POLISH ARCHIVES OF
Internal Medicine
POLSKIE ARCHIWUM MEDYCYNY WEWNĘTRZNEJ

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PUBLISHER
Medycyna Praktyczna

INDEXED IN
Chemical Abstracts Service (CAS), Crossref, Database of Abstracts of Reviews of Effects (DARE), EBSCO, EMBASE, GENAMICS, Geneva Foundation Free Medical Journals, Index Copernicus (IC), J-Gate, Master Journal List, MNiSW, National Library of Medicine (NLM), Polish Medical Library (GBL), PubMed/MEDLINE, Science Citation Index Expanded (SCI-Ex), SciFinder, Scopus, TUMS Digital Library, Web of Science Core Collection

Print ISSN: 0032-3772

Online ISSN: 1897-9483

Impact Factor 2018 = 2.882

The journal receives funding from the Ministry of Science and Higher Education for promoting scientific research (agreement no. 595/P-DUN/2019).

Jak zamawiać publikacje MP

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An official journal of the Polish Society of Internal Medicine
founded by professor Władysław Antoni Gluziński

MONTHLY VOL. 129 (Special Issue 3) 2019

ABSTRACT

The 3 leading causes of death in patients after solid organ transplantation (SOT) include cardiovascular diseases, malignancies, and infections. According to our current understanding, the latter play the key role in the pathogenesis of atherosclerosis. Similarly, infections (mainly viral) are implicated in the pathogenesis of at least 20% of known neoplasms. In other words, the implications of acute and chronic infectious diseases in modern medicine, not only transplantology, are significant and ever-increasing.

Immunosuppressive treatment impairs the immune function, which renders the patient more susceptible to infections. Furthermore, treatment of infections in immunocompromised patients poses a challenge and SOT.

The current publication provides a brief summary of the key information provided in 20 lectures on viral infections in patients after SOT delivered during the *9th Practical Transplantology Course* in Warsaw, Poland on September 15–16, 2017.

PART I

General overview of major viral infections

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Conflict of interest: none declared.
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Cytomegalovirus infection **Immune response to cytomegalovirus in solid organ transplant recipient** Cytomegalovirus (CMV) is one of the most common opportunistic pathogens in individuals with primary or secondary immunodeficiency, such as organ transplant recipients. Prior to the introduction of ganciclovir (GCV), which is commonly used nowadays, CMV pneumonia caused 50% mortality in kidney transplant (KTx) recipients and over 80% mortality in bone marrow transplant recipients.[1,2] With advances of medical sciences, detection and treatment of disseminated CMV infection have become easier. Currently, diagnosis of CMV infection involves genetic testing along with serology methods. The effect of CMV infection on organ transplant recipients, especially the immunomodulatory effect of CMV, has not been fully elucidated. New knowledge should be applied to developing safer prevention and treatment standards.

Clinical presentation CMV infection may present as flu-like symptoms, with fever, chills, and muscle aches, as well as leuko- and thrombocytopenia and elevated liver enzyme levels seen in laboratory test results. The condition was initially termed "a 40-day fever", due to an increase of body temperature with the onset on day 40 following transplantation, present in most cases. Depending on the transplanted organ, CMV infection may present as: a) bronchiolitis obliterans syndrome or interstitial pneumonia, sometimes leading to respiratory failure, b) esophagitis resembling Candida infection, c) colitis with persistent diarrhea, d) heart failure, or e) vanishing bile duct syndrome. Furthermore, biochemical signs of transplanted organ failure are often present, such as elevated serum creatinine levels in patients after KTx.

Risk factors The risk of CMV infection varies between the organs mostly due to the differences in lymphatic tissue content. With no prophylaxis, the highest incidence is seen in lung and pancreas transplant recipients (50%–75% and almost 50%, respectively). In kidney, liver, or heart transplant recipients, the incidence of CMV infection ranges between 10% and 30%, just as it is in allogeneic bone marrow recipients (30%).[3]

Seronegative recipients of organs from seropositive donors are known to have the highest risk of CMV infection. As mentioned earlier, a primary

infection can have a very severe course; therefore, in cases of serological mismatch between the donor and the recipient, prophylaxis based on GCV should be used.

Another risk factor is immunosuppression (IS) protocol, in particular induction with antilymphocyte therapies, for example, antilymphocyte globulin.[4] Everolimus-based IS regimens are considered to carry a lower risk of infection reactivation as compared with those based on, for example, calcineurin inhibitors.[5]

There are data suggesting that the human leukocyte antigen (HLA) matching status affects the incidence of CMV infections. Furthermore, the degree of HLA matching and reduction of $CD8^+$ T-lymphocyte count are important risk factors of graft loss due to CMV infection.[6] It is also true for children after bone marrow transplant, in whom HLA mismatch was associated with increased rates of new-onset CMV infection or its reactivation. Further research will help determine whether individual alleles, such as HLA-B44, significantly modify the risk of CMV infection.[7]

Long-term prognosis Cardiovascular diseases Cardiovascular diseases are one of the leading causes of mortality in transplant recipients. Patients with known hypertension were shown to be CMV--seropositive with higher CMV DNA load twice as often as healthy recipients.[8] In a study assessing different risk factors of hypertension, such as age, male sex, hyperlipidemia, body mass index, or diabetes, only seropositive CMV status, CMV DNA load, and miRNA-UL112 expression significantly increased the risk of hypertension.

Malignancies CMV alters the immune response by producing interleukin (IL)-10 homologue, which "blinds" the immune system. Indirectly, it also increases the production of vascular endothelial growth factor, which promotes carcinogenesis. Furthermore, inhibiting cyclooxygenase 2 involved in signal transduction to vascular endothelial growth factor resulted in decreased incidence of malignancies in animal models.[9] However, data on humans are conflicting. On one hand, a prospective study of CMV-positive patients demonstrated a significantly shorter time from transplantation to the onset of malignancy than CMV-negative individuals.[10] On the other hand, a study was published which not only failed to confirm such association, but actually refuted it.[11] The association between CMV status and the risk of posttransplant lymphoproliferative disease (PTLD) has not been unequivocally established, and an analysis of over 22 000 patients after different transplants did not confirm the association between CMV status and the incidence of malignancies.[12] Data on bone marrow recipients makes the analysis of CMV effect on carcinogenesis even more complex. The seronegative status of both the donor and the recipient is associated with 40% risk of recurrence of acute myeloid leukemia, as compared with only 30% risk in seropositive individuals. Therefore, it seems that the CMV infection itself does not increase the risk of malignancy but is rather a cofactor in a complex process. This conclusion is supported by the report indicating that the incidence of malignancies in KTx recipients may be associated with the number of HLA mismatches and CMV mismatch between the donor and the recipient.[13]

Immune tolerance CMV infection does not always adversely affect patients, as seen, for example, in bone marrow transplant recipients. Similarly, the γδ T cells, known to induce operational tolerance, were shown to be involved in the late acute rejection of liver transplant (LTx), which is a new finding. Interestingly, the population of the γδ T cells increases after primary CMV infection, which is potentially associated with a lower acute rejection rate. This, however, was only confirmed in patients after LTx.[14] Favorable characteristics of the γδ T cells include their almost complete lack of alloreactivity, a very wide spectrum of cancer-associated ligand recognition, or acting as a triggering factor for dendritic cell maturation. Unfortunately, the same cells also trigger endothelial immune response, which is the basic mechanism of antibody-mediated rejection in KTx.[15] This means that our current knowledge is not sufficient to fully utilize their potential beneficial effect.

Prophylaxis Pretransplant CMV serologic status in donors (D) and recipients (R) is used for stratifying the overall risk of infection. D+/R– mismatch is high, while D±/R+ is a moderate risk factor. R+ status itself is a risk factor for the reactivation of latent infection. D–/R– status is not a risk factor provided that blood products, administered during the transplant surgery, are screened for CMV and are leukodepleted.[16] Universal prophylaxis with oral valganciclovir (VGCV) is currently the most common protocol against CMV infection.[16,17] The optimal duration of prophylaxis varies between 3 and 12 months, depending on the risk level and the transplanted organ. Its duration should increase along with the risk. The recommendations are presented in TABLE 1. The incidence of CMV infection in high--risk patients decreases along with the increasing duration of prophylaxis.[16] The alternative option is a preemptive therapy, based on regular viral load monitoring and early introduction of anti–CMV therapy in relevant cases. Currently, universal prophylaxis is used more commonly, as a more simple and effective strategy.[16] There is also a "hybrid" approach, used mainly in high--risk patients, which includes initial prophylaxis continued for the first 3 to 6 months after transplantation, followed by the secondary preemptive protocol thereafter. VGCV is typically used at a dose of 900 mg/d; however, there are data to confirm the efficacy of half the dose (450 mg/d) in adult transplant recipients.[18] Dosing in children

TABLE 1 Recommended duration of cytomegalovirus infection prophylaxis, based on ganciclovir (intravenous)/valganciclovir (oral), depending on risk stratification and transplanted organ[17]

Status of D/R risk	Duration of prophylaxis				Comments
	Kidney	Liver	Heart Lung	Pancreas Islets Intestine	
D+/R– high	≤6 months	3–6 months[a]	3–6 months ≥6 months	3–6 months[b] 3 months ≥6 months[a,b]	Polyclonal induction may prolong the need for prophylaxis.
D+/R+ D–/R+ moderate	3 months	3 months	3 months ≥6 months	3 months 3 months 6 months[a]	Polyclonal induction may prolong the need for prophylaxis.
D–/R– low	–[c]	–[c]	–[c]	–[c]	Blood products transfused at the time of transplantation must be screened for CMV and leukodepleted.

a In children ≤12 months

b Adding anti–CMV antibodies suggested in children

c Regular CMV monitoring in children

Abbreviations: CMV, cytomegalovirus; D, donor; R, recipient

is determined using a special formula.[19] There are data on the beneficial effect of IS including mTOR inhibitors (everolimus) on lowering the risk of CMV infection (in combination with regular prophylaxis). The underlying mechanism is based on the involvement of the mTOR pathway in CMV replication.[20] Overall, medical anti–CMV prophylaxis is an inevitable part of early posttransplant management, required in patients at moderate or high risk of CMV infection.

Treatment Antiviral medications approved by the US Food and Drug Agency, which are included in the guidelines on treatment of CMV in patients after SOT, include:

1 Gancyclovir (GCV), an intravenous nucleoside analogue and its prodrug—oral L-valyl GCV ester (VGCV). It is phosphorylated by a viral kinase and cellular kinases to GCV triphosphate, which competitively inhibits deoxyguanosine triphosphate incorporation into DNA by viral DNA polymerase. As a result, it induces chain termination or limits chain elongation.

2 foscarnet, an intravenous pyrophosphate analogue, which inhibits viral DNA polymerase.

3 cidofovir, an intravenous nucleotide analogue, which inhibits viral DNA polymerase in a mechanism similar to the one of GCV.

Pharmacological armamentarium of controversial efficacy, considered experimental or alternative CMV treatments, include: leflunomide, anti–CMV hyperimmunoglobulin, polyvalent immunoglobulins, artesunate, and cyclopropavir.

Therapeutic strategies One of the therapeutic strategies in CMV infection is preemptive treatment, that is, treatment of asymptomatic CMV infection, which is practically started as soon as CMV DNA load has been confirmed, before the onset of any clinical symptoms.[16,21] To confirm virus replication in asymptomatic recipients, viral testing should be repeated regularly following transplantation. There are no specific guidelines on the threshold value of CMV DNA load for commencing treatment. Considering variable assay methodology and difficulties in achieving consistent results that would conform to the 1st International Standard by the World Health Organization (WHO), a threshold for intervention should be determined separately for each laboratory.

The other strategy is treatment of symptomatic CMV infection or CMV disease. In adult KTx recipients with severe course of CMV disease and in all children with CMV disease, regardless of its clinical course, treatment with intravenous GCV is indicated.[16,21] GCV or VGCV can be used in adult KTx recipients with CMV disease of a milder clinical course. Treatment should be continued for at least 2 weeks until the symptoms have resolved, and virus eradication has been confirmed in 2 subsequent assays. During treatment, viral load should be monitored every 7 days to assess treatment response. Additionally, kidney function (of the transplanted kidney in KTx recipients or own kidneys in recipients of other SOTs) should be monitored, so as to adjust the dosage to the current estimated glomerular filtration rate. GCV administered for 2 to 3 weeks clears the virus in 90% of cases. The recurrence rate in primary infection is 65%, and 20% in seropositive recipients.

Another essential element of CMV treatment is adjusting immunosuppressive therapy. Dose reduction or temporary discontinuation of antimetabolites are indicated in patients with confirmed, intensive viral replication, leukopenia, and severe CMV disease. The data on the dosage of calcineurin inhibitors remain unequivocal. There are case reports to suggest a beneficial role of mTOR inhibitors on decreasing the incidence of CMV disease.

TABLE 2 Risk factors for genetic resistance

D+/R– serologic status
Multiple weeks of drug exposure with continued CMV DNA replication
Very high CMV DNA load at baseline
Subtherapeutic drug concentration due to too low dose and/or malabsorption or impaired drug metabolism
CMV reactivation
Thoracic organ transplant
Strong immunosuppression
Noncompliance

Abbreviations: see **TABLE 1**

Over 90% of GCV-resistant CMV isolates contain HCMV UL97 mutations. The HCMV UL97 gene product, a protein kinase, is responsible for the phosphorylation of GCV in HCMV-infected cells. Less frequently, mutations can be found within the UL54- gene, which encodes the viral DNA polymerase. Genetic drug resistance occurs in 0.25% to 15.2% of treated SOT recipients, with a mortality rate of 17% to 32%. If not caused by genetic mutations, GCV resistance may stem from severe immunodeficiency of a recipient or suboptimum drug exposure.[16,21] Risk factors for genetic resistance are presented in **TABLE 2**.

GCV resistance should be suspected in nonresponders, recurrent CMV infection, and/or clinical symptoms of CMV disease during treatment continued for 6 weeks, especially if risk factors for resistance have been identified. IS should then be reduced to lowest possible doses and genetic testing for the UL97 mutation should be requested. If a mutation is confirmed, depending on the locus and its effect on effective treatment with GCV, the dose may be increased. Alternatively, foscarnet or cidofovir can be used. Unfortunately, the latter option is limited in KTx recipients by the risk of nephrotoxicity. In patients with multiple drug resistance, immunoglobulin, leflunomide, or artesunate can be used, although clinical data regarding their efficacy appear controversial. Early data on brincidofovir in SOT recipients did not support its efficacy against CMV infection. Currently, clinical trials are pending of maribavir or letermovir in SOT recipients. CMV-specific T-cells are currently evaluated in patients after hematopoietic stem cell transplantation as part of clinical research.

Severe refractory cytomegalovirus infection in heart transplant recipients There are data supporting the use of foscarnet in heart transplant recipients with confirmed drug resistance. Here, we present 2 case reports of orthotopic heart transplantation (OHT) recipients, both in a serious clinical condition at baseline. None of them needed mechanical ventilation prior to surgery. CMV prophylaxis was not used either, as both donor and recipient pairs were seronegative. Typical 3-drug IS protocol, based on tacrolimus (TAC), mycophenolate mofetil (MMF), and glucocorticoids, was used.

In the first patient, the onset was at 3 months following OHT. The infection was detected during a routine follow-up and the treatment was started immediately. Initially, VGCV was administered, and with no evidence of response, human immunoglobulin G containing a high amount of antibody to CMV was administered.

In the second patient, the onset was 2 years following OHT and the general practitioner as well as the consultant in an internal diseases ward at the local hospital, did not include CMV infection in the differential diagnosis, considering the clinical presentation. Unfortunately, empirical antibiotic therapy was started without assessing its potential interactions with IS, and without assessing blood levels of immunosuppressants. The diagnosis was made in our transplant unit, where the patient was referred to after 3 months of virtually symptomatic, ineffective treatment, having suffered from iatrogenic kidney failure along the way. GCV was used in combination with human immunoglobulin G containing a high amount of antibody to CMV.

The course of the disease at its advanced stages was severe and similar in both cases, with gastroenteritis and bone marrow suppression. Furthermore, patient 1 presented with resistance to GCV, as well as symptoms of severe foscarnet toxicity, whereas administered anti–CMV antibodies were not fully effective, either. In patient 2, severe gastrointestinal symptoms predominated, including intestinal obstruction secondary to paralytic ileus. Despite pp65-negative test results, the patient's clinical condition deteriorated, and he needed hemodiafiltration due to kidney failure. The dose of GCV was reduced for the same reason, which caused the recurrence of the active infection. Both patients died eventually, which illustrates how dangerous CMV infection can be in transplant recipients.

To sum up, when planning CMV prophylaxis and/or treatment, the following should be considered: a) transplanted organ; b) CMV status of the donor and the recipient; c) IS protocol; d) number of HLA mismatches; e) γδ T subpopulation (if possible); f) CMV strain, and g) individual immune profile of a given patient. Further research is needed to fully characterize interactions between latent CMV infection and human immune system (**FIGURE 1**).[22]

BK virus **Definition and clinical presentation** BK virus (BKV) is a member of the polyomavirus family. All polyomaviruses are structurally similar, with similar capsid size and high genetic homology. The name of the virus originates from the initials of the KTx recipient with ureteral stricture, in whom the virus was first isolated from urine in 1971.[23]

BKV infection is a relatively common early complication and an important contributor to the dysfunction of the transplanted kidney. As latent virus is reactivated in uroepithelial cells, BKV-associated nephropathy (BKVAN)

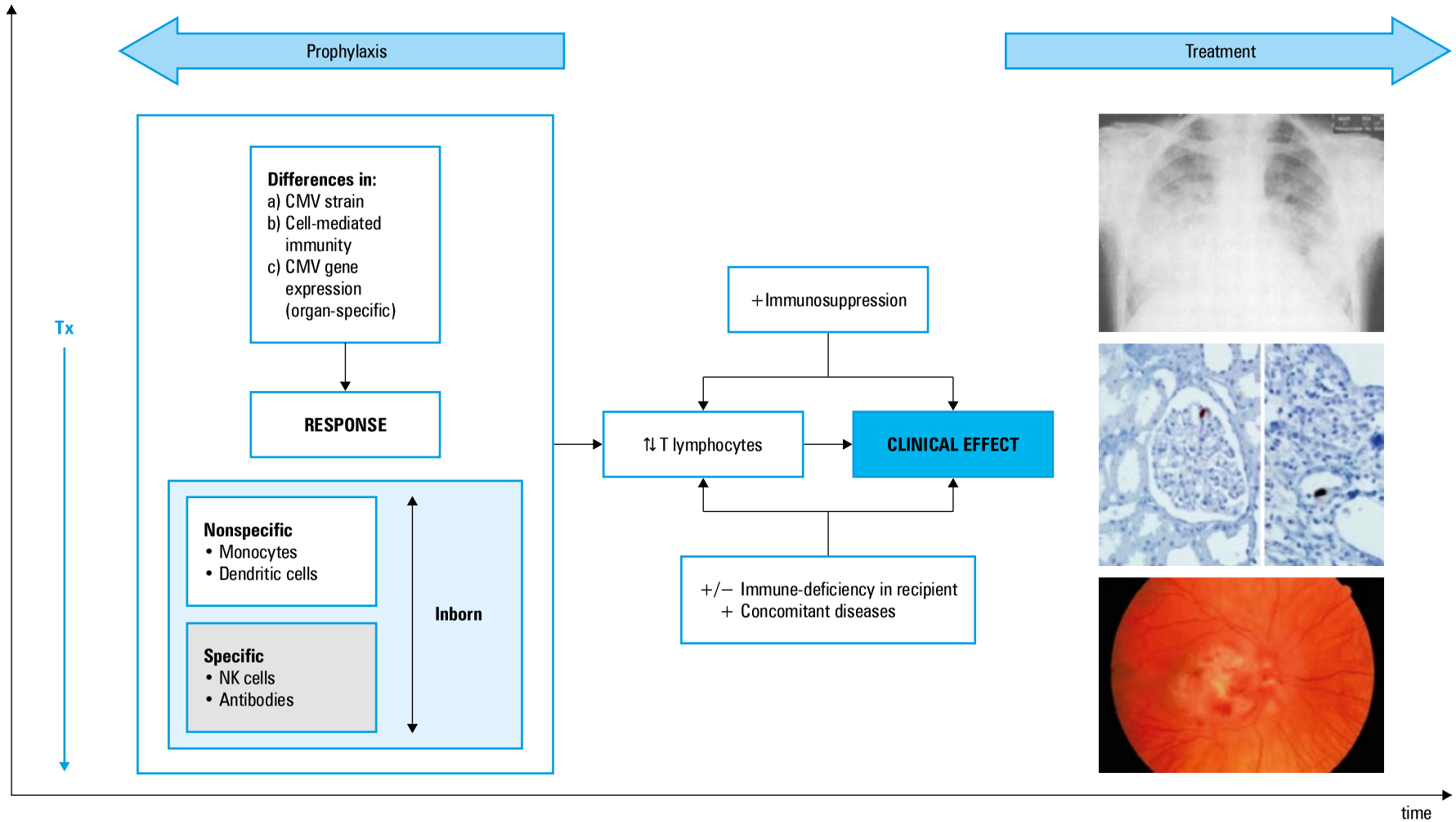

FIGURE 1 Summary of factors considered when deciding about the prophylaxis or treatment of cytomegalovirus (CMV) infection

TABLE 3 Risk factors for BK virus–associated nephropathy

Donor-specific	Recipient-specific	Procedure-specific	Virus-specific
• Number of HLA mismatches • Deceased donor • Seropositive donor with high BKV-specific antibody titer, suggestive of recent exposure • Presence of the virus in the transplanted organ • Female sex	• Older age • Male sex • Some HLA loci (absence of HLAC7 in a donor and/or recipient triples the risk of BKV reactivation in a recipient) • Ureteral injury • Diabetes • No or low activity of BKV-specific T cells (important especially in children due to possible primary infection), but also individual (genetic and epigenetic) differences in response to IS and genetic variability of BKV.	• Cold ischemia time • Delayed graft function • Graft origin (deceased/living donor)[a] • Quality and intensity of IS treatment (acute graft rejection and its treatment) • Ureteral stenting	• Polymorphisms of the polyomavirus major capsid protein gene VP1 and the noncoding control region sequences.

a Animal research demonstrated that virus replication was accelerated in injured kidneys.

b Total immunosuppressive load is the key determinant, although antithymocyte globulin, mycophenolate mofetil, and tacrolimus were also mentioned as important.

Abbreviations: BKV, BK virus; IS, immunosuppression

develops, which affects 5% to 10% of KTx recipients. The BKVAN-associated risk of graft loss within 2 years following virus identification is high (15%–50% of cases). Other complications of BKV infection include ureteral stricture seen in KTx recipients and hemorrhagic cystitis seen in hematopoietic stem cell transplantation recipients. Other possible manifestations affect respiratory, gastrointestinal, and central nervous systems. BKV is known for its oncogenic effect.[24]

Its replication initiates a cascade of events leading to cell lysis and viruria. The virus replicates in the interstitial cells, passes to the tubular vessels, which clinically manifests as viremia. Later, it causes tubulointerstitial injury, which leads to BKVAN. However, not each virus reactivation leads to this type of nephropathy. Therefore, both viruria and viremia indicate the risk of nephropathy, yet do not confirm its presence. On the other hand, BKV is confirmed in blood samples of most patients with active nephropathy.

Research has demonstrated a number of risk factors for BKVAN, dividing them into donor-, recipient-, procedure-, or pathogen-specific, as presented in TABLE 3.

It is still unknown whether BKVAN is caused by the reactivation of a latent infection or whether it is caused by suprainfection with polyomaviruses transmitted from the donor. Research has demonstrated that donor assessment for BKV may decrease the risk of BKVAN in KTx recipients.

Diagnosis and therapeutic recommendations Currently, preventive monitoring of the recipient is indicated, which is the key in detecting reactivation of latent infection and preventing its clinical consequences. The diagnosis of BKV is based on serology testing.

Viral load determination has a low predictive value. According to the guidelines, urine cytology (decoy cells) or urinary VP1-mRNA titer measurement may be performed as screening tests. If positive, a test should be repeated within 4 weeks, while the presence of one of the following findings constitutes an indication for kidney biopsy, which confirms the diagnosis: a) urinary BKV DNA $>10^7$ copies/ml; b) urinary V1mRNA $>6.5\times10^5$ copies/total mRNA, or c) blood BKV DNA $>10^4$ copies/ml. It is recommended to perform screening at least once a month over the first 3 to 6 months following transplantation and then every 3 months until the end of year 1, plus every time with elevated creatinine levels or acute graft rejection (or both).

The cornerstone of BKV infection and BKVAN therapy is to minimize IS.[25] According to the 2010 Kidney Disease: Improving Global Outcomes guidelines, IS should be reduced with BKV load over 10 000 copies/ml (10^7 copies/l). It should involve halving the MMF dose and reducing calcineurin inhibitor (CNi) doses, so that the serum cyclosporine A (CsA) level does not exceed 60 to 100 ng/ml and the serum TAC level does not exceed 3 to 5 ng/ml. MMF should be discontinued in cases of persistent viruria.

The alternative strategy assumes an initial reduction of the CNi dose by 25% to 50%, followed by reducing the MMF dose by half and further reductions until discontinued. TAC can also be replaced with CsA, which additionally decreases the MMF level, whereas CNi can be replaced with mTOR inhibitor. However, this approach has not been supported by research findings. Similarly, oral prednisone dose should be reduced to 5 mg/d, whereas creatinine levels should be reassessed every 1 to 2 weeks, and viral load, every

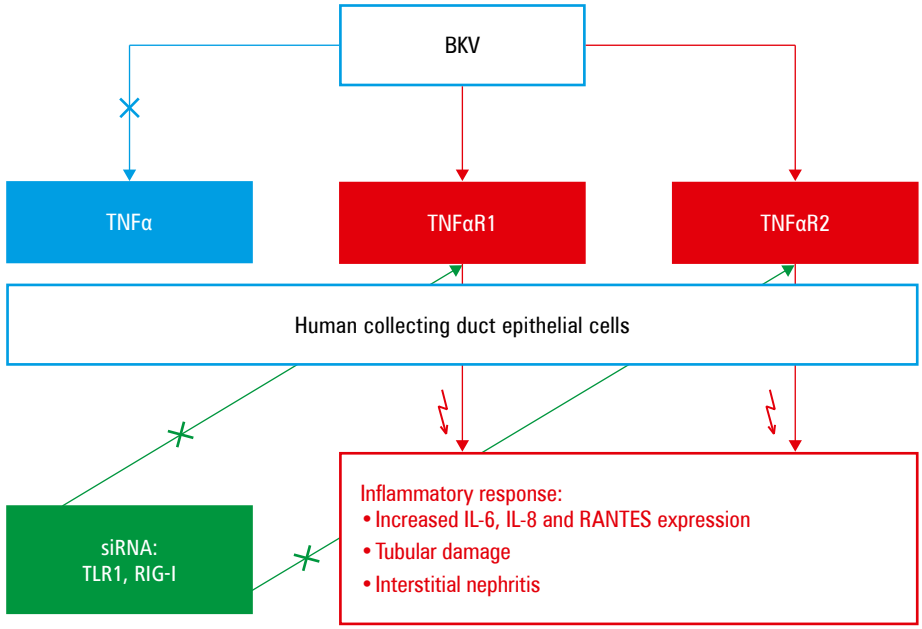

FIGURE 2 Tumor necrosis factor (TNF)/TNF receptor (TNFR) system involvement in BK virus (BKV)-associated nephropathy.[27] BKV may downregulate TNF-α and upregulate both TNFαR1 and TNFαR2 in human collecting duct epithelial cells. Such a response could be effectively blocked by small interfering RNA (siRNA) to toll-like receptor 1 (TLR1) and retinoic acid inducible gene-I (RIG-I), 2 double-stranded RNA receptors of the innate immune system.[28] Abbreviations: IL, interleukin; RANTES, regulated on activation, normal T-cell expressed and secreted

2 to 4 weeks. Regardless of the treatment approach, a rapid reduction of viral load improves or at least stabilizes the estimated glomerular filtration rate.

Despite the risk of increased alloactivation and acute rejection, such management significantly decreases the risk of graft loss. Nevertheless, studies with a longer follow-up are still needed to assess the management strategies for chronic allograft dysfunction (chronic allograft nephropathy).

Although antivirals (cidofovir), antibiotics (fluorochinolones), as well as leflunomide and intravenous immunoglobulins show some antiviral activity, their benefit in BKVAN has not been established yet. At the same time, they are associated with severe adverse effects.

Future treatment options may include T-cell immunotherapy (transfer of BKV-specific T cells), which has demonstrated promising results in vitro.[26] Further research of BKV-specific immune response is necessary, as it may lead to the development of effective treatments and vaccines (FIGURE 2).[27,28]

After allograft loss due to BKVAN, retransplantation is possible unless the virus continues to replicate. Transplant nephrectomy of allografts lost due to BKVAN does not affect long-term treatment outcomes.[29]

Impaired renal allograft function The 2 main endpoints in assessing the efficacy of KTx are recipient and allograft survival. While there is no significant effect of BKV infection on recipient survival, allograft function and survival are significantly impaired in patients with confirmed BKV replication and infection progressing to BKVAN.[30] Importantly, allograft function in seronegative recipients was significantly better than in seropositive ones after effective treatment.[31] This observation strongly suggests that from the perspective of allograft function it is better to prevent infection than to diagnose and treat.

To develop effective BKV preventative strategies, the risk factors should be identified and eliminated. These include, for example, cold ischemia time and KTx from a deceased donor, which cannot be modified after transplantation. The risk of BKVAN increases with male sex and biopsy-confirmed severe interstitial lesions, which cannot be modified, either. The only known modifiable risk factor is IS. The risk of early BKV infection (<6 months following KTx) was shown to increase the most in patients treated for acute rejection (hazard ratio [HR], 3.5), with concomitant CMV replication (HR, 2.3) and those receiving IS induction with T cell–depleting agents (HR, 1.9). The risk factors for late BKV infection (>6 months following KTx) include chronic inflammation, repeat transplantation, presensitization, presence of panel reactive antibodies, presence of donor specific antibodies, and previous infections.[30] Undoubtedly, all these risk factors for early and late BKV infection are IS-dependent. According to the available literature, it is a cumulative dose of immunosuppressants rather than individual agents that increases the risk of BKV infection. The main drugs used in KTx recipients are TAC and mycophenolic acid (MPA) derivatives. There is no specific TAC dose suitable for all patients,

and the discrepancies between the daily dose and serum levels may be significant. There is, however, a simple arithmetic parameter, referred to as the concentration/dose (C/D) ratio, where D is a daily dose of TAC, and C is its trough concentration measured at 12 hours following the last dose. Thus, a patient who needs 10 mg of the drug to achieve a blood level of 10 ng/ml has a C/D ratio of 1. Such patients were classified as intermediate metabolizers. A C/D ratio exceeding 1 means that even a small drug dose is sufficient to achieve its target concentration. Such patients are slow metabolizers and have low risk of drug-specific adverse effects. On the other hand, those with a C/D ratio below 1 are fast metabolizers, and even though they need high drug doses, they achieve low blood concentrations.[32] These patients constitute a group at particular risk of TAC nephrotoxicity and BKV infection. Therefore, IS conversion should be considered in each patient with a C/D ratio below 1.[32] It should also be noted that TAC increases the exposure to MPA. The early pharmacokinetic research from the late 1990s demonstrated that when combined with TAC instead of CsA, a half dose of MPA was sufficient to achieve an identical area under the curve in a plot of MPA concentration in blood vs time. This means that some patients treated with TAC and MPA may be overexposed to MPA and need dose reduction. It was also demonstrated that high MPA dose upon onset of BKV replication is an independent risk factor of BKVAN.[30] Furthermore, when used in combination with steroids, MPA was associated with significantly higher risk of BKV infection and BKVAN than comparable treatment algorithms based on everolimus and CsA.[33] Therefore, to minimize the risk of BKV infection, drug exposure of patients treated with TAC and MPA derivatives should be monitored, with dose adjustments or conversion if feasible and clinically justified.

Epstein–Barr virus **Virus structure** Currently, the name Epstein–Barr virus (EBV) is used interchangeably with human herpesvirus (HHV)-4. The latter originates from the fact that EBV is the 4th on the list of human herpesviruses which contain double DNA strand. EBV is highly infectious and it enters the B cell using the CD21 complement receptor type 2. When viral DNA linearizes, replication and transcription occur, which leads to the lytic phase ending with the breakdown of an infected cell and release of a new generation of virions. When inside a cell, viral DNA becomes a circular, extrachromosomal episome and the virus is latent—the cell is infected, but its function is fairly stable. It turned out that the virus is capable of manipulating the host immune system, causing the effect referred to as "immune evasion". Two main mechanisms of this effect have been established. Firstly, the EBV genome contains a gene encoding a protein which closely resembles human IL-10. Furthermore, viral IL-10 has an identical biological mechanism of action as human IL-10. Additionally, EBV triggers the production of 17 viral miRNA, which affect cell proliferation and alter their apoptotic potential. At the same time, EBV induces a generation of host miRNA (147a and 155), which modulate immune response.[34-36]

Diagnosis Virologic diagnosis is complex and depends on the stage of infection. Historically, detecting antibodies against virus-specific antigens was the first method to be used. The presence of IgM antibodies against viral capsid antigen and Epstein–Barr nuclear antigens 1, 2 or 3 was to confirm active infection, whereas detecting immunoglobulin G antibodies confirmed past infection without the ability to indicate how long ago the patient was infected. Obviously, antibody titer and its increase mattered, as well. The detection of the Epstein–Barr nuclear antigen was considered a confirmation of viral DNA presence in cells.[37]

However, serology methods have some serious limitations. Antibody production following an exposure to an antigen may take days or even weeks, which means that if an individual is tested directly following infection, then the virus is actively replicating itself and clinical symptoms are likely to develop, whereas serology test results remain negative. Immunocompromised patients are likely to have lower antibody levels and their production will be slower. Also, in chronic kidney or liver disease there is an associated immune dysfunction, and aging alters specific antigen-induced serologic response. As a result, these patients are poor responders to both vaccination and infection. Newer diagnostic methods enable direct identification of viral genetic material. Polymerase chain reaction (PCR) is used for detecting viral DNA, and real-time PCR—for quantifying its copies. Clinical samples may include serum (it determines the free virus present in circulation), full blood (it determines the virus present in the immune cells), or tissue specimens. For the latter, the in situ hybridization can be used to stain and identify the virus, or alternatively, the technique developed by Epstein and Barr, including electron microscopic assessment of the specimens, can be applied. These 2 methods, however, are not routinely used in clinical diagnosis. Here, the results of a study on next generation sequencing should be mentioned, carried out in samples collected from 8240 healthy individuals. The analyses showed that 42% of them contained genetic material of 94 viruses (including 19 DNA viruses). HHV-4 (ie, EBV) was present in 14.45% of healthy population.[38]

These findings can be looked upon and interpreted differently. When assessed using serology methods, 70% to 90% of all adults, including 14.5% of all healthy population carry genetic material of EBV in their lymphocytes. Unfortunately, it is impossible to determine the form in which the virus is present in cells or the percentage that undergoes replication.[39]

Oncogenesis and other clinical sequelae Fifty years ago, Burkitt and Epstein were the first to suggest the possibility of a causal relationship between infections and malignancies. Currently, it is thought that 20% of malignancies are caused by only 7 viruses, namely, EBV, human papillomavirus (HPV), human T-lymphotropic virus 1, hepatitis C virus (HCV), hepatitis B virus (HBV), Kaposi's sarcoma-associated virus, and Merkel cell polyomavirus.[40] Their epidemiology changes dynamically and due to the changes in prevention and treatment strategies of HBV and HCV, the incidence of selected malignancies may decrease. Unfortunately, the above list includes EBV, for which there is currently no vaccine and the available treatments are only partly effective. According to the WHO, EBV may be responsible for 200 000 cases of malignancies each year. Transplant recipients are at risk of PTLD, which is a form of lymphatic malignancy and as such may be linked to viral infection. It should be noted that not all cases of PTLD are associated with EBV, and that EBV-negative cases, albeit very rare, were also reported.[41,42] Currently, increasingly more data points to the involvement of EBV in the development of nasopharyngeal cancer, cervical cancer (along with HPV), as well as breast, prostate, and gastric cancer.[43,44]

The effect of EBV on the immune system causes additional pathologies. EBV has been implicated in systemic lupus erythematosus and multiple sclerosis. In lupus, there are several causal mechanisms, particularly altered apoptosis of infected B cells. In multiple sclerosis, EBV cooperates with another representative of the Herpesviridae family, HHV-6A, inducing abnormal response to myelin proteins.[45] Coinfection with 2 herpesviruses, for example, EBV and CMV or EBV and HHV-6 or HHV-7, is common. It should also be noted that the immune evasion is also induced by CMV. Coinfection alters the course of the disease making it atypical and more severe, which poses a diagnostic and therapeutic challenge.

Treatment The course of disease in immunocompetent patients is usually mild and does not require specific treatment, so symptomatic treatment is used. However, in immunocompromised patients, the disease can take a dramatic, life-threatening course, so it requires intensive treatment. Adenine nucleoside analogues, which insert themselves into the viral genome thus stopping its replication, include acyclovir, GCV, and their derivatives Val-acyclovir and VGCV(offering 3 times better gastrointestinal absorption). The mechanism of action of ACV involves inhibiting protein kinase of the EBK virus (EBV-PK, encoded by the *BGLF-4* gene). Since ACV is a prodrug which requires phosphorylation by EBV-PK, theoretically only EBV-infected cells can activate it. Practically, though, noninfected cells are also capable of ACV phosphorylation (and, in turn, activation), but the rate is 100-fold slower than in the infected cells. As mentioned above, up to date, there is no vaccination available.[46]

Drug-induced inhibition of DNA synthesis may cause adverse effects. All rapidly dividing cells quickly "sense" the presence of false adenine derivatives, which causes gastrointestinal and bone marrow adverse effects. Treatment of EBV infection as described above (ACV is typically used) is usually effective. However, it only inhibits virus replication rather than eradicates it completely. It should also be noted that such treatment is only slightly effective (or plainly ineffective) in malignancies. Although the treatment inhibits virus replication, it does not affect the neoplastic disease itself. In immunocompromised patients, the possibility to reduce the dose of IS should always be considered. Such patients may benefit from human immunoglobulin therapy, although the effect will be significantly worse than the one seen in CMV infection.

Influenza **Epidemiology** Influenza is an acute infectious respiratory disease caused by orthomyxoviruses known for their high antigenic variability. There are 3 types of influenza virus (A, B, and C), which are then subclassified according to the hemagglutinin (ha) and neuraminidase (n) subtype combinations, which are both immunogenic surface antigens.

The main route of transmission is human-to-human via direct contact or the droplet route. In temperate climate zones, a seasonal increase in the incidence of influenza is observed between November and April. At the same time, every dozen years highly virulent strains emerge, which cause pandemic outbreaks. According to the Polish National Hygiene Institute, the incidence rate of influenza in Poland is 8000 to 10 000 per 100 000 population, with the mortality below 0.5% The annual incidence of influenza in transplant recipients is 1% to 4%.[47] The clinical symptoms manifest after an incubation period of 1 to 3 days. However, the infection can be transmitted onto others from even 1 day before the onset of clinical symptoms until day 3 to 5 of the disease, or even for several weeks in immunocompromised individuals. "Seasonal" influenza is a self-limiting upper respiratory tract infection. Typical symptoms include fever with chills, muscle pain, and nonproductive cough. The recovery takes 1 to 2 weeks. Pandemic influenza (AH1N1), on the other hand, more often leads to the involvement of the lower respiratory tract and complications, including death.

Due to an impaired lymphocyte function, SOT recipients on IS are susceptible to a more serious clinical course. They also present more often with drug resistance. The risk factors for severe clinical course include early period after transplantation, high-dose IS, and type of the allograft (lung allograft recipients are particularly vulnerable).[48] Immunocompromised individuals more often develop viral pneumonia (25%-40% of cases), secondary bacterial pneumonia (17% to 68%), or fungal pneumonia.[48,49] In these patients, the infection may also present atypically (eg, with isolated fever

TABLE 4 Recommended dosage and duration of influenza antiviral medications for treatment or chemoprophylaxis (adults)

Chemoprophylaxis (after known exposure)			
Oseltamivir	75 mg once daily		10 days
Treatment			
Zanamivir	10 mg twice daily		5 days
Oseltamivir	ClCr >60 ml/min	75 mg twice daily	5 days
	ClCr >30–60 ml/min	30 mg twice daily	5 days
	>10–30 ml/min	30 mg once daily	5 days
	<10 ml/min	NA	5 days
	HD	30 mg	After HD
	CAPD	30 mg	Single dose
Resistant to oseltamivir	Zanamivir	10 mg twice daily	5 days
	Oseltamivir + amantadine	75 mg twice daily + 100 mg twice daily	5 days
	Oseltamivir + rimantadine	75 mg twice daily + 100 mg twice daily	5 days

Abbreviations: CAPD, continuous ambulatory peritoneal dialysis; ClCr, creatinine clearance; HD, hemodialysis; NA, not available

or rhinitis with no fever). Gastrointestinal symptoms (nausea, vomiting, diarrhea) may predominate in even one-third of patients. Up to 65% to 68% of transplant recipients with influenza require inpatient treatment, of whom 13% to 14% require intensive care.[50] The mortality rate in transplant recipients is 4% to 8%, and even 14% in patients with AH1N1 infection.[49,50] Other complications of influenza are observed in the respiratory system (pneumonia, exacerbation of other chronic respiratory diseases up to respiratory failure), cardiovascular system (myocarditis and pericarditis, decompensated chronic heart failure), and nervous system (encephalitis, meningitis, Guillain– Barré syndrome, Reye syndrome). The need to reduce IS in severe influenza increases the risk of acute graft rejection.

Detection and prophylaxis Virus isolation, positive antigen assay, viral DNA in a clinical specimen, or specific serologic response are needed to confirm the diagnosis. The use of serum antibody assay is limited in transplant recipients due to potential failure to trigger the full serologic response. Viral RNA assay is the most effective diagnostic method. Although high genome variability may cause false negative results, real-time PCR is a preferred diagnostic approach in immunocompromised patients, in whom molecular tests offer too low sensitivity.[47] Nasopharyngeal swabs, sputum, nasopharyngeal or bronchial aspirate, and pericardial fluid or cerebrospinal fluid samples can be used for testing.

Prevention of influenza involves annual vaccinations and preventive use of antiviral medications. Trivalent seasonal influenza vaccination is recommended in all SOT recipients and transplant candidates, as well as all individuals that are in direct contact, including health care professionals. Only inactivated vaccines should be used in transplant recipients. The vaccine can be administered at any time starting at 3 to 6 months following transplantation.[51] There are no known reports of acute or chronic allograft rejection triggered by the vaccination. The risk factors of incomplete immune response to vaccination include high doses of IS, early posttransplant phase, and recent treatment with antilymphocyte globulins. Chemoprophylaxis of infections currently involves the use of neuraminidase inhibitors (oseltamivir). Due to the fear of increasing drug resistance, they cannot be used as substitutes of vaccinations. Neuraminidase inhibitors are indicated only in patients with expected low-level response to vaccination and contraindications to immunization.[51] Once the contact with influenza virus is confirmed, antiviral treatment should be started immediately after the onset of first clinical symptoms. The dose of neuraminidase inhibitors should be adjusted to creatinine clearance values. M2 inhibitors (amantadine, rimantadine) have been almost completely removed from treatment algorithms due to increasing drug resistance (TABLE 4). Unlike immunocompetent individuals, transplant recipients benefit from antiviral treatment even if influenza symptoms persist for 48 hours. Treatment should be continued until complete virus eradication has been achieved in the upper and lower respiratory tract (real-time PCR once a week until negative).[51] If no clinical improvement after 7 to 10 days of treatment is observed, testing for pathogenic variant genes should be carried out. When drug resistance is confirmed, treatment should be converted into one of acceptable efficacy.[47]

The need to ensure safety of immunocompromised patients obligates transplant centers to undertake actions aiming at limiting influenza virus transmission at peak season. As part of such actions, all transplant procedures should be temporarily suspended, and the patients admitted for diagnostic assessment or as elective admission—discharged. The number of outpatients seen should be reduced to a minimum. Staff members should be sent home immediately after the onset of the first respiratory symptoms.

Hepatitis C virus Recent years have witnessed revolutionary changes in the treatment of hepatitis C, owing to the introduction of interferon-free therapies based on DAAs. These changes are particularly important for LTx recipients, in whom the efficacy of interferon and ribavirin therapy was low and the adverse effects of interferon were particularly pronounced. Treatment efficacy measured as sustained virologic response at 24 weeks following treatment completion (SVR24) ranged between 12% and 40% for genotype 1 and the incidence of interferon-induced liver allograft rejection was up to 25%.[52] Effective HCV replication depends on the production of viral RNA-encoded proteins in ribosomes of infected cells. HCV translation yields a single polyprotein precursor of approximately 3000 amino acid in length, which is

further cleaved into structural proteins composing form the newly formed virion (p7, E1, E2, c) and nonstructural (NS) proteins, which perform various roles in new viral RNA strand replication and forming new virus particles. Primary protein is cleaved by cellular proteases and NS3/4A viral protease. The NS5A protein participates in forming new virus particles, whereas RNA-dependent NS5B polymerase is responsible for new viral RNA strand replication.

Currently available DAAs can be classified into 3 categories, depending on the target viral protein: NS3/4A protease inhibitors (simeprevir and paritaprevir, grazoprevir), NS5A polymerase inhibitors (daclatasvir, ledipasvir, ombitasvir, elbasvir, velpatasvir) and NS5B polymerase inhibitors (sofosbuvir and dasabuvir). Interferon-free treatment shows very high efficacy with SVR24 over 90% in genotype 1 and 4 HCV. The efficacy in genotype 2 and 3 HCV is lower; however, the research of new viral protein inhibitors and new therapeutic algorithms is still under way.

Treatment algorithm depends on a number of factors, such as HCV genotype, cirrhosis, comorbidities (especially kidney failure), previous failure of IFN or DAA treatments, and potential drug interactions. They are discussed in detail in the guidelines published by the Polish Group of Experts for HCV in 2017.[53] Treatment algorithms based on simeprevir, paritaprevir, and elbasvir with grazoprevir should not be used in patients with decompensated cirrhosis, due to the risk of exacerbating liver failure. In patients with stage 3 chronic kidney disease (glomerular filtration rate >30 ml/min/1.72 m^2), DAA dose adjustments are not needed. In patients with more advanced kidney failure, elbasvir with grazoprevir, or ombitasvir, paritaprevir, ritonavir combined with dasabuvir should be used. In patients on renal replacement therapy with genotypes 1 or 4, elbasvir with grazoprevir is recommended, whereas pegylated interferon should be used in other genotypes. It should be noted that ribavirin dose needs adjustment in patients with kidney failure. In LTx recipients with genotype 1 HCV, regardless of the severity of fibrosis and decompensation, sofosbuvir and ledipasvir should be used for 12 weeks. In patients with compensated cirrhosis, daclatasvir with sofosbuvir can also be used for 12 weeks. Ombitasvir, paritaprevir, ritonavir combined with dasabuvir can be used for 24 weeks only in patients with fibrosis Metavir grade F0–F2. In patients after LTx, DAA should be used in combination with ribavirin. In genotype 3 HCV, daclatasvir should be used for 12 weeks in combination with sofosbuvir and ribavirin. Unfortunately, in genotype 3 the Polish National Health Fund only reimburses the therapy with sofosbuvir and ribavirin, the efficacy of which measured as SVR24 is below 70%.

Interferon-free treatments are safe and do not usually cause significant adverse effects. However, it seems that rapid inhibition of viral replication may alter the immune system, which is reflected in 2 phenomena observed in DAA-treated patients: activation of HBV infection in patients with chronic hepatitis B, and the increased risk of hepatocellular carcinoma (HCC) recurrence in patients with a history of effective HCC treatment. Decreased activity of $CD8^+$ T cells in chronic hepatitis C is caused by an increased expression of inhibitory receptors, absence of $CD4^+$ T-helper cells, and increased activity of regulatory T cells. In most patients, effective 12-week DAA treatment leads to reconstitution of HCV-specific $CD8^+$ T-cell response. It is not associated with increased activity of $CD8^+$ T cells against other viruses, which supports a specific effect of HCV elimination rather than immunomodulatory effect of the administered medications. In chronic hepatitis C, natural killer (NK) cells become dysfunctional and activated. Their functional polarization involving increased cytolytic activity and decreased interferon-γ production is associated with chronic exposure to interferon α and may make it impossible to eradicate the virus leading to chronic hepatitis. DAAs reduce the activation of NK cells in the liver and peripheral blood, thus decreasing their cytolytic activity and restoring normal phenotype within 8 weeks of treatment, with the effect sustained afterwards. Decreased cytolytic activity of NK cells may unlock immune regulation of cancer.[54,55] Research has confirmed increased risk of HCC recurrence after DAA treatment in patients after successful treatment of primary HCC, including LTx recipients.[56,57] However, there is also evidence that the risk of recurrence may be comparable to the one found in patients previously treated with interferon.[58] Apparently, there is no consensus on DAA use in patients with a history of HCC. Such patients should be closely monitored (magnetic resonance or computed tomography imaging are recommended) before and after treatment. There have also been reports suggesting lower efficacy of interferon-free therapies in patients with known HCC metastases identified prior to DAA administration.[59]

Our own experience supports very high efficacy of interferon-free therapy in 110 LTx recipients with genotype 1 HCV. Treatment failure was noted in 3 cases only (SVR24 = 97%), including 2 cases of extrahepatic HCC metastases found prior to treatment, and 1 case of adrenal HCC metastasis found on magnetic resonance imaging following treatment completion. In 7 patients with genotype 3 HCV, sofosbuvir and ribavirin were ineffective in 2 cases (SVR = 71%), including 1 case of new HCC lesion seen following treatment completion.

HIV **History of battle against HIV** AIDS was first described 38 years ago, in 1981. Gottlieb et al[60] reported cases of pneumocystis pneumonia in the *New England Journal of Medicine*. Over the next year, it became clear that a causative agent was transmitted by sexual intercourse. In 1982, many cases were discovered among blood recipients. Most of them were adult patients with hemophilia, but some children were infected, as

well. The next risk group were intravenous drug users who used nonsterile syringes and needles. The other identified route of infection was a vertical mother-to-child transmission.

In 1980, Robert Gallo et al[61] discovered T--lymphotropic retrovirus, the first virus known to affect human CD4 cells. They hypothesized that this virus was also responsible for the newly discovered syndrome. However, after months of intensive research all agreed that HIV was identified in 1983 by Francoise Barre-Sinoussi and Luc Montagnier from Pasteur Institute in Paris, who were awarded Nobel Prize a few years ago, in 2008.

It became clear that HIV epidemic was a challenge for public health systems worldwide. A number of organizations initiated vigorous action to find an effective solution. First, the route of infection was established. A few weeks after HIV discovery, routine enzyme-linked immunosorbent assays were manufactured and introduced to clinical practice. Donor testing in blood banks reduced the number of transfusion-transmitted infections to almost zero. Community campaigns played the crucial role in combating the epidemic. Individuals above the age of consent were continuously educated how to avoid HIV infection. A number of harm reduction programs for intravenous drug users were developed in many countries, demonstrating their effectiveness.

Public authorities along with researchers and pharma industry set another important direction. Their main effort focused on developing the HIV vaccine. Unfortunately, despite a number of promising concepts and a development of almost-ready vaccines, which entered phase III clinical trials, there is still no vaccine available today. Furthermore, there is little chance for the effective vaccine to become available in the nearest future. The protection rate of an effective vaccine should be close to 100%, whereas a prototype HIV vaccine only offers a 50% protection rate.

Therefore, medical therapy plays the key role in HIV infection. The first drug, azidothymidine, was introduced as monotherapy. A few years later, though, its efficacy in monotherapy was shown to be of limited duration, rendering it ineffective after a couple of years. Additionally, resistance has been shown to result from a set of mutations, which was another disadvantage of monotherapy. Fortunately, protease inhibitors, a newer class of antiretroviral drugs, were discovered at around the same time. As a result, triple-drug HAART was introduced in 1994. Also, in 1994, the efficacy of antiretroviral therapy in preventing vertical transmission of HIV was shown, reducing the infection rates in children born from HIV-positive mothers from 30% to less than 1%.

It was possible owing to numerous grants given for basic science research, which significantly accelerated scientific progress and expanded the existing knowledge. Owing to the effort of researchers working in drug development, newer treatments were discovered, more effective and much safer than previous generations, giving the patients a chance for longer survival. New gold standard of antiretroviral treatment involves treating everybody as early as possible to achieve undetectable viral load. The goal of care is now defined by WHO as 90-90-90, which means that 90% of diagnosed patients receive the care, 90% of them are treated with antiretrovirals, and 90% of them have undetectable virus.

If a patient with HIV infection is treated early enough, full immune reconstitution is almost certain. In many developed countries, these patients are also considered eligible for organ transplant, should a need arise. The outcomes of transplantation in these patients are the same as in an HIV-negative population.

HIV and liver transplantation Until recently, HIV infection was considered a significant contraindication to LTx. HIV-positive patients were not considered eligible for LTx not only due to active and poorly controlled infection. Other concerns were alcohol or other substance abuse and psychosocial problems, as a result of which successful treatment after LTx was highly unlikely. Another relevant issue was a suggestion that IS could accelerate HIV/AIDS progression and increase mortality. In 1996, HAART was introduced in developed countries, which revolutionized treatment approaches and outcomes in HIV-positive patients to an extent where over 50% of all-cause mortality in HIV-positive population are not directly related to HIV/ AIDS. However, liver diseases have become the leading cause of death in this population, even exceeding the share of cardiovascular diseases. Approximately two-third of deaths in HIV-positive patients were caused by chronic HCV infection, followed by 17% of deaths caused by HBV infection and 3% caused by HAART-induced liver injury. Other causes of liver failure include alcoholic fatty liver disease, nonalcoholic fatty liver disease and HCC. Over 30% of HIV-positive patients have HCV coinfection, and the vast majority of deaths in HIV--positive population with advanced liver disease are caused by HCV.[62,63]

Many studies demonstrated that the mortality rates, as well as the incidence of malignancies and opportunistic infections, in HIV-positive patients with T-cell reconstitution after HAART are comparable to those seen in HIV-negative population. Therefore, LTx is a valuable treatment option in this particular group. In the years from 2003 to 2011, many reports have been published on LTx in HIV-positive recipients, mostly in the United States, France, Italy, and Spain. The reported patient groups consisted of dozen to 60 recipients, with the majority presenting with HIV (62%–100%) and HBV (29%–100%) coinfection. The follow-up period ranged between 1 and 6 years, and the survival rate was 48% to 87%. Considering such promising results, HIV infection was no longer considered a contraindication to LTx. Three groups of criteria for transplant eligibility have been identified, as shown in **TABLE 5**.

TABLE 5 Criteria for liver transplant eligibility in human immunodeficiency virus

Stage of liver disease (a hepatologic criterion identical as in HIV-positive patients)	Stage of HIV infection	Social and psychological criteria
• Liver failure (HCV, less often acute and chronic HBV, HCC) • Acute liver failure • Decompensated liver disease (ascites, encephalopathy to be differentiated with HIV-associated encephalopathy) • Bleeding esophageal varices • Functional liver impairment (albumin <30 g/l, INR >1.5, bilirubin >450 mmol/l) • HCC (Milan/Mazzaferro criteria)	• Possibility to continue effective, safe, and long-term antiretroviral treatment after LTx • Ideally, HIV RNA load <50 copies/ml • CD4 >100 cells/ml • CD4 >200 cells/ml in patients with a history of opportunistic infection (in Spain) • CD4 >200 cells/ml (CD4 >100 cells/ml in case of decompensated cirrhosis or portal hypertension) (in Italy)	• Positive psychosocial evaluation: assessment of social network support (acceptance, family and friend support) • Patients with active psychoactive substance dependence are not considered eligible for LTx • Stable patients on methadone maintenance therapy are considered eligible for LTx • In some countries, additional requirements have been introduced, such as 2-year abstinence from heroin and cocaine and 6-month abstinence from other psychoactive substances, including alcohol

Abbreviations: HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; INR, international normalized ratio; LTx, liver transplant

In recent years, the reports of successful LTx in HIV-positive recipients with long-term survival have been published, with primary HCC being the main indication,[64] as well as the reports of LTx from HIV-positive donors to HIV-positive recipients.[65]

In 2011, in the Department of General, Transplant and Liver Surgery, Medical University of Warsaw, in cooperation with the Department of Hepatology and Acquired Immunodeficiency (Prof. Alicja Wiercińska-Drapało), a successful emergency LTx was performed in a 20-year old HIV-positive recipient with HBV and end-stage liver failure (encephalopathy and hepatic coma), which made it the first liver graft transplanted to the HIV-positive recipient in Poland. The patient survived for over 1 year despite noncompliance. He died due to multiorgan failure, secondary to another sexually transmitted disease.

Considering the above information, it seems that chronic nature of HIV disease, along with an increasing number of patients with end-stage liver disease and complications of HAART, will likely result in an increasing demand for LTx in this population. Assessing patient eligibility, including virologic, immune, and psychosocial factors, is the key to a safe and effective transplant. Furthermore, posttransplant care is a multidisciplinary challenge and effort due to issues related to surgical complications, immunosuppressive treatment, HAART, and compliance. Long-term results in patients with HIV and HCV coinfection are worse than in patients with HCV, especially with the same Model for End-Stage Liver Disease scores, although newer DAAs will likely improve treatment outcomes. It should also be noted that HIV-positive donors can be considered an alternative source of organs for HIV- and HCV-positive recipients.

HIV and kidney transplantation Renal involvement is becoming increasingly more prevalent in HIV-positive patients, leading to increased mortality and morbidity. The most common conditions include acute kidney injury, chronic kidney disease, HIV-associated nephropathy and other glomerulonephritides as well as complications from toxicity of antiretroviral and other drugs (FIGURE 3).

In aging population of HIV-positive individuals, renal involvement could also be associated with HBV or HVC coinfection, as well as other comorbidities, such as diabetes and hypertension. Outcomes of HIV-positive patients changed dramatically owing to the appropriate antiretroviral therapy, and patients on renal replacement therapy (dialysis, transplantation) have similar survival rates to those with other comorbidities, such as diabetes. Traditionally, HIV infection was considered an absolute contraindication to KTx. It was due to the concern that IS would accelerate AIDS progression and increase mortality. The concern came from the published reports on some transplant recipients unknowingly infected with HIV, and individuals who received transplant from HIV-positive donors and then acquired HIV infection. Nowadays, most centers still exclude patients who test positive for HIV. However, controlled HIV infection is not a contraindication to KTx, and these patients could be effectively managed in units with relevant expertise and experience. As HAART became available, the prognosis of HIV infection has dramatically improved, making HIV infection a chronic, yet manageable, disease. It also triggered changes to assessment protocols for HIV-positive KTx candidates.[66] Several studies demonstrated excellent outcomes in HIV-positive subjects who underwent KTx while on HAART therapy.[67,68] One-year survival rates appear comparable between HIV-positive and

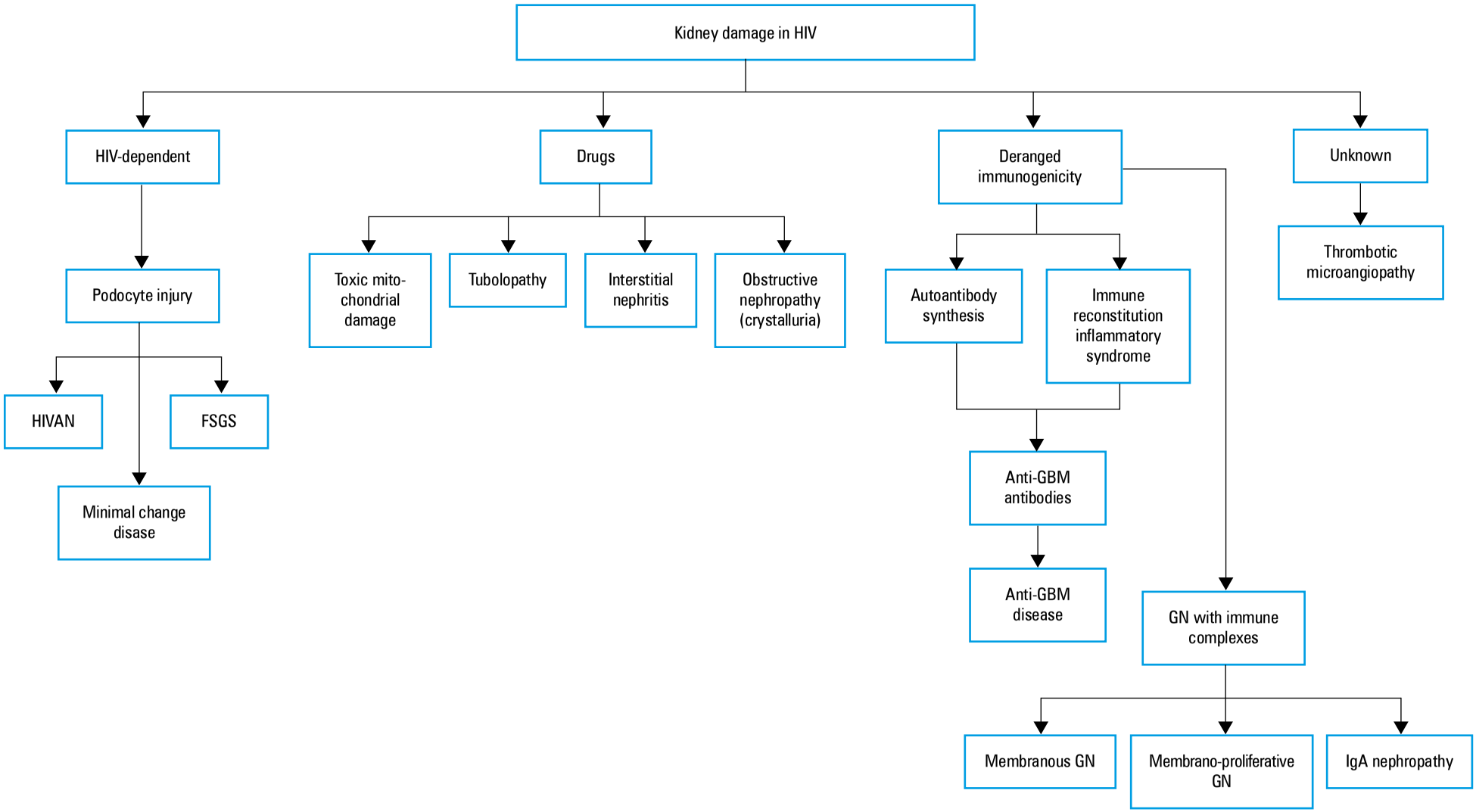

FIGURE 3 Renal involvement in HIV

Abbreviations: FSGS, focal segmental glomerulosclerosis; GBM, glomerular basement membrane; GN, glomerulonephritis; HIVAN, HIV-associated nephropaty; IgA, immunoglobulin A

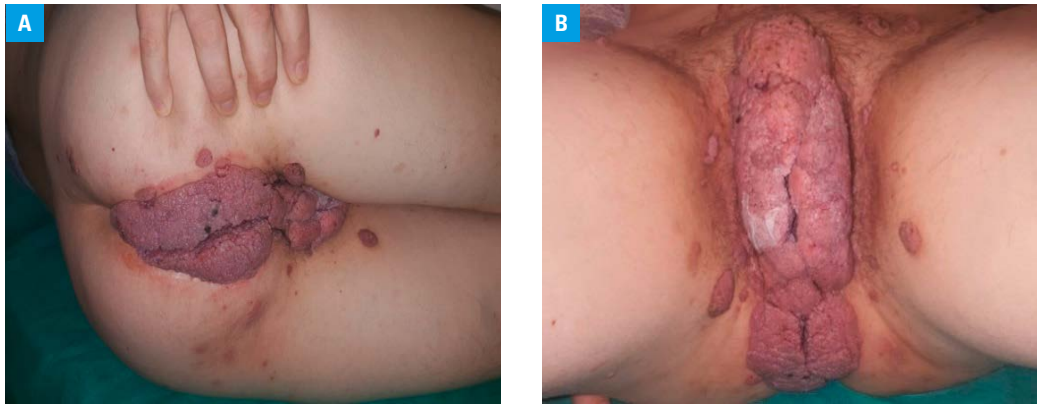

FIGURE 4 Anogenital lesions in a woman treated with long-term immunosuppression, with a 20-year history of type 1 diabetes; 2 years following kidney transplant from a multiorgan donor. High-grade squamous intraepithelial lesions classified according to the International Society for the Study of Vulvovaginal Disease and International Society of Gynecological Pathologists: **A** - vulvar intraepithelial neoplasia (usual-type VIN or VIN 3); **B** - anal intraepithelial neoplasia (AIN 3)

HIV-negative recipients, and the 3-year survival rates are significantly lower as compared with living-donor or standard-criteria-donor recipients. However, the incidence of acute rejection is markedly higher in HIV-positive recipients.[69] In countries with a high prevalence of HIV, such as South Africa, successful KTx from HIV-positive donors to HIV-positive recipients has been reported.[70] According to the European Renal Best Practice (ERBP) guidelines,[71] HIV-positive kidney transplant candidates should be waitlisted if they are compliant with treatment (particularly HAART therapy), their $CD4^+$ T-cell counts exceed 200/μl, they have been stable over the previous 3 months, HIV RNA was undetectable over the previous 3 months, no opportunistic infections occurred over the previous 6 months, and they show no signs of progressive multifocal leukoencephalopathy, chronic intestinal cryptosporidiosis, or lymphoma (grade 1C). Although the ERBP did not refer to exclusion criteria, it seems reasonable to exclude patients with a history of opportunistic infections (unless immune reconstitution has occurred), major neoplasms which might require systemic chemotherapy, or end-stage liver disease. The ERBP also suggest discussing the most appropriate antiretroviral therapy with the infectious diseases team to anticipate potential drug interactions after transplantation (ungraded statement). It is of utmost importance, since HIV therapy includes a combination of at least 3 fully active drugs from the currently available classes of antiretroviral agents: nucleoside analogue reverse transcriptase inhibitors (NRTIs), non-NRTIs (NNRTIs), HIV-protease inhibitors, entry inhibitors, and integrase inhibitors. Protease inhibitors, especially ritonavir, inhibit P450 cytochrome isoenzymes, and one such isoenzyme, CYP3A4, is critical for the metabolism of calcineurin inhibitors.[72] It was reported that protease inhibitors increased through levels of cyclosporine, TAC, and sirolimus, compared with those on NNRTIs alone or with patients not on antiretroviral therapy, requiring either a dose reduction or an increase in dosing interval.[73] As proton pump inhibitors are frequently used in patients after transplantation, caution should be exercised with atanazavir, as these drugs interfere with its absorption. Efavirenz, an NNRTI, exerts a mixed inhibitory and inducing effect on some P450 isoenzymes. Antiproliferative drugs, such as mycophenolate mofetil, may interact with nucleoside analogues, like zidovudine and stavudine, limiting their efficacy. On the other hand, mycophenolate enhances the effect of abacavir. Currently, due to low potential for drug interactions, raltegravir and dolutegravir are more frequently used. Antithymocyte globulin, an agent frequently used in induction or to treat acute rejection, may be associated with marked CD4-positive depletion. Therefore, it should be used with caution in this population, and prophylaxis against opportunistic infections should be reinitiated if not used or discontinued earlier. It is difficult to separate the complications of particular opportunistic infections associated with HIV progression from the complications associated with the IS used for transplantation. Prophylaxis against opportunistic infections is certainly indicated in patients after transplantation, as they need lifelong primary and secondary trimethoprim and sulfamethoxazole-based prophylaxis against *Pneumocystis jiroveci* (*P. jiroveci*).[74]

Human papilloma virus The introduction of primary prevention and continuous monitoring in patients with known high-risk HPV, enabled not only early diagnosis and treatment but also prevention of malignancies in transplant recipients using long-term immunosuppressive therapy.[75,76]

HPV belongs to DNA viruses, and protein E6 and E7 transformation is directly associated with HPV infection, which may have different presentations (subclinical infection, active clinical stage and latent stage). Clinically, we typically diagnose preexisting HPV infection already after transplantation, or de novo infection after transplantation, which manifests as Kaposi sarcoma, lymphoma, or skin papillomata including anogenital region and/or oral cavity (FIGURES 4 and 5). Malignancy may develop at any time following transplantation, with the mean time of 47 months, and constitutes a cause of death in transplant recipients. The incidence of malignancies at 30 years following transplantation is 75% for skin cancer and 33% for other cancers.[77,78]

FIGURE 5 Lesions in a single-organ–transplant recipient treated with long-term immunosuppression, 6 months following liver transplant from a multiorgan donor. **A** – scar after liver transplantation; **B** – low-grade squamous intraepithelial lesion of the vulva (also classified as VIN 1, VIN 2 or mild, moderate dysplasia); **C** – low-grade anal squamous intraepithelial lesion (also classified as AIN 1 or mild dysplasia)

TABLE 6 Management of human papillomavirus infection in organ / cell transplant recipients on long-term immunosuppression

Multidisciplinary care since detection of HPV infection (especially if oncogenic HPV is involved): dentist, dermatologist, general practitioner, gynecologist, and surgeon
IS monitoring and changes to treatment algorithm if clinically justified
Diagnosis and decision on management option: conservative vs surgical
Molecular HPV diagnosis and immunohistochemical evaluation of biopsy specimen or resected lesion
If malignancy found: chemotherapy and radiotherapy, and regular follow-up
Testing sexual partners as a source of infection
Choosing optimum management strategy in pregnant women
Monitoring of children born from mothers with known HPV infections
Immunotherapy as primary HPV prevention to be considered

Abbreviations: HPV, human papillomavirus

Cancer markers are used increasingly often at present nowadays in monitoring IS in transplant recipients and in patients treated for high-risk HPV–dependent malignancies. One of such markers is tumor protein p53, which plays a role key role in prognosing recurrence or death in HPV-positive patients after transplantation on long-term IS. Surgical management of basal cell carcinoma or squamous cell carcinoma with full histological diagnosis helps determine appropriate treatment and prognosis. Clinical experience demonstrated that high expression of p53 correlates with increased risk for the recurrence of oral and anogenital cancers.[79,80]

Treatment algorithm and multidisciplinary medical care not only improved survival in allograft recipients, but also significantly improved and prolonged the allograft function with long-term IS, decreasing the risk of many malignancies, directly linked to oncogenic viruses, including HPV. The management of HPV-infected transplant recipients on long-term IS, requires multidisciplinary care of different specialist care (TABLE 6).

Viruses versus myocarditis and acute coronary syndromes Myocarditis is an inflammation involving cardiomyocytes, interstitium, blood vessels, and sometimes also the pericardium. The etiology cannot be determined in the majority of cases. Apart from de novo infection, reactivation of latent infection is also likely. Basic classification includes acute, giant cell, and eosinophilic myocarditis. Classification by the course of disease includes fulminant, acute, and subacute / chronic myocarditis. In 2013, *European Heart Journal* published the most current position statement of the European Society of Cardiology on the etiology, diagnosis, management, and therapy of myocarditis.[81]

The diagnosis of myocarditis is based on the specific criteria including acute chest pain, rapid progression to heart failure, fatigue, exercise dyspnea, arrhythmia, or even cardiogenic shock of unknown origin. The second group of criteria include abnormal pattern on electrocardiogram, and abnormalities in diagnostic imaging and laboratory tests. Myocardial biopsy plays the key role in diagnosing myocarditis and determining its etiology as well as prognosis. The most common causes of myocarditis

PART II

Specific issues in management of viral infections in transplant recipients

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include viral (both DNA and RNA viruses), bacterial, fungal, protozoan, and parasitic infections. Other causes include immune and toxic factors. Over the last 60 years, the profile of viruses responsible for myocarditis has changed significantly: the share of enteroviruses and adenoviruses has decreased, while parvovirus B19, HHV-6, as well as HCV, EBV, and influenza viruses have emerged as the key contributors.[82] It should be noted that from 0.4% to 13% of adults hospitalized with confirmed influenza infection develop myocarditis, and as many as 68% of myocarditis cases secondary to influenza are seen in adults below 40 years of age.[83]

Acute myocarditis is a dangerous condition, which may lead to chronic cardiac failure in 25% of cases. The acute phase may also lead to death or severe dilated cardiomyopathy in 12% to 25% of cases.[84] The immune mechanism is the key contributor to myocardial damage due to myocarditis. The NK cells, which exert cardioprotective effect by inhibiting virus replication, are the first to reach the infected myocardium. The next immune stage involves cytokine production: IL-1β, tumor necrosis factor α (TNF-α), IL-2β, and interferon γ. The immune response is followed by T-cell influx, reaching the peak between day 7 and 14 of the infection. It is also the phase where the largest damage to the myocardium occurs, as molecular mimicry mechanisms cause T cells to damage infected cardiomyocytes.[85] Nitric oxide also plays an important role in the pathomechanism of myocarditis. In myocarditis, the expression of induced nitric oxide synthase is upregulated by TNF-α and IL-1β originating from both inflammatory cells and myocytes. This increases the production of NO and toxic peroxynitrite, causing oxidative stress that leads to apoptosis and necrosis as well as impairs myocardial function. Treatment of myocarditis is limited to symptomatic options available for heart failure and arrhythmia or involves implantation of implantable cardioverter-defibrillators. Antiviral drugs are currently in clinical trials.

According to current definitions, acute coronary syndromes (ACSs) can be classified into ST-segment elevation myocardial infarction ACS (in which the blockage of the coronary artery causes

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Conflict of interest: none declared.
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the blood flow to stop, thus leading to myocardial necrosis) and non–ST-segment elevation (NSTEMI) ACS (unstable angina [UA] / NSTEMI). UA / NSTEMI is referred to as a clinical syndrome caused by a new or persistent, yet increasing, blood flow reduction in the coronary artery (UA), which leads in some patients to myocardial necrosis presenting as elevated blood levels of necrosis markers, without signs of new ST-segment elevation on electrocardiogram.[86]

The key viruses implicated in ACS include HCV, HIV, and CMV. However, it should be noted that parvovirus B19 is the most common etiological factor of acute myocarditis presenting as ACS with generalized ST-segment elevation. HIV leads to a faster and more aggressive development of coronary atherosclerosis in affected patients, both directly by inducing permanent inflammation and coagulation disorders and indirectly through adverse effects of antiretroviral agents and higher risk of coronary artery disease (CAD) in HIV-positive population.[87] HCV infection, on the other hand, causes chronic, smoldering inflammation, which along with dyslipidemia, hepatic steatosis, and insulin resistance increase the risk of CAD, which is typically already more advanced on diagnosis. The adverse effects of antiviral agents should also be considered in this context, as it is with antiretrovirals used in HIV-positive patients. Additionally, HCV increases the risk of stroke by 27%. Among all HHVs, only CMV has been confirmed to increase the risk of CAD and ACS.[88] CMV infection is also a key contributor to ACS in patients after OHT.[89,90] It affects the development of graft vascular disease associated with chronic graft rejection, thus decreasing survival.

Viruses and kidney transplant eligibility **General recommendations** Patients awaiting KTx should be free of infections. Active acute infection is an absolute contraindication to a transplant surgery. Patients with chronic infections may only be considered eligible after the infection is resolved or well-controlled with treatment. Therefore, each patient on a transplant waiting list should be monitored for chronic infections and should undergo the following virologic tests: HCV PCR, HCV genotype, hepatitis B surface (HBs) antigen (HBsAg), HBV DNA, as well as antibody titers against CMV, EBV, HIV, HCV, and HBV (HBs and hepatitis B core [HBc]).[91,92] If clinically indicated, antibody titers against herpes simplex virus (HSV), Varicella zoster virus (VZV), HPV, and West Nile virus should be determined along with serology testing for HHV-8 and BKV. Additionally, both the donor and the recipient should be tested for CMV and BKV directly prior to transplantation, so that their serological status is known. It is essential for choosing the optimal IS protocol and determines potential need for CMV prophylaxis.

Vaccinations are a means for preventing infections.[93] A good example is HBV vaccination, which virtually eliminated new cases of HBV in patients on renal replacement therapy. Vaccinations should be administered prior to transplantation, which is an absolute necessity in case of attenuated vaccines. The most common of them are measles, mumps, rubella, and varicella zoster vaccines. A 4-week interval between attenuated vaccine administration and transplantation is indicated. There are no vaccines available for CMV, EBV, HCV, HSV, and BKV.

Viral infections in kidney transplant candidates Hepatitis B and hepatitis C virus Although HBV and HCV infections are not contraindication to KTx, HBV- and HCV-positive recipients are at higher risk of posttransplant complications. Therefore, each patient eligible for KTx should undergo virology testing prior to the procedure.

Currently, HBV and HCV infections are increasingly less problematic due to availability of effective HBV vaccines and effective treatment of HCV. Anti–HBs antibody titer should be reassessed every 6 months and a decision of potential booster vaccine should be guided by its results. In nonimmunized patients, anti–HBc antibody titers should also be determined. In patients positive for anti–HBc antibodies, HBV DNA load should be determined.

Obviously, transplant candidates positive for HBV or HCV (or both) should be treated preferably prior to transplantation, although the results of HBV treatment are usually unsatisfactory. On the other hand, owing to the availability of effective antiviral treatment, HCV has virtually been eradicated in affected patients. HCV-infected patients should be treated prior to transplantation, as most direct-acting antivirals (DAAs) used for HCV treatment interfere with IS, especially with calcineurin inhibitors. Patients without serologic and virologic features of HBV or HCV infections (or both), should be tested for the presence of HBsAg, HCV RNA, and anti–HBc and anti–HCV antibodies every 6 months.

Human herpes virus 8 HHV-8 infection is usually a reactivation of a latent infection. It is responsible for Kaposi sarcoma in transplant recipients. Some guidelines—especially in endemic countries—provide for routine anti–HHV-8 antibody testing in potential transplant recipients.

Human papilloma virus A significant number of transplant recipients develop papillae (warts) on the skin and in the genital area, which are not cancerous but are prone to malignant transformation. Their presence increases the risk of cervical, vulval, rectal, and skin cancer. Therefore, vaccinating transplant candidates without anti–HPV antibodies seems clinically justified, especially in young women.[92] However, there are no guidelines, not even in the development stage, on preventing HPV infections in male transplant recipients.

Herpes simplex virus 1 and 2 Both HSV infection and its reactivation in allograft recipients are common. The reactivation typically occurs within

the first month following transplantation. Apart from insignificant mucositis, HSV may also cause severe infections, such as encephalitis. On enrollment on a transplant waiting list, potential recipients are not routinely tested for the presence of anti–HSV antibodies. However, some centers in the United States test their patients for the presence of anti–HSV-2 antibodies.

Human polyomaviruses BK and JC BKV is not routinely tested when assessing patient eligibility for transplantation, except in those who need another transplant and lost the first kidney allograft due to BKV infection. Repeat transplantation is possible in such patients. However, it should be performed at least 6 months following a complete discontinuation of IS, when BKV is not present in the blood and only low viruria is allowed.[92] Graft removal does not prevent future BKV infection. However, it should be considered if IS cannot be discontinued (double transplant) or with persistent high virus load.[92] JC virus is only a rare cause of nephropathy. Due to its special affinity to nervous tissue, it usually causes progressive multifocal leukoencephalopathy. When cured, and the patient is eligible for another transplant, the risk of reactivation should always be considered. Therefore, such patients should be tested for JC virus before and after transplant.[92]

Varicella zoster virus About 90% of transplant recipients test positive for anti–VZV antibodies. Reactivation after transplant usually presents as shingles, and in seronegative recipients as chickenpox, often following a severe clinical course. Serologic assessment of recipients for VZV is usually recommended. If seronegative, they should be immunized when on dialysis.[91,92] As only live attenuated vaccines are available, they cannot be administered after transplant.[93]

HIV HIV is discussed in greater detail in the subsequent part of this paper. Here, we only present basic information on the eligibility of HIV-positive patients for transplantation. HIV infection does not constitute a contraindication to transplantation if the patient meets the following criteria:[94] 1) CD4 count greater than 200 cells/μl over the last 6 months; 2) undetectable HIV viral load (<50 copies of HIV-1 RNA/ml) over the last 6 months; 3) documented, regular highly active antiretroviral therapy (HAART) over the last 6 months; 4) no signs or symptoms of acquired immune deficiency syndrome (AIDS); 5) available antiretroviral treatment after transplantation.

Anti–HIV antibody titer should be determined in potential transplant recipients every 6 months.

Treatment of hepatitis B virus infection in transplant recipients The majority of general population with chronic HBV infections undergo the so called immune-tolerant phase of infection (very high HBV-DNA load, no immune response to the virus, alanine transaminase (ALT) levels and liver histology close to normal), and during this period such patients are positive for hepatitis B e-antigen (HBeAg) and HBsAg. The second phase is immune clearance of infection (the immune system recognizes the virus and attempts to clear it from the liver, which results in ALT flares and a decrease of HBV-DNA load). Subsequently, most patients remain in the immune control or non-replicative phase, and they are negative for both HBeAg and HBsAg, and also positive for anti–HBe, anti–HBs, and anti–HBc antibodies. However, most patients with end-stage renal disease remain in the so called inactive carrier phase with normal ALT levels and either low or undetectable HBV-DNA due to the attrition of the immune system. They remain positive HBsAg-positive and HBeAg-negative, although some patients may proceed to clear HBsAg and be negative for HBsAg and positive for anti–HBc antibodies, and sometimes even positive for anti–HBs antibodies. Do the patients ever get rid of the HBV? The traces of HBV-DNA (cccDNA, covalently closed circular DNA) insert themselves in an episomal form, similar to chromosomal DNA of the host, into the nuclei of hepatocytes and remain there as long as the cell survives, probably surviving the mitosis and transferring to daughter cells. Therefore, it is immune control rather than clearance that takes place in chronic HBV infection, even in patients with the so called resolved HBV. However, the problem starts when IS is achieved with T-cell inhibition, because there is less control of viral replication, which facilitates HBV reactivation resulting in a dramatic increase of viral load. With downregulation of IS, immune reconstitution occurs. T cells "wake up", recognize the abundance of HBV and while clearing HBV from the liver, they simultaneously destroy hepatocytes. This leads to flares of hepatitis, which may result in liver failure and patient death even despite continued antiviral therapy.

Depending on the HBV profile at baseline, HBV reactivation is defined as: 1) a marked increase in HBV replication or a new onset of HBV-DNA in a HBsAg-positive individual or 2) a reverse seroconversion defined as a reappearance of HBsAg and HBV-DNA in a previously HBsAg-negative patient, without detectable HBV-DNA load. The main causes of reactivation include cancer chemotherapy, immune modulation for autoimmune conditions (use of TNF inhibitors in inflammatory bowel disease or rheumatoid arthritis), HIV, solid organ transplantation (kidney, heart, lung), bone marrow transplantation, and antiviral HBV therapy. The key risk factors for reactivation can be classified broadly into the 3 categories of host-related factors, virus-related factors, and type or degree of IS. Host-related factors include male sex, older age, presence of cirrhosis, and type of disease needing IS (eg, lymphoma). Virus-related factors associated with an increased risk of reactivation include high baseline HBV-DNA levels, HBeAg positivity, chronic hepatitis B, non-A HBV genotype, treatment of

coinfected patients with antivirals such as DAAs for HCV, and non-B antiretroviral therapy for HIV. The assessment of host- and virus-related risk factors should be important caveats to help decide whether to initiate preventative therapy before initiating IS. To properly assess the risk of reactivation, the following serological markers should be tested: HBsAg, anti–HBc antibody, anti–HBs antibody, and HBV-DNA in all HBsAg--positive patients. The risk of HBV reactivation can be divided into high (>10%), moderate (1%–10%), and low (<1%) based on the type of immunosuppressive therapy stratified by the HBV serology. The risk is considered high to moderate in patients positive for anti–HBc but negative for anti–HBs antibodies, whether HBsAg-positive or HBsAg-negative. It is lower in patients negative for HBsAg and positive for anti–HBc anti–HBs antibodies. The risk of reactivation is high in all patients treated with B cell–depleting agents, including rituximab, and may be high or moderate in those treated with high doses of steroid and calcineurin inhibitors.

In many patients, exposure to immunosuppressive therapy early in HBV reactivation, induces the asymptomatic phase, which provides a window of opportunity to initiate treatment. In HBsAg--positive patients, this asymptomatic phase is characterized by a rapid increase in HBV-DNA, followed by a rapid increase in ALT levels. In HBsAg-negative patients, this asymptomatic phase is characterized by the reappearance of HBsAg, and then a sudden increase in HBV-DNA, followed by an increase in ALT levels. Taking the above into consideration, prophylaxis should be ideally started 2 to 4 weeks before the initiation of immunosuppressive therapy and maintained for at least 6 to 12 months after the last dose of the therapy.

The recommendations for the management of hepatitis B reactivation are as follows (FIGURE 6): antiviral therapy should be started in patients with chronic hepatitis B (HBsAg-positive, HBV--DNA level of ≥2000 IU/ml, increased ALT levels). Inactive HBV carriers (HBsAg-positive, HBV-DNA <2000 IU/ml, normal ALT levels) exposed to high- and moderate-risk immunosuppressive therapy should undergo prophylaxis. Inactive HBsAg carriers exposed to low-risk immunosuppressive therapy, as well as HBsAg--negative and anti–HBc-positive patients, should be monitored (ALT and HBsAg, plus additionally HBV-DNA in HBsAg-positive individuals). HBsAg-negative and anti–HBc-positive patients exposed to high-risk regimens should receive routine prophylaxis.

HBsAg-negative, anti–HBc-positive and anti–HBs-positive patients have a lower risk of reactivation than those without anti–HBs antibodies. When exposed to moderate risk of reactivation, anti–HBV prophylaxis should be considered in these patients. Alternatively, they can be monitored (serum ALT and HBsAg levels) every 3 months until 6 months after the last dose of immunosuppressive therapy. HBV reactivation is likely to occur up to 1 to 2 years after the last dose of rituximab. Therefore, anti–HBV prophylaxis can be continued for up to 2 years after the last dose of rituximab in all exposed patients.

The European Association for the Study of the Liver 2017 Clinical Practice Guidelines on the management of HBV in renal transplant recipients recommend entecavir or tenofovir as prophylaxis or treatment. Lamivudine is not considered the drug of choice because it has a lower genetic barrier to resistance; especially when administered for over 12 months. HBV serology, including HBsAg and anti–HBs antibody levels, should be monitored after transplantation. Vaccination pre- and posttransplantation is also recommended in all patients, including those with resolved hepatitis B, with the aim to maintaining HBsAb level above 100 U/l. HBsAb is an important natural antibody to prevent HBV infection; it may also sufficiently prevent HBV reactivation in occult HBV carriers after KTx.

After vaccination, more patients become HBsAb-positive, which reduces the risk for HBV reactivation or HBV reinfection after KTx. Although the efficacy of vaccination is lower in immunosuppressed patients, a booster dose may induce an anamnestic response in patients with a history of HBV infection and decreasing HBsAb levels, so vaccination may prove useful in a reasonable proportion of such patients. In patients who do not exhibit an anti–HBs antibody response, monitoring viral serology remains mandatory to detect reactivation.

In summary, it should be emphasized that the need for prophylaxis or treatment of HBV infection depends on viral and host factors, as well as the risk of HBV reactivation based on the type of immunosuppressive therapy.

Viral infections in kidney and upper extremity transplant recipients

Human adenovirus infections

In patients on IS, adenovirus may cause hemorrhagic cystitis and—less often—other organ involvement, typically within the first 3 months following transplantation.[95]

Case report 1 A 45-year-old man previously treated with hemodialysis received a kidney allograft from a deceased donor. The IS regimen included TAC, MMF, and glucocorticoids. On day 10 following transplantation, the patient presented with dysuria, hematuria, and pain within the bladder area which required opioid analgesics. The decision was made to remove the ureteral catheter (on day 19 following transplantation), which did not improve the symptoms. Urinalysis demonstrated red blood cells and macroscopic hematuria. On day 25 following transplantation, adenovirus infection was confirmed with viruria of 1.27×10^6 copies/ml, and viremia of 1.65×10^5 copies/ml.

MMF dose was reduced and GCV was administered for 14 days. The viral load in blood became undetectable with the significantly reduced

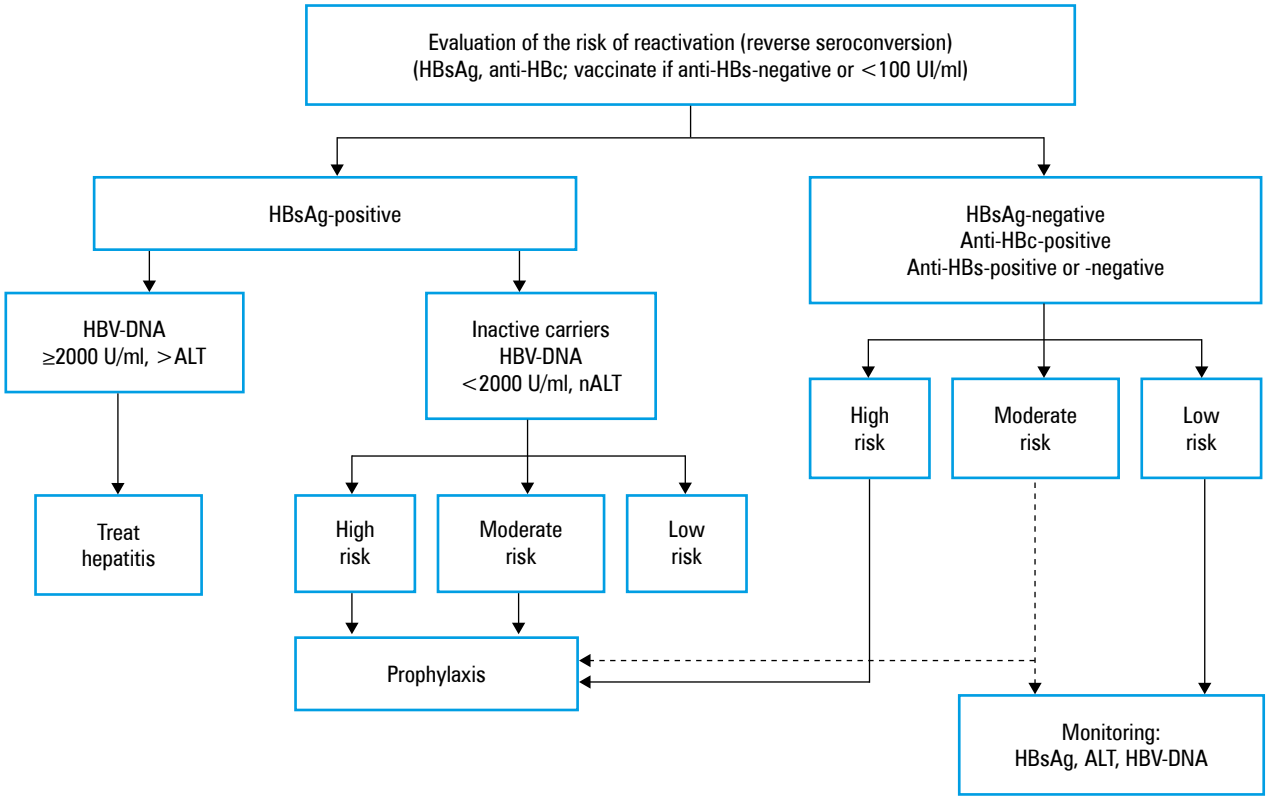

FIGURE 6 Algorithm for management of hepatitis B reactivation (modified from Loomba and Liang. Gastroenterology. 2017; 152: 1297-1309.)

Abbreviations: ALT, alanine transaminase; anit-HBc, antibody to hepatitis B core antigen; anti-HBs, antibody to hepatitis B surface antigen; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen

viruria (2.3×10^5 copies/ml). After the completion of intravenous treatment, VGCV prophylaxis was continued until the end of month 6 following transplantation. At 3- and 6-month follow-up, viruria or clinical symptoms did not recur, and the function of the allograft was stable with a serum creatinine level of 1.5 mg/dl.

Case report 2 A 62-year-old male KTx recipient presented to the emergency department at 24 month following transplantation with fever (40°C), hematuria, and dysuria. He was on chronic IS with TAC, MMF, and glucocorticoids. Diagnostic imaging (abdominal ultrasound and computed tomography) was requested due to significant hematuria, and massive interstitial nephritis of the allograft was diagnosed. Cystoscopic examination demonstrated blood-stained urine leaking from the allograft, along with hyperemia and inflamed mucosal membranes of the bladder. Urine cultures were negative; however, adenovirus was detected in blood (3.28×10^2 copies/ml) and urine (3.35×10^6 copies/ml) samples. MMF was temporarily discontinued. The patient was started on GCV (for 18 days) and VGCV was continued. Such treatment resolved clinical symptoms and cleared the virus from the blood and urine. The patient was restarted on MMF 3 months later. Follow-up assessment at 12 months and 3 years did not demonstrate reactivation of infection.

Parvovirus B19 infection In SOT recipients, parvovirus B19 infection leads to anemia and reticulocytopenia in 99% of cases. Other symptoms may include fever, joint pain, rash, leukopenia, thrombocytopenia, as well as chronic or acute allograft dysfunction. The treatment involves immunosuppressant dose reduction and intravenous immunoglobulins.[96]

Case report A 45-year-old woman with a long history of IS due to focal segmental glomerulosclerosis received KTx from a deceased donor. The kidney immediately assumed its function, and the early postoperative course was uneventful. IS included induction with basiliximab, followed by TAC, MMF, and glucocorticoids. Six weeks following TAC, she was admitted to our department on an emergency basis due to anemia, stenocardia, and exercise dyspnea. The follow-up hemoglobin level was 7.4 g/dl; leukoreduced red blood cell (RBC) unit were administered and MMF dose was reduced. The patient was readmitted 3 weeks later due to significant anemia and leukopenia (hemoglobin, 6.9 g/dl; white blood cell count, 2.74 K/μl). The graft function was normal. Virology testing confirmed very high blood B19 parvovirus levels ($>10^7$ copies/ml). Leukoreduced RBC unit and 10 g of intravenous immunoglobulin were administered and MMF was discontinued. Another 3 weeks later, the patient was hospitalized again

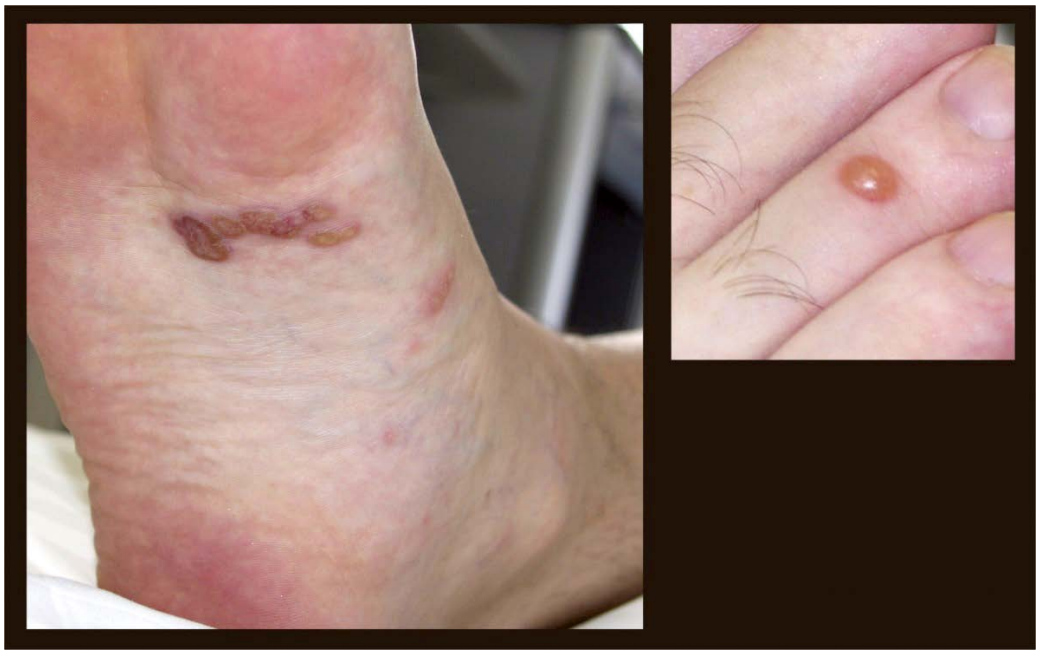

FIGURE 7 Vesicular lesions of varicella zoster virus infection on the sole of the patient's foot.

due to urinary tract infection caused by *K. pneumoniae*. Along with antibiotics, she was administered 20 g of intravenous immunoglobulin and 3 packs of leukoreduced packed red blood cells. The parvovirus B19 viral load still remained high (>10^7 copies/ml). Additionally, TAC was switched to CsA. Later, the patient experienced similar incidents of urinary tract infection and anemia twice at 1-month interval. From month 7 following the onset of symptoms, anemia was controlled, viral load was undetectable, and the function of the allograft kidney was normal.

Varicella zoster virus infections VZV causes the occurrence of serous blisters and vessicles on an erythematous base within the involved segment of the nervous system. In immunocompetent individuals, the face, torso and chest are typically affected. In SOT recipients, the infection can be diffuse.[97]

Case report A 40-year old male upper extremity allograft recipient presented to the emergency department with severe pain in his right hip and sacral bone. The symptoms, which were not associated with other clinical symptoms or abnormal laboratory test parameters, left him immobile and he needed opioid analgesia. For 74 months following upper extremity transplant, the patient was on long-term IS based on TAC, MMF, and glucocorticoids. The results of abdominal ultrasound, chest X-ray, lumbosacral spinal X-ray, and hip X-ray were insignificant. After 3 days following admission, the linear vesicular lesions on the sole of the patient's foot were noticed, consistent with VZV infection (FIGURE 7). ACV treatment was started, initially intravenously, followed by oral formulation (for a total of 17 days), and the dose of MMF was reduced. The treatment led to gradual symptom resolution.

Histological diagnosis of BK virus and cytomegalovirus infections Even though CMV rarely causes microscopically detectable lesions in transplanted kidneys, the *in situ* hybridization techniques confirmed the CMV presence within the tubular epithelium of about 40% of kidney allografts.[98] Classic microscopic manifestation of CMV infection includes cytopathy affecting epithelium, less often endothelium (FIGURES 7 and 8), and (the least often) inflammatory mononuclears.[99] Among CMV-induced cellular changes, the most characteristic are the oval inclusions, situated centrally within the nucleus, with darker center and bright, crescent-shaped periphery (thus resembling an "owl's eye" appearance). Apart from those intranuclear inclusions, tiny basophilic cytoplasmic inclusions can also be seen in some cases. Along with cytopathy, cellular inflammatory response may be present, sometimes with the formation of epithelial granulomas or tiny parenchymal abscesses. Due to the nonspecific morphology of interstitial inflammatory infiltrate the diagnostic differential must include an acute tubulointerstitial rejection. Apart from CMV early nuclear protein, CMV mRNA, or CMV protein revealed by immunohistochemical or *in situ* hybridization assays of help may be immunomorphological evaluation of major histocompatibility complex class II antigens (HLA-DR). The demonstration of HLA-DR expression on the surface of tubular epithelium is suggestive of allograft rejection. There have been reports indicating potential involvement of CMV in the post-transplantation thrombotic microangiopathy, that could be triggered by CMV-induced production of anticardiolipin antibodies. In rare cases, CMV infection can lead to acute glomerulopathy (FIGURES 8 and 9).[100]

The only method to confirm BKV-associated nephropathy is a core needle biopsy of the kidney. However, it should be noted that the infection-related lesions are focal, which increases the risk of sampling error if only tiny, single tissue fragments from the superficial renal cortex are collected during the biopsy.[101] BKVAN is diagnosed

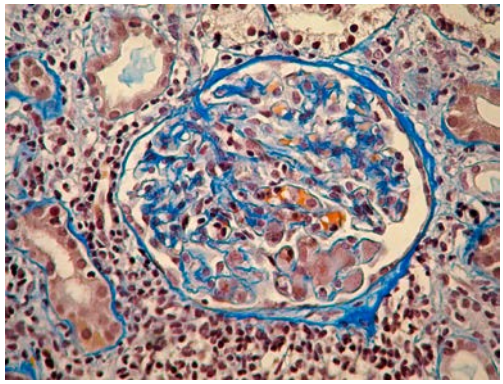

FIGURE 8 Acute cytomegalovirus glomerulopathy (acid fuchsin orange G staining). Cytopathic lesions (hypertrophy and nuclear enlargement) in endothelial cells.

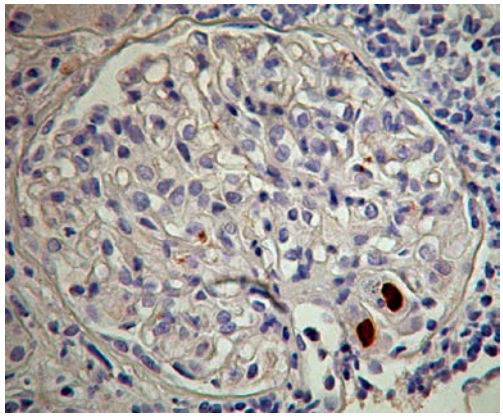

FIGURE 9 Acute cytomegalovirus (CMV) glomerulopathy, CMV nuclear viral inclusions in endothelial cells (immunohistochemical staining with anti–CMV antibodies).

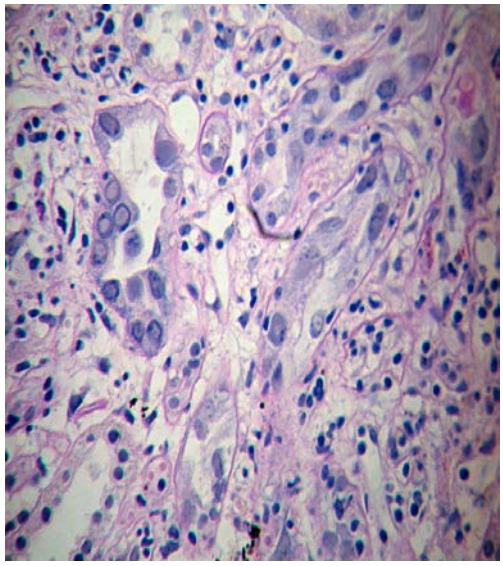

FIGURE 10
BK virus–associated nephropathy; intraepithelial viral inclusions and acute tubular necrosis (HE)

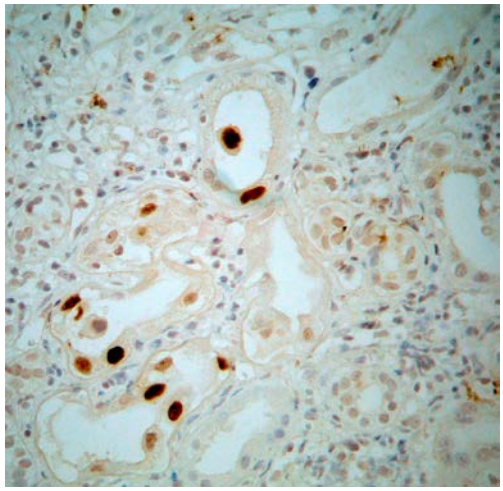

FIGURE 11
BK virus–associated nephropathy. Nuclear viral inclusions in tubular epithelium marked by brown coloration in the immunohistochemical reaction with anti-SV40 antibody.

based on virus replication in tubular epithelial cells and/or the Bowman's capsule epithelium which may be revealed by immunohistochemistry or *in situ* hybridization methods. A characteristic, but not always present feature that accompanies virus replication, is the presence of intranuclear inclusion in the tubular, as well as parietal (Bowman's capsule) epithelial cells (FIGURE 10).

In early stages of the infection, the only manifestation of virus replication may be necrosis of single tubular epithelial cells.[102] In some cases, tiny focal areas of polymorphic inflammatory infiltration or fibrosis can be present within the interstitium, often only within the medulla. At early stages of BKVAN, intranuclear viral inclusions may be unnoticeable; which makes a diagnosis fully dependant on viral antigen detection (FIGURE 11) in tubular or glomerular parietal epithelial cells. Another sign of BKV-associated nephropathy is the presence of virion clusters forming structures that may be visualized in urine samples by electron microscopy. Late-stage BKVAN is characteristically manifested by the degeneration and necrosis of infected epithelial cells as well as tubulointerstitial nephritis and tubulointerstitial scarring. Along with the presence of lymphocytes and—less often—neutrophils, another feature of BKVAN is a significant concentration of plasma cells in the interstitium; some of which may also invade tubular epithelium. In some cases, the described lesions may be concomitant with less specific morphologic abnormalities such as pseudo crescents formation (associated with BKV-induced damage and proliferation of the parietal glomerular epithelium), as well as immune complexes deposition within tubular basal membranes.[103]

The coexistence of BKV intraepithelial replication with endarteritis, capillarities (glomerular and/or peritubular) or microscopic signs of complement activation within the allograft tissue implicates the recognition of concomitant BKVAN and acute rejection. Due to identical morphologic presentation in the interstitial compartment, differentiation between isolated BKVAN and BKVAN superimposed on acute tubulointerstitial rejection is much more challenging. However, the distinction between these two clinical situations has the key significance, due to different management algorithms.

Viral diarrhea Gastrointestinal symptoms are present in 40% to 70% of transplant recipients. They are typically ascertained from the history. According to the United Network for Organ Sharing data, 11.5%, 17.5%, and 22.6% of KTx recipients experience diarrhea within 1, 2, and 3 years after transplantation, respectively.[104] WHO defines diarrhea as the passage of 3 or more loose or liquid stools per day, whereas persistent diarrhea lasts for over 1 month. Diarrhea in transplant recipients causes increased morbidity, need for inpatient treatment, acute kidney injury due to dehydration, variable exposure to immunosuppresants

causing nephrotoxicity or acute rejection, and impaired survival and quality of life. Diarrhea in transplant recipients can have an infectious or noninfectious etiology. Infectious diarrhea can be caused by bacteria, viruses, fungi, and parasites, whereas noninfectious one can be induced by immunosuppressants, other medications, and comorbidities, such as graft versus host disease, PTLD, inflammatory bowel disease, colon cancer, and malabsorption syndrome.[105]

Among viruses, CMV, norovirus, rotavirus, sapovirus, and adenovirus are the most common causes of diarrhea in transplant recipients. CMV is responsible for 5% to 20% of diarrhea in transplant recipients. Clinically, such infection presents as gastroenteritis with symptoms from the entire gastrointestinal tract (esophagus, stomach, small intestine, and colon). The diagnosis of gastrointestinal CMV disease should be based on viral load testing (preferably using quantitative PCR assay) and histological confirmation of infected cells (preferably immunohistochemistry or in situ hybridization). It should be noted that the viral load may be undetectable in about 15% of cases of gastrointestinal CMV disease. Gastrointestinal CMV disease should be treated in line with the general guidelines on CMV treatment (GCV is the first-line treatment). However, some patients may need longer treatment.[106] Other viruses can be detected in stool samples using molecular biology assays. Modern diagnostic techniques significantly increased the detection of viral diarrhea (from 23% to 72%), and demonstrated that a significant percentage (39%) of diarrhea have a mixed etiology. Multiplex PCR platforms are capable of multiple matrix amplification during a single polymerase chain reaction, which can detect over 20 pathogens in a stool sample within 1 hour.[107]

Norovirus (single-stranded RNA virus) causes outbreaks of acute diarrhea resolving after 2 to 3 days in immunocompetent individuals. In transplant recipients, norovirus is responsible for 17% to 26% of diarrhea, which can be acute or chronic, and the virus is excreted in stool for a relatively long time. Almost 80% of hospitalized patients present with acute kidney injury reversible after rehydration therapy. In transplant recipients, the course is usually 2-stage, with acute symptoms followed by a cycle consisting of a period of normal stools and a period of abnormal stools. Clinical observation supports the adverse effect of chronic noroviral diarrhea on the function of kidney allograft. Treatment is usually symptomatic, and there have been attempts to reduce IS and use oral or intravenous human immunoglobulins as well as antiparasitic nitazoxanide. There is no available vaccine.[108] Sapovirus (identified during an outbreak of gastroenteritis in Sapporo in 1982) causes milder diarrhea than norovirus. The symptoms resolve within 7 days and treatment should be symptomatic.

Rotavirus infections (1%-15% of all posttransplant diarrhea cases) have the most severe clinical course. The incubation period lasts 2 to 4 days, the onset is acute, and the symptoms include fever, vomiting, and watery diarrhea lasting 3 to 8 days. The virus remains present in the stool for 1 to 3 weeks. The infection usually develops in recipients of intestinal or liver transplant and in children recipients. The treatment is symptomatic. The attenuated vaccine is available yet contraindicated in transplant recipients.

Among 7 DNA groups (A-G) of adenoviruses, groups F (AdV 40 and 41) and G (AdV 52) are known to cause gastroenteritis, manifesting as fever, vomiting, and diarrhea. The infection in transplant recipients may develop as a reactivation of a latent infection or donor-derived infection. The infection usually develops within the first few months following transplantation, often with an involvement of an entire allograft. If diarrhea is the only symptom, the treatment should be symptomatic. If involvement of at least 2 organs is confirmed, antiviral treatment with cidofovir, ribavirin, or brincidofovir should be attempted.

Ground glass opacification "Ground glass opacification (GGO)" is a descriptive term used in radiology, referring to an area of increased attenuation in the lung on computed tomography with preserved bronchial and vascular markings.[109] It is a nonspecific sign with a wide etiology including pathologies with alveolar or interlobular septal involvement. It is seen when the alveoli are incompletely filled with transudate, exudate, blood, or cellular infiltration, or when the pulmonary interstitium thickens as a result of fibrosis, edema, or cellular infiltration. The described lesions can be chronic or acute. In transplant recipients (but not only), clinical information regarding the patient's immune status, nature, and duration of symptoms, smoking status and comorbidities are the key to accurate differential diagnosis. Viral, bacterial, and fungal infections, malignancies and drug-induced abnormalities may all present as GGO.

One of significant pathologies seen in transplant recipients is angioinvasive aspergillosis, which typically manifests as consolidated nodules surrounded by the GGO forming a crescent shape (halo sign)[110] (**FIGURE 12**) Although GGO can be seen in other pathologies (eg, granulomatosis with polyangiitis, malignancies, or viral infections), in antibiotic-resistant cases, it is considered pathognomonic for *Aspergillus fumigatus* infection.[111] Treatment and improving immune status of a patient results in separation of necrotic parenchyma from healthy parenchyma, which presents as the "air crescent sign."[112]

Other pathologies present in transplant recipients include CMV and *P. jiroveci* infections. Radiographic features of CMV infection (just as it is with other viruses) are nonspecific and typically involve bilateral blot-like or confluent areas of GGO, tiny nodules, and alveolar consolidation[113,114] (**FIGURE 13**). Radiographic features of

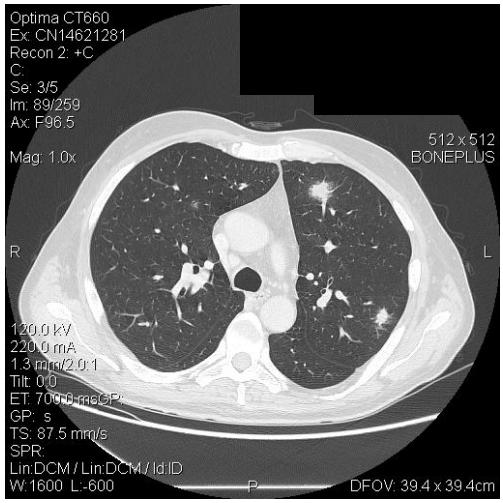

FIGURE 12 Halo sign in angioinvasive pulmonary aspergillosis

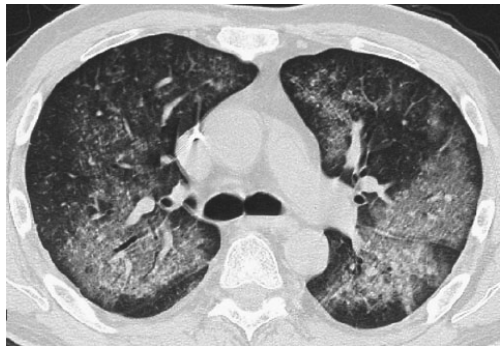

FIGURE 13
Cytomegalovirus infection manifested by bilateral ground glass opacity areas on computed tomography

P. jiroveci infection also include GGO, forming a pattern of reticular opacities caused by interlobular septal thickening. Clinical features of *P. jiroveci* infection include pneumatocele (which—if ruptured—may lead to pneumothorax), peripheral subpleural sparing, and infrequent occurrence of mediastinal lymphadenopathy and pleural effusion (up to 10% of cases).[115-117] GGO may also be seen in cases of drug-induced (eg, by everolimus) nonspecific interstitial pneumonia.[118,119] The drug-induced character of GGO is confirmed with its resolution following drug discontinuation.

Eventually, there are patients with persistent GGO in radiographic imaging. Long-term IS increases the risk of malignancy; therefore, differential diagnosis of persistent GGO should include adenocarcinoma. In such cases, long-term radiographic monitoring is indicated to assess the lesion for potential growth.[120,121]

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The art of immunosuppression in liver transplantation: personal reflections based on a 40-year career

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Introduction Since the introduction of liver transplantation (LTx) in clinical practice by Th. E. Starzl in 1963, major progress has been made in short--term outcomes. Indeed, organ procurement and preservation techniques, transplant surgery itself and perioperative care have all improved substantially. Despite this progress, long-term survival rates remained almost unchanged, which can be easily noticed when comparing the survival rates over the two last decades, having first eliminated the early mortality (6-month post-LTx) data. This is explained by the fact that many recipients die—most of them with a functioning graft—due to cardiovascular, infectious and oncologic (de novo tumor formation) complications, all of them linked directly or indirectly to the life-long intake of noxious immunosuppressants. With 85 to 90% of recipients surviving the early post-LTx period, the transplant community should focus on these complications and thus on the quality of life of liver allograft recipients. A thorough reflection about the use of immunosuppressants is, therefore, more than welcome in the beginning of this millennium (TABLE 1)

Fifty years ago, clinicians developed immunosuppression (IS) schemes, which aimed at eliminating acute cellular rejection (ACR) of liver allograft at any cost. It all started in the early 1960's with the "secret cocktail" of steroids and azathioprine. This rather simplistic IS scheme enabled stable 25% survival, despite almost unsurmountable technical and peri-operative problems at that time. Later on, antilymphatic sera were added to this cocktail aiming essentially at reducing numerous physical and psychological side effects of chronic steroid intake. The introduction of calcineurin inhibitors (CNi) in the early 1980s, first cyclosporine A and later on tacrolimus, represented a major breakthrough paving the way for selective IS resulting in a 80% survival of both patients and grafts.

Although the immunopriviledged status of a liver was proven a long ago, both in the laboratory and in clinical practice, clinicians were pushed by numerous industry driven studies to use increasingly stronger IS schemes in order to virtually eliminate ACR. With more experience, a curious constellation has become noticeable that the incidence of ACR remained similar despite using much stronger CNi-based IS schemes. It was only trough a better understanding and uniformization of transplant pathology (cf. Banff classification and classification of antibody mediated or humoral rejection) that the rejection process became considered a physiological event inherent to the mixing of the donor and recipient immune systems (cf. chimerism). It was understood that this interaction should be allowed to take place under the umbrella of an ideally individualized IS rather than forcefully suppressed. Both experimental and clinical studies of SOT confirmed the importance of this interaction, providing an explanation so as to why recipients who had a controlled ACR may have a significantly better long-term outcome.

Along with an obvious immunologic advantage, a number of other reasons support minimal IS schemes such as high risk of cardiovascular (20%), oncologic (10 to 20%) or infectious complications, kidney failure (20%) and metabolic syndrome (40%). All these complications significantly impact on long-term survival rates, some of them becoming relevant only after 10 years. Every effort should, therefore, be undertaken to counteract such evolution. Reducing the immunosuppressive burden should nowadays be the priority of every transplant center.

How to proceed to fulfil all these requirements? IS reduction should start by avoiding or eliminating steroids, which are the most noxious of all immunosuppresants. Whereas eliminating CNi

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Conflict of interest: none declared.

TABLE 1 Dogmas (or fixed beliefs) on immunosuppression (IS) to be reversed in liver transplantation

Every moderate or severe (Banff score >6) rejection needs to be treated.
Multidrug antirejection therapy is better than monodrug calcineurin inhibitor–based IS.
Steroid containing IS is better than steroid free IS.
Mycophenolate mofetil is more effective than azathioprine.
m-Tor inhibition–based IS is better than calcineurin inhibitor minimization in relation to renal sparing.
m-Tor inhibition–based IS improves results of transplantation for hepatocellular cancer.

has been shown to be a risky undertaking, steroid withdrawal or avoidance has been proven to be feasible and safe, since the seminal paper by Padburg et al. After a long period of hesitation, IS without long-term (ie, months) steroid use has become a common practice, especially in Europe. If the liver tests remain stable, one can take one more step towards subtherapeutic monotherapy. The next step of the minimization philosophy is to go for a monodrug CNi-based therapy. At long--term, IS monotherapy can consist of either CNi or an antimetabolite, depending on potential neuro- and especially nephrotoxicity. The subtherapeutic monotherapy IS strategy again has been proven to be feasible as well as safe. These three steps have without any doubt led to a marked improvement in the quality of life of the patients as well as an improved renal profile.

Consequently, the question should be put forward whether it is desirable to further walk down the road towards clinical operational tolerance (COT). The COT road has undoubtedly been violated frequently by the IS studies, those poorly designed or even excessive, and by numerous changes to IS care of a given recipient. Indeed, one should opt for a continuity of care and vision in the follow-up of transplant recipients, a condition very frequently lacking in daily clinical practice. Each change in the assumptions of the IS plan of an individual patient inevitably disrupts their tolerogenic process. Just like it is seen in modern traveling, a destination (a city or region) can be reached using different means of transportation (walk, car, train, boat and plane) using different routes (over sea, over land, heading north or south). Successful management of immunosuppression therapy should, therefore, involve a team's sticking to a uniform scheme, based on the current, reliable knowledge in the field.

In the light of all this reasoning, doubts regarding the use of anti-lymphocytic sera (no proven benefit in a large recent Cochrane review), mofetil mycophenolate (no proven benefit compared to the old azathioprine) or m-Tor inhibitors (no proven benefit compared to low IS load in transplant oncology, and no proven benefit in relation to renal function sparing compared to low dose CNI use) in clinical practice become easily understandable. All these considerations should also lead to a modified definition of the end-points for any IS study. Instead of being a specific parameter, such as acute (cellular and/ or humoral) rejection or renal insufficiency, a combined end-point should rather be assumed comprising patient and graft survival, as well as the incidence of chronic rejection. It also implies developing a long-term strategy for protocol liver biopsy.

Many publications, even in the highest impact factor transplantation journals, omitted the comparison of immunosupressive schemes with the real standard of care. This strategy has hampered major improvements in relation to long--term patient outcomes over the last two decades. It is now the task of all transplant professionals to take up this challenge seriuosly and to embark on well designed, investigator-driven studies.

A plea should also be made to reorganize transplantation clinics in order to centralize care provided for transplant recipients, often presenting with numerous comorbidities. Such centralization will not only contribute to improved efficiency; it will also be of utmost importance in the expanding field of transplant oncology. Indeed, widening the inclusion criteria of primary and secondary liver tumors implies a very good interaction between immunology and oncology in order to achieve a maximum possible reduction of tumor recurrence. Evidently, the minimization approach is also of importance in reducing the risk for bacterial fungal as well as viral diseases.

Conclusion Despite major progress in early outcomes, long-term outcomes after liver transplantation remain compromised by the development of many side-effects linked to the chronic use of immunosuppressants. Based on the immunoprivileged status of liver allograft, the transplantology community should wholeheartedly support immunosuppressive minimization protocols. Good and consistent results can only be obtained by standardization and uniformization of liver transplant clinics. Part of the success will depend on the set up of well-performing, outpatient liver biopsy clinics.

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