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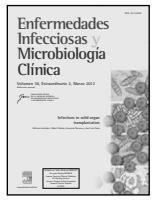

The role of the clinical microbiology laboratory in solid organ transplantation programs

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A B S T R A C T

Infections remain a major complication of solid organ transplantation. For this reason, the clinical microbiology laboratory plays a key role in the success of transplant programs, which must have the support of a qualified laboratory, both technically and professionally. Transplant programs strongly condition the structure and functionality of microbiology laboratories, but at the same time, benefit greatly from the knowledge generated from these programs. The laboratory must make a special effort to implement rapid methods that can respond to the broad spectrum of potential pathogens in solid organ transplant patients. The integration of microbiologists in multidisciplinary teams is highly recommended, as only then can they obtain the highest quality and efficiency in the diagnostic process. This article provides an updated review of the techniques to be used once transplantation has occurred. The role of the microbiologist is also crucial in the pretransplant period, as good microbiological candidate evaluation at this time strongly conditions the success of the transplantation program.

Papel del laboratorio de microbiología clínica en los programas de trasplante de órgano sólido

Palabras clave:
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Papel del laboratorio de microbiología
Trasplante de órgano sólido

R E S U M E N

Las infecciones siguen siendo una importante complicación del trasplante de órgano sólido. Por esta razón, el laboratorio de microbiología clínica desempeña un papel clave en el éxito de los programas de trasplante. Estos programas deben tener el apoyo de un laboratorio cualificado, tanto técnica como profesionalmente. Los programas de trasplante condicionan fuertemente la estructura y funcionalidad de los laboratorios de microbiología, pero al mismo tiempo se benefician enormemente del conocimiento generado alrededor de dichos programas. El laboratorio debe hacer un esfuerzo especial en la puesta en marcha de métodos rápidos que den respuesta al amplio espectro de potenciales patógenos en los pacientes de trasplantes sólidos. La integración del microbiólogo en los equipos multidisciplinares es muy recomendable y sólo así se puede obtener la calidad y mayor eficiencia del proceso diagnóstico. En este artículo se lleva a cabo una puesta al día de las técnicas utilizables una vez que se ha realizado el trasplante. No obstante, el papel del microbiólogo es también crucial en el período previo al trasplante, ya que una buena evaluación microbiológica del candidato en este momento condiciona fuertemente el éxito del programa de trasplante.

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Introduction

Despite recent advances in transplant medicine, infections (along with rejection) remain the most important cause of failure after solid organ transplantation (SOT). Better understanding of risk factors, the advent of more potent and safer immunosuppressive drugs and therapeutic advances have improved overall prognoses. However, the wide spectrum of potential pathogens and non-specific clinical symptoms require the establishment of etiology in order to administer the most adapted therapy. Moreover, most of these infections are preventable and their consequences can be reduced with the careful selection of donors and the correct evaluation of candidates in the pretransplant period.

The microbiology laboratory therefore plays a crucial role in SOT programs, whose success depends considerably on the laboratory's technical capabilities. The spectrum of tests should cover all classical areas of clinical microbiology, and must be guided mainly by practical approaches, particularly with a wide assortment of rapid diagnostic techniques. The existence of a transplant program strongly affects the strategy, internal organization and resource utilization of laboratories. They, in turn, will benefit from the enormous knowledge and technical skills generated from transplantation.[1] The integration of microbiologists in multidisciplinary teams is highly recommended, not only to provide the best patient care but also to allow the laboratory's diagnostic options to fit changing scenarios. Special emphasis should be made on taking advantage of its experience in selecting the most appropriate tests and, more importantly, in interpreting results. Rationale evaluation and adaptation of rapidly-evolving technologies must also be among the main objectives of microbiology laboratories in SOT settings.[2]

The present article is an overview of current knowledge on clinical microbiology in transplantation, with a special focus on diagnostic methods in SOT recipients after transplantation. As mentioned earlier, the role of the microbiologist is not limited to this particular period, and we refer readers to the guidelines of the *Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica* (Spanish Society of Clinical Microbiology and Infectious Diseases, SEIMC) for more information.[3]

Diagnosis of Bacterial Infection in Solid Organ Transplant Recipients

SOT recipients have many of the requisites for developing bacterial infections. Advances in prophylaxis have lessened their clinical consequences, but both morbidity and mortality remain high. Because of the broad spectrum of potential pathogens, the correct laboratory approach must be guided by information provided by clinicians. In turn, microbiological findings strongly affect the management of SOTs by clinicians. Close contact between professionals is essential for best patient care. For more information, we refer readers to the article on bacterial infections published in this supplement of *Enfermedades Infecciosas y Microbiología Clínica*.[4]

Bacterial infections have a higher incidence in the first month after transplantation, including those caused by multiresistant pathogens prevalent in the institution. Clinical microbiologists should promote a continuous and careful epidemiological surveillance program, facilitating the adoption of rational control measures in case of outbreaks of these dangerous pathogens. Special mention should be given to *Acinetobacter baumannii*, methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci and multiresistant *Pseudomonas aeruginosa* strains. Systematic investigation of these pathogens in both SOT recipients and health care personnel is not recommended on a routine basis, unless a specific outbreak was detected in these units.

Diagnosis of acute bacterial infections is based on risk factors and on maintaining a high index of clinical suspicion. However, signs and symptoms of infection may be less evident in SOT recipients than in immunocompetent patients. A broad microbiological approach should be initiated, with serial blood cultures (at least three sets), and cultures of catheters, wound swabs, respiratory specimens or of any anatomical area suggesting the possibility of infection. Care should be taken to obtain these specimens under the best conditions, using adequate containers and ensuring correct labeling and rapid transport to the laboratory. Sample processing should follow standard procedures, with special attention to difficult-to-treat organisms, such as multiresistant pathogens and those with a higher incidence among SOT patients, such as *Listeria*, *Salmonella*, *Mycobacterium*, *Nocardia* and *Legionella.* Attempts to establish the etiology of the infection by using rapid and simple techniques (conventional staining, antigen detection, etc.) should always be made, even when information provided by these tests are not completely accurate. In addition, more sophisticated strategies should be implemented, and the laboratory that serves an institution running a SOT program must show both high technical and professional skills.

Tuberculosis is a relatively frequent complication in Spain and elsewhere, with special significance in lung transplant recipients.[5,6] A thorough investigation to rule out this infection in candidates is the cornerstone of preventing this serious complication. However, if tuberculosis is suspected after transplantation, special efforts should be made by the laboratory to establish rapid diagnosis and susceptibility tests, given the overall poor prognosis, the spread of resistance facilitated by globalization and the interactions between antituberculous and immunosuppressive drugs. Diagnostic strategies and methods used in SOTs should not differ from those used in other patients. The recent introduction of integrated devices providing a rapid, sensitive and easy to perform diagnosis by nucleic acid amplification (GeneXpert® MTB/RIF assay) may be useful when either pulmonary or extrapulmonary tuberculosis is suspected in a SOT patient.[7]

SOT recipients are particularly susceptible to developing episodes of *Clostridium difficile*-associated diarrhea. A rapid etiological diagnosis is crucial for starting therapy early and for controlling the spread of the pathogen to other patients. Laboratories that support transplantations should offer rapid techniques for both A and B toxins, supplemented with isolation of bacteria in culture and followed by verification of their toxin-producing capacity (toxigenic culture). The possibility of performing epidemiological characterization of isolates should also be of interest, due to the spread of hypervirulent strains belonging to 027 and 078 ribotypes in some countries.[8]

The incidence of legionellosis in SOTs varies depending on the epidemiological situation of each institution. Clinical presentation is less defined, which makes necessary to maintain a high suspicion index. Antigen detection in urine, although restricted to serogroup 1, is the best approach to rapid diagnosis. Isolation in selective media is recommended for epidemiological purposes.

Laboratory Diagnosis of Fungal Infections

Fungal infections remain one of the most serious complications following SOT. Steps taken in recent years towards a better understanding of pathology and risk factors, as well as the introduction of new therapeutic approaches, have improved the poor prognosis associated with these infections.[9] Lack of both sensitivity and specificity of classical methods has spurred research into new alternative diagnostic tests focusing on antigen detection and molecular techniques.[10] However, improvements are insufficient, and rapid and accurate techniques are clearly warranted in this field.

Conventional diagnostic methods

In spite of its well-known limitations, conventional diagnosis has a series of advantages (simplicity, low cost, etc.) that makes its use

advisable when a fungal infection is suspected in a SOT patient. A good laboratory diagnosis starts with sampling a specimen representative of the fungal infection being diagnosed. When feasible, specimens should be obtained directly from the affected organ. Serial specimens may partially overcome the lack of sensitivity of conventional tests. Since most fungal pathogens (with exceptions, such as *Histoplasma*) are not primary human pathogens, repeated detection may improve the predictive value.

Microscopic examination provides a presumptive diagnosis of fungal infection in cases where some fungal elements are visible. Generally, sensitivity is insufficient but its simplicity may be extremely useful in some circumstances, such as the observation of the *Cryptococcus neoformans* capsule, or the presence of small intracellular yeast suggesting *Histoplasma capsulatum*. A careful microscopic exam allows a rapid and accurate diagnosis in up to 75% of *C. neoformans* infections.

Isolation in culture provides identification of a broad spectrum of fungal species and allows us to perform susceptibility tests and epidemiological characterization. Accurate species identification should be attempted in all instances due to its importance from a therapeutic standpoint. Sequencing techniques have emerged as a useful tool for this purpose, due to the limitations of morphological and biochemical identification methods. Blood cultures should always be performed when disseminated fungal infection is suspected, and isolation of *Aspergillus*, *Fusarium*, *Scedosporium*, *Paecilomyces*, etc., should be interpreted within the patient's clinical context. However, blood culture sensitivity is clearly insufficient, as some fungal species do not grow in media, and only half of candidemias can be diagnosed in blood culture, even when culturing several specimens.

Serological tests lack both sensitivity and specificity in SOT patients. Their utility is restricted to recipients living in or travelling to regions with endemic histoplasmosis and coccidioidomycosis, and results interpretation should always be cautious.

Detection of fungal antigens

Joint detection of both mannan antigen and antibodies seems useful in cases of hepatosplenic candidiasis in hematologic patients, with higher sensitivity than that of culture,[11] but experience with SOT recipients is currently limited.

(1,3)-β-D-glucan

This cellular wall component of some fungi may be detected in cases of invasive infections caused by yeast and molds (*Candida*, *Aspergillus*, etc.). Zygomycota and *C. neoformans* produce lower quantities of this compound, making detection of this antigen less useful for laboratory diagnosis.[10,12,13] The European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) has included β–glucan detection in the case definition of probable aspergillosis, although with a lower evidence level than that of galactomannan antigen detection.[14] There are 3 commercial techniques, each with different cut-off values for result interpretation. Sensitivity and specificity depends on the technique, study population and design. There is little experience with SOT recipients and both false positive and negative results have been reported.[11] Overall, β-glucan detection should only be used as an additional diagnostic tool along with other clinical, radiological and microbiological data.

Galactomannan antigen

Serum detection of this fungal antigen has become one of the most important advances in the diagnosis of invasive aspergillosis in immunosuppressed patients, including SOT recipients.[10,11] Accordingly, the EORTC/MSG has included galactomannan detection as one of the microbiologic criteria for this disease.[14] Sensitivity and specificity values are variable, depending on the study population, sampling frequency, type of specimen, technical procedure and cut-off used for interpretation.[14] The ELISA Platelia® Aspergillus (BioRad) is the most widely used commercial method with the highest sensitivity.[15] Galactomannan detection is useful for following up patients at risk of invasive aspergillosis, monitoring treatment and, for some authors, as an indication for starting a rescue therapy.[10] Cut-off values may be interpreted both in a static (index ≥0.7 ng/ml in one sample) and dynamic manner (index ≥0.5 ng/ml in two consecutive samples).

Most clinical experience with galactomannan antigen detection comes from hematologic patients.[11] In SOT recipients with less severe neutropenia, sensitivity and specificity results, although acceptable, are not as good for the diagnosis of invasive aspergillosis. Several authors have also reported promising results with galactomannan detection in bronchoalveolar lavage (BAL) specimens and in cerebrospinal fluid (CSF). There is insufficient experience with cut-off values for assay interpretation in these specimens, but values of ≥1 ng/ml for BAL and ≥0.5 ng/ml for CSF are currently accepted by some experts.[15,16] Sensitivities higher than 80% have been reported for galactomannan detection in BAL of neutropenic patients[16] and from critically-ill patients with immunity disorders.[17]

Both false positive and negative results have been reported in the literature. Dietary interactions, use of piperacillin-tazobactam or amoxicillin-clavulanate as antibiotic treatment and cross reactions with other molds and yeast (*Penicillium*, *Paecilomyces*, *Cryptococcus*, etc.) are among the most frequent causes of false positive results. We refer the reader to the well documented review articles.[10]

Molecular diagnosis

Molecular methods, mainly real-time PCR techniques, have emerged as highly promising diagnostic tools, due to the limitations of conventional approaches and the severity of fungal infections in immunosuppressed patients. However, despite concerted efforts, standardization of these techniques remains elusive. Several variables may affect standardization of these tests and, eventually, their clinical utility. For this reason, molecular methods should be considered as a support for clinical and laboratory diagnosis. Readers are referred to comprehensive reviews on this subject.[18,19] In general, two issues concerning molecular tests remain unresolved: a) distinguishing simple colonization or external contamination from true disease attributable to the fungus, as occurs with respiratory specimens, and b) the lack of sensitivity in specimens with low fungal DNA content, as is the case with blood. More experience in test interpretation and selection of the most appropriate diagnostic specimen for each process is crucial. For instance, input of higher blood volumes for nucleic acid extraction is recommendable, as is the use of biopsies or BAL specimens instead of sputum for performing PCR. However, in spite of this lack of standardization, some studies show 90% sensitivity and 75% specificity on respiratory specimens for diagnosis of invasive aspergillosis,[19] and many laboratories are currently implementing molecular assays.

Finally, susceptibility studies for antimycotic drugs are increasingly necessary for managing SOT patients with fungal infections. Standardized methods have been proposed by both EUCAST (European Committee on Antimicrobial Susceptibility Testing) and CLSI (Clinical Laboratory Standards Institute),[20,21] and represent a remarkable advance in managing fungal infections. Current development of new drugs is an additional justification for this need.

Laboratory diagnosis of other less frequent mycoses in SOT patients

Although infrequent, infections caused by *C. neoformans* should be considered in SOT recipients. Clinical presentation and diagnostic

methods do not differ from those used in other immunosuppressed patients. India ink microscopic examination and culture should be performed when this infection is suspected, although their sensitivity may be unsatisfactory. Antigen detection in CSF or serum is a valuable tool for the diagnosis of meningeal cryptococcosis, as well as for monitoring treatment, but sensitivity is lower (65%) in respiratory disease.[11,22] Commercial methods are based on latex agglutination or enzymoimmunoassays. Low titer, false positive results have been reported in the presence of rheumatic factor, *Trichosporon* colonization, systemic bacterial infections, neoplasms, etc. Assay interpretation in the solid transplant setting arises from experience with other immunocompromised patients. Molecular techniques for *C. neoformans* detection have been developed, but are not currently available in most laboratories.

Similar problems occurred with some fungal infections, such as mucormycosis, or those caused by *Fusarium* or *Scedosporium*. Due to their clinical severity and poor prognosis, maintenance of a high suspicion index and careful clinical evaluation are crucial for requesting the most appropriate complementary diagnostic tests, which at this time should be based on conventional methods. Microscopic examination is cheap, rapid and easy to perform. Presence of cutaneous lesions in neutropenic patients with fever may be indicative of *Fusarium* infection, and blood cultures should be drawn (up to 60% of fusariosis cases can be diagnosed by this method). Culture of respiratory specimens is useful in pulmonary infections caused by these molds, but their slow growth may delay diagnosis and, more importantly, the early start of treatment. Detection of β-D-glucan is usually positive in these patients.

Use of prophylaxis with cotrimoxazole has made *Pneumocystis jiroveci* pneumonia a rare event in SOT. Fluorescent staining with specific antibodies is currently the most widely used method for detecting this organism. Real-time PCR techniques have been described, but are not readily available and experience in the SOT setting is limited. Some studies have reported lower sensitivity in SOT recipients compared to that of human immunodeficiency virus type 1-infected patients.[23] According to other reports, positive predictive values may be unsatisfactory due to genome detection in patients with low level infections,[24] thus suggesting the need for quantitative methods. Finally, quantitation of β-D-glucan has shown promising results in cases of *P. jiroveci* infection in immunocompromised patients.[25]

Parasitic Infections

Heart transplant patients are at a higher risk of developing clinical episodes of toxoplasmosis, due to parasite tropism of the transplanted organ's striated muscle. Toxoplasmosis may also reactivate from a latent infection. Cotrimoxazole prophylaxis is mandatory for preventing toxoplasmosis,[26] and this practice has drastically reduced the number of cases in SOT recipients. Serological diagnosis in this setting is likely to be difficult due to both the technical limitations of the assays and poor antibody response by SOT patients. Seroconversion may establish the diagnosis in primary infections, but there is no direct relationship between antibody titer and risk of immediate onset of toxoplasmosis. Direct detection of the parasite in blood, fluids and tissues establishes the diagnosis but culture is not readily available for many laboratories. Quantitative real-time PCR techniques allow for direct diagnosis and disease monitoring.[26]

Leishmaniasis is a significant disease in Spain due to the existence of endemic areas with several cases observed in SOT patients. Antibody titration by indirect immunofluorescent assay may be used when this infection is suspected. However, due to imperfect antibody response, direct diagnostic tests are preferable. Microscopic examination should be attempted due to its simplicity and rapidity. Culture is feasible but has been progressively replaced by molecular techniques. New real-time PCR methods may be used both for diagnosis and monitoring purposes.[27,28] As for the most appropriate specimen, bone marrow aspirates are recommended for microscopic examination and culture,[27] due to the contraindication for performing spleen biopsies in many SOT patients. Interestingly, some authors have reported similar positive detections for both bone marrow aspirates and blood specimens when using nested or real-time PCR techniques in immunosuppressed patients.[28] If confirmed, they could facilitate surveillance of this infection in SOT patients.

Due to globalization (travel, immigration, etc.), parasitic diseases prevalent in certain geographic areas, such as malaria and Chagas disease (trypanosomiasis),[26,29] should be considered in SOT patients. Universal blood screening has reduced the risk of transmission of these parasites in Spain, but acquisition through donated organs or, in the case of Chagas disease, reactivation after latent infection in the recipient produces cases within the SOT setting. Special diagnostic efforts should be made before transplantation. Serological screening of patients and donors coming from or living in endemic areas, although imperfect, may reduce the risk of transmission. There are commercial techniques, some of which are suitable for the immediacy of a donation. When acute malaria is suspected after transplantation, careful microscopic examination of multiple blood smears is the preferred method for diagnosis in SOT recipients,[26,29] and more sensitive assays, such as PCR methods, are not usually required. Diagnosis of symptomatic trypanosomiasis after transplantation is more difficult, although seroconversion may be useful in previously seronegative patients. When disease arises from reactivation of a latent infection (the most common scenario), molecular amplification techniques are preferred. However, a negative result does not exclude the diagnosis due to the intermittent presence of the parasite in the bloodstream.

Strongyloides stercoralis may cause life-threatening hyperinfection syndromes in SOTs, usually after reactivation of a chronic infection in patients coming from endemic areas (some regions of Spain were once endemic, and cases in SOT recipients have been observed). A careful evaluation after transplantation, with the possibility of treatment, is crucial.[30] However, laboratory diagnosis is difficult due to the limited presence of larvae in stool samples, especially in chronic infections with extended evolutions. Repeated serial examinations (at least 6 different stool specimens) are recommended. Serological tests may be useful when strongyloidiosis is suspected and direct examinations remain negative, for instance in patients with high-count eosinophilia and epidemiological antecedents. False positive (cross reactivity with other parasites) and negative (poor antibody response in immunocompromised patients) results are possible with serological tests.

Laboratory Diagnosis of Viral Infections

Herpesviruses

Members of the *Herpesviridae* family are the most prevalent viruses infecting SOT recipients. Although these viruses share many common biological aspects, their clinical features, risk factors, diagnostic approaches and antiviral susceptibility vary significantly. For more information on herpesvirus infections in SOT patients, we refer to the articles of Aguado et al[31] and Carratalà et al[32] published in this supplement. All of these variables affect the diagnostic strategy, which will always be adapted to the particular circumstances of the patient (Table 1). The integration of microbiologists in multidisciplinary transplant teams is crucial for providing the best care for SOT recipients.

Pre-transplant serological screening of both candidate and donor is an important task for the clinical laboratory so as to adopt the best prophylactic and control measures, and also to obtain useful information that may be crucial after transplantation.[3] For instance, vaccination against varicella-zoster virus (VZV) with attenuated OKA

Table 1
Specimens and techniques recommended for laboratory diagnosis of members of the *Herpesviridae* family*

Virus	Cutaneous lesions	PBL	Whole blood	Plasma	Cerebrospinal fluid	Biopsies	BAL	Urine
HSV1	IFA				Real time PCR			
	PCR				Nested PCR			
	Cell culture							
HSV2	IFA				Real time PCR			
	PCR				Nested PCR			
	C							
VZV	IFA				Real time PCR			
	PCR				Nested PCR			
	Cell culture							
CMV		IFA	Real time PCR	Real time PCR	Nested PCR	Nested PCR	Real time PCR	Cell culture
					Real time PCR	Real time PCR	Cell culture	
EBV			Real time PCR	Real time PCR	Real time PCR			
					Nested PCR			
HHV6			Real time PCR	Real time PCR	Real time PCR			
					Nested PCR			
HHV7			Real time PCR	Real time PCR	Real time PCR			
					Nested PCR			
HHV8			Real time PCR	Real time PCR		Nested PCR		
						Real time PCR		

BAL: bronchoalveolar lavage fluid; IFA: antigen detection by immunofluorescence; PBL: Peripheral blood leukocytes; PCR: polymerase chain reaction.
*See abbreviations for virus species into the text.

strain may be indicated during the pretransplant period, but contraindicated for the post-transplant period. Cytomegalovirus (CMV) seronegativity in a candidate is a well-known risk factor for further development of CMV disease after transplantation. Conversely, serology is not useful in the post-transplant period.

Infections caused by herpes simplex type 1 and 2 viruses (HSV1 and HSV2, respectively) are frequent in the SOT setting, although both morbidity and mortality are moderate, due to prophylactic therapeutic measures.[32] Most of these infections are diagnosed on clinical grounds, and laboratory tests are reserved for atypical or severe clinical cases. The same diagnostic approaches apply to VZV. Members of the *Alphaherpesvirinae* subfamily (HSV1, HSV2 and VZV) show tropism for skin and mucous membranes. In these cases, antigen detection by immunofluorescent assay (IFA) is the method of choice for laboratory diagnosis. Accurate results for the IFA rely on good specimen sampling, taking care to obtain high cell content. For specimens with reduced viral loads, as occurs in the diagnosis of central nervous systems infections in CSF, real-time PCR techniques with specific probes for all three viruses are the best option. Both in-house and commercial methods are used in different laboratories, and the choice of either strategy will depend on the particular conditions of each laboratory.

In specialized laboratories, alphaviruses can be isolated in culture (fibroblasts, Vero cells, etc.). Sensitivity is good for HSV from cutaneous specimens, and somewhat lower for VZV. Cell culture can isolate other viruses that may be responsible for infections (e.g., enteroviruses in some cutaneous lesions), as well as obtain viral strains for further study and characterization. Culture is not useful for isolation of these viruses in CSF in cases of suspected meningoencephalitis, and only PCR techniques should be used.

Human CMV is the most relevant viral agent and a significant cause of morbidity and mortality in SOT patients. Monitoring the course of this infection is essential, both for diagnostic purposes and for starting early therapy.[31] However, the particular diagnostic approach may vary depending on the type of SOT, patient's clinical condition (e.g. mismatch D+/R–, etc.) or the prophylactic strategy adopted for each patient. In general, quantitative techniques in blood (viral load) are essential for CMV monitoring, with some exceptions.

CMV viral load determination is particularly useful for guiding preemptive therapy. Most laboratories use plasma samples, although some authors call for quantifying viral load in whole blood.[33,34] In any case, cut-off reference values for starting therapy are difficult to obtain. Major efforts have been made in recent years, but standardization is far for being achieved.[35] Many variables influence viral load values: type of SOT, patient's serostatus and clinical condition, immunosuppressive protocol, type of specimen, technique used for viral load determination and the variability associated with each laboratory. Generally, cut-off values range around 3 and 4 $\log_{10}$ copies/ml for plasma and whole blood, respectively, and 3 $\log_{10}$ copies/10^5 leukocytes when using this cellular fraction. The recent introduction of an international standard and use of validated commercial methods may help to establish more precise cut-off values. Still, for the reasons mentioned above, each transplant program should obtain its own reference values.

Before the advent of real-time molecular techniques, the antigenemia assay (quantitative detection of pp65 antigen in peripheral blood leukocytes) was considered the gold standard for controlling and monitoring CMV infection and disease in SOT patients. This technique requires relatively high blood volumes (5 ml), is labor intensive and its interpretation is subjective. Moreover, it requires sample processing within the first 4-6 h after collection, which makes antigenemia inappropriate for referring samples from outpatients. However, this method is fast (results can be available within 2 hours from the arrival of the sample at the laboratory), flexible (not forced to work in batches) and less costly than molecular assays. It can be used for monitoring SOT patients provided it is performed under standard conditions; however, most laboratories have progressively replaced antigenemia by molecular methods for

organizational reasons.[33,36] There are several commercial systems for determining CMV viral load. In general, all are valid for this purpose, but it should be noted that the values obtained from these different systems are not interchangeable. There are also separate commercially available reagents (primers, specific probes, control plasmids, etc.) for in-house determination of viral load, which reduce assay costs considerably.

Viral load detection in blood may be insufficient for diagnosing focal CMV disease. In these cases, CMV detection in other samples, such as biopsies, CSF or BAL, is recommended. If encephalitis is suspected, the most sensitive diagnostic technique (nested or real-time PCR) should be used due to the limited DNA content. For gastrointestinal tract biopsies, qualitative detection by sensitive PCR assays does not establish the diagnosis of focal disease, which should be made by histopathological examination. A quantitative technique is recommended for respiratory samples, where detection of CMV is not able to distinguish between simple colonization and virus-attributable disease. These specimens can also be inoculated in cellular systems in specialized laboratories, which allows for a broader etiological diagnosis. Culture is also necessary for further phenotypic and genotypic studies and for epidemiological characterization. Urine may be a good source for this culture.

Although the incidence of post-transplant lymphoproliferative disorders (PTLD) associated with Epstein-Barr virus (EBV) in SOT patients is low and associated with well-defined risk factors (see the article from Carratalà et al[32] in this supplement), experts agree on monitoring the presence of EBV by quantitative methods.[37] The appropriate strategy for monitoring (the SOT patients to be included, guided or not by risk factors, type of clinical specimen, etc.) should be established for each center. Interpretation of EBV viral load results is more difficult than in CMV. Controversy persists on the most appropriate clinical sample (plasma, whole blood or lymphocyte fraction) to be used. In addition, there is considerable overlap of viral load values between patients who develop further PTLD and those who do not, which complicates the determination of cut-off values for guiding control measures.

Human herpesviruses 6 and 7 (HHV6 and HHV7) have been involved as etiologic agents of some diseases in SOT patients[32] and as possible cofactors of CMV reactivation in the so-called betaherpesvirus syndrome.[38,39] However, this association is unclear. They have also been involved in some neurological diseases where no other known pathogen is detected. In general, there is insufficient information to recommend the monitoring of these viruses in SOT recipients, and diagnosis should be guided by clinical suspicion. The same is true for the human herpesvirus 8 (HHV8), which is responsible for cutaneous and visceral Kaposi's sarcoma, and for other less common diseases in immunocompromised patients.[40]

The diagnosis of HHV 6, 7 and 8 can be done with blood, CSF (in case of neurological involvement) and with biopsies from suspicious lesions. Detection and quantification by real-time PCR is the method of choice. Interpretation of results may be difficult due to the lack of well-designed studies and the variety of clinical situations. These viruses do not grow in normal cell lines. HHV6 is the exception (it may be detected in shell vial cultures using MRC-5 fibroblasts), but this is not a practical approach for clinical laboratories and has been displaced by molecular techniques. Isolation of these viruses is reserved for specialized laboratories for research purposes.

Common respiratory viruses

Transplant recipients are also exposed to respiratory viruses affecting healthy people, but the prognosis is poor and the lower respiratory tract is more frequently involved. Rhinoviruses, respiratory syncytial and influenza viruses and adenoviruses are the most prevalent etiologic agents.[41]

Real-time PCR techniques are the methods of choice for laboratory diagnosis of respiratory viruses in SOT patients. Sensitivity, speed (less than 3 h) and the detection of a broad spectrum of viruses using multiplexed assays are among the most important performance characteristics of these techniques.[42] In contrast, antigen detection tests and cell cultures lack sensitivity, delay diagnosis and detect a reduced spectrum of viruses.

Upper respiratory tract specimens are typically used for diagnosis, with nasopharyngeal aspirate/lavage and the joint sampling of pharyngeal and nasal swabs performing the best. However, it should be noted that these specimens are only indirect markers of what actually happens in the lower respiratory tract.[43,44] In critical patients, more representative specimens, such as BAL and biopsies, should be obtained.

Use of molecular assays for respiratory infections, including those in SOT patients, pose some still unsolved problems, and has generated a degree of controversy. The first problem is how to distinguish between colonization and true infection when a respiratory virus is detected in the upper respiratory tract, especially since mixed viral detection occurs frequently with molecular techniques in immunocompromised patients.[45-48] The second problem occurs when both viral and bacterial respiratory pathogens are present, so it is difficult to determine if the former is the causative agent, a trigger or just a simple bystander. The third problem is determining the primary viral pathogen and its relationship with clinical severity and prognosis. Molecular assays do not necessarily detect viable viruses and, despite their longer persistence in immunosuppressed patients in comparison with immunocompetent hosts, controversy still persists. Well-designed prospective and controlled studies using both conventional and molecular diagnostic methods are currently in progress trying to answer these questions for SOT patients.

Finally, in case of administration of specific antiviral treatment, as occurs with neuraminidase inhibitors against influenza viruses, it is important that the laboratory is capable of detecting resistance mutations selected during treatment. Gene sequencing or allele-specific PCR assays should be employed when a persistent viral detection is observed in patients on treatment.[49]

Hepatitis B and C, human immunodeficiency and human T-cell lymphotropic viruses

Laboratory methods and biological markers that are useful in SOT settings are the same for other hosts, either for new diagnoses or follow-up of previous infections. It should be noted that serological tests may give erroneous results because of the immunosuppressed status of the patient. Quantitative molecular techniques (mostly real-time PCR) must be used in this setting due to their high performance for both diagnostic and treatment monitoring purposes. It is recommended that the laboratory be able to perform antiviral resistance studies, and apply them to SOT patients who do not respond as expected to antiviral treatment.

Human polyomaviruses

Human polyomaviruses JC and BK (JCV and BKV, respectively) are prevalent in the general population. Specific antibodies can be detected in up to 70% of people aged 5-8 years. Both JCV and BKV persist in a latent stage in epithelial cells of the renal and urinary endothelium from where they reactivate intermittently in 0.5%-20% of immunocompetent individuals. JCV can also persist in B-lymphocytes where it can spread to the central nervous system.

BKV causes interstitial nephritis (BKVIN), which is clinically indistinguishable from rejection in renal transplant recipients and, rarely, in other SOT patients.[50,51] BKV has also been associated with asymptomatic hematuria, ureteral stenosis and hemorrhagic cystitis, mostly in hematopoietic stem cell transplantation, but less frequently

in SOT recipients. JCV is the causative agent of progressive multifocal leukoencephalopathy (PML).

Because active (replicative) BKV infection is the only common factor in renal transplant patients with BKVIN, periodic screening of patients with laboratory tests has been suggested in order to initiate early clinical intervention aimed at reducing graft loss.[50,51] Screening tests show a 99% negative predictive value (NPV) for further development of this complication. Urinary cytology and quantitative molecular assays in urine or blood are techniques that have demonstrated higher precocity and better clinical utility. Viruria precedes viremia by an average 4 weeks, and precedes BKVIN by 12 weeks. A positive screening test in urine should be repeated within 2-4 weeks and, if persistent, careful clinical patient follow-up along with confirmatory blood tests and/or examination of renal biopsies is recommended.

Urinary cytology (microscopic examination of the sediment by phase contrast or Papanicolaou staining) requires processing the specimen before degeneration of cell morphology. The so-called decoy cells, that is, transitional epithelial cells with enlarged nuclei and intranuclear basophilic inclusions, are characteristic of polyomavirus infection. Sensitivity of cytology is close to 100% (NPV 99%-100%), but its positive predictive value (PPV) for BKVIN is less than 30% due to its inability to differentiate between BKV and JCV, and also because it can be confused with the cytopathic effect of adenoviruses, cytomegalovirus and malignant cells. Presence of >10 decoy cells/smear, along with inflammatory cells would increase specificity.

Asymptomatic urinary excretion of BKV is frequent in transplant patients: 10%-45% in renal transplant, more than 50% in hematopoietic stem cell transplantation. Nevertheless, urinary viral loads correlate reasonably with BKVIN. Persistent values of $>10^7$ copies/ml for 3 or more weeks are associated with further development of BKVIN.[50] Several variables may influence quantifications, and therefore each laboratory should obtain its own cut-off values.

BKV viremia correlates better with BKVIN in that it is a marker of tissue lesions, but time to development is lower than in viruria. Levels maintained above 10^4 copies/ml for 3 weeks are associated with BKVIN, with a NPV close to 100% and a PPV of 50%.[51] Episodes of BKVIN with negative viremia but positive viruria seldom occur. In some instances, a low level of viremia persists while examination of renal biopsy is negative for BKVIN. In these cases, closer clinical and laboratory follow-up with repeated biopsy examinations is recommended. Plasma or serum specimens should be used for viral load, but the same type of specimen must be used for following up a particular patient. Significant overlapping in viral load has been observed between patients who eventually develop BKVIN and those who do not.

Screening of all renal transplant patients every 3 months during the first 2 years after transplantation, and then yearly, has been recommended by some experts.[50] In addition BKV should be investigated when graft dysfunction occurs (rising creatinine levels) or when a biopsy is performed for any reason. However, the cost/benefit of this strategy should be evaluated at each institution depending on the particular prevalence of BKVIN.

Definite diagnosis of BKVIN lies in the histological and immunohistochemical examinations of renal biopsies. Differential diagnosis should be made mainly with rejection, in that this complication requires rising immunosuppression while the contrary is true for managing BKVIN. Efficacy can be monitored in urine or plasma at 2-3 week intervals. A viral load decrease of >2 $\log_{10}$ in 8-12 weeks is indicative of efficacy.

PML is diagnosed on clinical grounds with radiologic findings (nuclear magnetic resonance or computerized tomography scans). Confirmation lies in the histopathology of brain biopsies. Sensitivity of JCV DNA detection by PCR is only moderate, around 50%-75%. However, this approach should initially be attempted because it can prevent more invasive procedures.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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