS/CDC high-risk donors, and with a revised list of risk categories [65]. All parties in this discussion are interested in protecting potential organ recipients from harm; the differences in opinion arose in balancing the risks of donor-derived transmission versus the risks of disqualifying donors through false-positive testing.

Multiplex Versus Single-Virus Testing

The fact that there are multiple transplant-associated viral infections, which are amenable to serial monitoring, has given rise to the development of multiplex assays that allow for the detection of more than one virus at any given time point from a single blood sample [2]. Viruses which are frequently serially monitored posttransplant, such as CMV, EBV, and BKV in kidney recipients and adenovirus particularly in pediatric HSCT recipients, would be candidates for inclusion in a blood multiplex viral molecular detection panel. In addition, the existence of a large number of respiratory viruses that produce similar symptomatology makes the use of a respiratory virus multiplex a natural one [2]. Potential advantages of a multiplex assay on blood or plasma would include the following: less blood drawn from the recipient for blood assays, detection of unsuspected coinfections, and rapid and sometimes quantitative results to facilitate preemptive strategies. The cost-effectiveness of multiplex testing has been evaluated in a study by Mahony et al., in which four strategies were compared for diagnosis of respiratory viral infections in pediatric patients (direct fluorescent antibody or DFA alone, DFA plus shell-vial culture, the xTAG RVP test alone, or the xTAG RVP test plus DFA) [66]. These authors reported that the least costly strategy was the xTAG RVP

multiplex test alone when the prevalence was >11% and was DFA alone when the prevalence was <11% [66].

Disadvantages of some multiplex tests have included occasional lower sensitivity for one or more individual viruses on the panel, although that finding has led to alterations of the multiplex test such as the RespPlex test vis-à-vis adenovirus testing [2]. Potential disadvantages of multiplex testing of disparate viruses, for example, a panel that includes CMV, EBV, and BK virus and others, also include the clinical quandary of what to do with low-level positive results or results in a subgroup of patients in whom a particular virus is of less clinical importance like BKV in non-kidney organ transplant recipients. For certain subgroups of patients, all of the assays on a multiplex test might provide valuable information; but for others, the clinicians may be interested in only one or two viruses. In that situation, should the information on detection of the other viruses be routinely provided in laboratory reports? These and other questions remain to be fully addressed. The issue of cost also needs careful scrutiny. Costs could potentially decrease because of ordering fewer tests if the clinicians intended originally to monitor more than one virus, but costs could also increase if the cost of the multiplex assay exceeds that of the single-virus assay for clinicians who intended to monitor only one virus. Mahony et al. reported the cost-effectiveness may also depend on the prevalence of the viral infections being tested and so may vary from one region to another or one season to another [66].

Conclusion

Molecular testing for transplant-related viruses has revolutionized posttransplant care and is having a significant impact on pretransplant testing of donors. Serial monitoring for CMV, EBV, and BKV has become a cornerstone of management, as this monitoring allows for early detection and intervention in appropriate subsets of transplant recipients. The utility of quantitative molecular testing is supported by a variety of studies and facilitates the timing of starting and stopping antiviral therapy, assessing the effectiveness of therapy, monitoring for recurrences of viremia, and deciding when to test for antiviral resistance. Multiplex testing for panels of respiratory viruses has demonstrated utility and cost-effectiveness in certain scenarios. The use of NAT in potential deceased and living donors is promising for reduction of donor-derived transmission from donors in the window period of HIV, HBV, and HCV infection, but the recent vigorous national debate, regarding whether all or a subset of potential donors should be subjected to NAT, reflects the complexity of the issues involved. Finally, the availability of both multiplex and single-virus molecular tests will present challenges to the clinician as to how best to utilize the additional information provided by these tests.

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