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# Complications of Post-Transplant Immunosuppression

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Additional information is available at the end of the chapter

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## 1. Introduction

Transplantation is one of the revolutionary fields in modern medicine that has saved thousands of lives. The continuous refinement of surgical techniques and the availability of potent immunosuppressive drugs have made transplantation the most effective treatment option for patients with end stage organ failure. Over 25 000 organ transplants are performed in the USA each year and survival rates following transplantation are now approaching 90% at 1 year and 75% at 5 years, depending on the organ transplant (kidney, liver, pancreas, heart, lung, intestine). Central to this success was the introduction of drugs that suppress the immune system and prevent rejection. Indeed, across organs, the use of current immunosuppression *regimens* effectively prevents acute rejection in the majority of patients. As a result, the incidence of graft loss due to acute rejection has decreased dramatically compared to the early era of transplantation.

This success of organ transplantation has led to a growing population of immunosuppressed transplant recipients with prolonged survival with a functioning graft, but also with prolonged exposure to the side effects and complications of chronic immunosuppression. Indeed, the burden of chronic immunosuppression post-transplant has become a growing concern among transplant physicians, although its impact is currently smaller compared to two decades ago, following the introduction of new and less-toxic immunosuppression *regimens* (see below). However, chronic immunosuppression remains associated with significant morbidity: as an example, the majority of patients treated with calcineurin inhibitors develop some degree of renal function impairment and up to 10% progress to kidney failure requiring dialysis or kidney transplant (see below).

The consequences of chronic immunosuppression on multiple organ systems are becoming increasingly evident and often new symptoms or disorders develop post-transplant as a consequence of the side-effects of immunosuppressive drugs (ie opportunistic infections,

malignancies, diabetes mellitus, hypertension and others, see below). The onset of new post-transplant conditions requiring treatment has multiple implications in terms of function of the graft, patient compliance and cost. Therefore, strategies to limit and prevent the complications of prolonged immunosuppression post-transplant are needed.

Here we present briefly the most common immunosuppression *regimens* currently used in abdominal organ transplantation and we review current major complications and challenges of prolonged immunosuppression after transplant. We will discuss issues common to abdominal organ transplants including liver, intestine, pancreas, kidney, without entering into organ-specific issues. The discussion is limited to adult transplant recipients, since pediatric transplantation raises a number of issues specific to this age group in terms of immunosuppression *regimens* and its complications including growth-related issues and compliance. In addition, a pediatric transplant recipient has potentially an expected more prolonged exposure to chronic immunosuppression than an adult and, as a consequence, more time to develop complications.

We will also present current strategies in the management of complications of immunosuppression and ways to limit the burden of immunosuppression. Finally, we report on current research and indicate future directions to improve post-transplant immunosuppression.

## **2. Current immunosuppression *regimens* in abdominal organ transplantation**

Compared to the early era of transplantation, a substantial progress has been made since the early 1990s in developing effective immunosuppressive agents to prevent allograft rejection. As a result, graft and patient survival rates have dramatically improved. In addition, highly immunogenic organs such as heart, lungs and intestine, previously characterized by a high incidence of failure due to rejection, are now being successfully transplanted since more potent immunosuppressive drugs have become available.

Most immunosuppressive agents target T lymphocytes, which are primary mediators of the alloimmune response and effectors of the rejection process. Current immunosuppression protocols usually include two or more agents to target different steps or mechanisms of the alloreactive immune response. The combination of multiple drugs not only increases the efficacy of the immunosuppression *regimen* but also often allows dose reduction of one or more of the drugs in an attempt to limit the associated toxicity (see below). Recently, other agents have been introduced that target B lymphocytes and other mechanisms involved in the alloimmune response including complement and others mechanism of the innate immune system (see below). As a result, an increasing number of immunosuppressive agents are now available (Table 1).

There are also new drugs being evaluated in clinical trials that target novel mechanisms and pathways of the immune response in attempt to reduce the burden of side effects and complications of agents currently available [1,2].

Antibodies	Alemtuzumab, Atgam, Basiliximab, Daclizumab, OKT3, Thymoglobulin
Antimetabolites	Azathioprine, Mycophenolate mofetil, Mycophenolate sodium
Calcineurin-inhibitors	Cyclosporine, Tacrolimus, Voclosporine
Corticosteroids	Methylprednisolone, Prednisone
Co-stimulation blockers	Belatacept
Proliferation- inhibitors	Everolimus, Sirolimus
Others	Bortezomib, Infliximab, Rituximab

**Table 1.** Immunosuppressive agents currently available

Immunosuppression is usually heavier in the peri-operative period and early post-transplant (induction) when the risk of rejection is higher due to a number of factors including preservation injury of the graft and sudden exposure of the recipient immune system to a load of foreign antigen. Later, depending on graft function and tolerability, immunosuppressive doses are gradually reduced (maintenance) to levels adequate to prevent rejection and avoid toxicity. Although there are reports of “tolerant” patients, who maintain a functioning graft after discontinuation of immunosuppression (see below), these are rare and exceptional cases and immunosuppression needs to be continued lifelong, inevitably exposing the recipient to the long term effects of chronic immunosuppression. Since there is no single optimal immunosuppression *regimen*, post-transplant care strives to achieve the delicate balance between effective prevention of rejection and avoidance of toxicity. The doses of immunosuppressive drugs are usually adjusted according to target trough levels, which vary among organs and among transplant programs.

## 2.1. Antibodies

Polyclonal antithymocyte globulins (Atgam, Thymoglobulin) are prepared from the serum of rabbits immunized with human thymocytes. Antithymocyte globulins contain cytotoxic antibodies that bind to CD2, CD3, CD4, CD8, CD11a, CD18, CD25, CD44, CD45 and HLA class I and II molecules on the surface of human T lymphocytes. The mechanism of action of depleting antibodies is to reduce the number of circulating lymphocytes by direct cytotoxicity, both complement and cell-mediated. Anti-CD3 monoclonal antibodies (OKT3) is a mouse monoclonal antibody against CD3. It binds to T-cell receptor-associated CD3 complex and depletes and alters T-cells. Its use has declined since newer immunosuppressive drugs have reduced the incidence of rejection episodes.

Non-depleting antibodies (Basiliximab, Daclizumab) block lymphocyte function by binding to cell surface molecules involved in the regulation of cell function. The main uses of antibodies in post-transplant immunosuppression are during induction and for the treatment of severe or steroid-resistant rejection (review in [3]). The risk of opportunistic infections (viral, fungal) is higher after profound T cell depletion, especially if prolonged, compared to the use of non-

depleting agents. Adverse effects include fever, chills, thrombocytopenia, leukopenia, hemolysis, respiratory distress, serum sickness, and anaphylaxis.

## 2.2. Antimetabolites

Azathioprine, a derivative of 6-mercaptopurine functioning as an antimetabolite to decrease DNA and RNA synthesis, has been used for many years since the early era of organ transplantation in combination with corticosteroids. The mechanism of action of azathioprine is to incorporate into and to halt DNA replication by blocking the *de-novo* purine synthesis in lymphocytes. Adverse effects include myelosuppression (leukopenia, thrombocytopenia), nausea, vomiting, diarrhea, hepatitis, cholestasis and alopecia. In the last 10 years azathioprine has been largely replaced by mycophenolate mofetil and mycophenolate sodium (two preparations of mycophenolic acid) in many transplant programs.

Mycophenolic acid. Unlike other cell types that can “recycle” purines from the process of cell turnover, lymphocyte proliferation and responses are dependent on the *de novo* purine synthesis; mycophenolic acid blocks the action of the key enzyme inosine monophosphate dehydrogenase (IMPDH), a rate limiting step in the biosynthesis of purines crucial to cell cycling in T and B lymphocytes. Consequently, the proliferation and clonal expansion of T and B lymphocytes is prevented, with the effect of reducing the alloreactive immune response, including antibody production and the generation of cytotoxic T cells and other effector cells. In addition, mycophenolic acid suppresses the glycosylation and the expression of adhesion molecules, thereby decreasing recruitment of lymphocytes and monocytes into sites of inflammation and graft rejection.

Two formulations of mycophenolic acid are now available, mycophenolate mofetil and mycophenolate sodium. Both formulations are non-nephrotoxic and are being used in calcineurin-inhibitors sparing regimens in attempt to reduce the risk of renal failure (see below). The main side effects of mycophenolate mofetil are gastro-intestinal intolerance (diarrhea), reported in up to 45% of patients and often requiring dose reduction or discontinuation. The enteric-coated mycophenolate sodium was designed to reduce the mycophenolic acid-related gastro-intestinal adverse effects: the enteric coating dissolves at pH levels  $\geq 5$ , thus delaying the delivery of the active compound mycophenolate acid until the small intestine.

## 2.3. Calcineurin-inhibitors

The most commonly used class of immunosuppressive drugs currently used in organ transplantation are calcineurin inhibitors (CNIs). Indeed, calcineurin inhibitors (cyclosporine and tacrolimus) are main immunosuppressive agents in use today in virtually every transplant program. Their immunosuppressive effect results from the blockage of the production of pro-inflammatory cytokines including IL-2, INF- $\gamma$ , TNF- $\alpha$  and from inhibition of T cell activation and proliferation. Their mechanism of action is to inactivate calcineurin, an essential enzyme for the function of T cell lymphocytes. Calcineurin, an intracellular calcium/calmodulin phosphatase triggered by the engagement of T cell receptor by donor MHC, dephosphorylates

nuclear factor for activated T cells (NF-AT) which in turn promotes the transcription of cytokine genes. The main adverse effect associated with the use of CNI is renal function impairment: virtually all people who take a CNI will develop some degree of kidney toxicity and up to 10% will progress to kidney failure. With more people taking CNIs for longer and longer periods of time the consequences of calcineurin inhibition on other organ systems - particularly kidney function - have become a growing concern. In addition to nephrotoxicity, other adverse effects of CNI include hyperkalemia, hypomagnesemia, nausea, vomiting, diarrhea, hypertrichosis, hirsutism and gingival hyperplasia. Tacrolimus, a more potent CNI compared to cyclosporine, shares the same mechanism of action and the same risk of nephrotoxicity. Tacrolimus binds to a cytoplasmic protein FK506-binding protein 12 (FKBP12) to create a complex that inhibits phosphatase activity of calcineurin. Tacrolimus, like CsA, inhibits signal transduction pathways linked to the T-cell receptor for antigen at the level of JNK and p38 kinase. While the abnormal cosmetic side effects (hypertrichosis and hirsutism) are less frequent with tacrolimus compared to cyclosporine, glucose intolerance and neurotoxicity (headache, seizures) are more common. Voclosporin, a cyclosporine analog with reduced nephrotoxicity, is a novel calcineurin inhibitor being developed for organ transplantation and currently in clinical trials: preliminary results showed a reduced risk of post-transplant diabetes compared to tacrolimus while maintaining the same efficacy in preventing rejection in kidney transplantation [4].

#### **2.4. Corticosteroids**

Corticosteroids (methylprednisolone, prednisone) were the first immunosuppressive drugs to be used in transplantation and remain today first line treatment across organs for both prevention and treatment of rejection. The multiple anti-inflammatory and immunomodulatory effects on a wide variety of cells including lymphocytes, granulocytes, macrophages, monocytes and endothelial cells are well known and the molecular mechanisms of action of steroids have been described extensively [5]. Briefly, corticosteroids down regulate cytokine gene expression in lymphocytes, antagonize macrophage differentiation, inhibit neutrophil adhesion to endothelial cells thereby decreasing their extravasation to the site of inflammation, decrease circulating eosinophil and basophil counts, inhibit IgE-dependent release of histamine and leukotriene from basophils and inhibit degranulation of mast cells. Additionally, glucocorticoids downregulate endothelial cell function including expression of class II MHC antigen and expression of adhesion molecules. Based on these multiple effects on different cellular components of the immune response corticosteroids are very effective in preventing and treating acute allograft rejection, although there are instances of steroid-resistant rejection episodes. The multiple side effects of steroids are also well known and include impaired wound healing, increased risk of infection, hypertension, weight gain, hyperglycemia, osteoporosis, fluid retention, hirsutism, acne and cataracts. Side effects may have an important impact especially in the long term and in children (ie growth pattern), therefore multiple trials of steroid withdrawal and steroid-free regimens have been designed in an attempt to limit the side effects of corticosteroids.

## 2.5. Costimulation blockers

Costimulation blockers represent a new class of immunosuppressants with a different mechanism of action compared to calcineurin inhibitors [6]. Costimulation (or signal 2) refers to the amplifying signal received by the T lymphocyte after interaction with ligands presented by antigen presenting cells. This costimulation amplifies the initial T cell activation event (or signal 1) resulting from the engagement of T cell receptors with donor antigens. Indeed, T cells undergoing signal 1 without signal 2 become unresponsive and undergo apoptosis. Several costimulatory pathways mediate the interactions between the surface of T cells and antigen presenting cells. One of the most studied pathways involves the surface molecule CD28 on lymphocytes and the B7 family of molecules on antigen presenting cells. This signaling pathway has become an attractive target for the development of novel immunosuppressive drugs. Two humanized fusion proteins have been developed to inhibit costimulatory signaling, abatacept and belatacept. The latter has been used in clinical kidney transplantation [7].

## 2.6. Proliferation inhibitors (mTOR-inhibitors)

This group includes everolimus and sirolimus, two of the most recently introduced immunosuppressive agents in clinical transplantation, acting with a mechanism of action different from calcineurin-inhibitors and from antimetabolites. Sirolimus will be discussed first, being the first mTOR inhibitor to be used in clinical transplantation. Sirolimus (also known as rapamycin) is a bacterial macrolide antibiotic produced by a strain of *Streptomyces hygroscopicus* isolated from a soil sample collected from the island Rapa Nui, commonly known as Easter Island. Although originally an antifungal agent with potent anti-candida activity, side effects precluded its use as an antifungal, and it has since been used primarily as an immunosuppressant. Sirolimus and everolimus are members of a newer class of immunosuppressive agents called inhibitors of the mammalian target of rapamycin. Sirolimus binds the intracellular immunophilin FKB12, the same intracellular binding protein of tacrolimus, but with different mechanism of action. After binding the immunophilin, the complex sirolimus-immunophilin inhibits a protein called mammalian target of rapamycin (mTOR). Inhibition of mTOR results in selective inhibition of synthesis of new ribosomal proteins which are essential for progression of the cells from the G1 to the S phase. This results in blockage of T cell activation. In addition, sirolimus has been associated with inhibition of fibroblast growth factors required for tissue repair. The half life of sirolimus is 60 hours which allows single daily dose unlike other agents given twice daily and this has an important impact on patient compliance to immunosuppression *regimens*. Everolimus is a modified form of sirolimus to improve its absorption. Its half life is shorter and is administered twice daily. Everolimus is currently undergoing clinical trials in transplantation in attempt to reduce the nephrotoxicity of calcineurin inhibitors [8,9]. The adverse effects of mTOR inhibitors include thrombocytopenia, leukopenia, anemia, arthralgias, hyperlipidemia, pneumonitis, and diarrhea. There have also been reports of wound complications (delayed wound healing, incisional hernia) in the post-transplant period, an affect probably secondary to its antiproliferative effects on fibroblasts. Oral ulcers were seen with the liquid preparation; however, this seems to be less frequent with the use of the pill preparation.

## 2.7. Other novel immunosuppressive agents

In this group we include antibodies that act on different targets than T cells. Bortezomib is an antineoplastic agent originally developed for the treatment of multiple myeloma. It is a proteasome inhibitor that induces apoptosis in rapidly dividing cells with active protein synthesis like plasma cells. In kidney transplantation it has been reported to revert antibody-mediated rejection [10]. Anti-tumor necrosis factor (TNF) reagents (Infliximab) are monoclonal antibodies that bind with high affinity to TNF-alpha and prevent the cytokine from binding to its receptors. It is approved for treating the symptoms of rheumatoid arthritis. In transplantation it has been investigated in the treatment of severe rejection after intestinal transplantation [11]. Rituximab is a monoclonal antibody directed against the CD20 antigen on B cells. It is approved for the treatment of certain types of non-Hodgkin lymphoma and to reduce the signs and symptoms of moderate to severe rheumatoid arthritis. In transplantation its use is currently being studied in treating some forms of antibody-mediated rejection [12] and as part of desensitization protocols in highly sensitized transplant recipients [13].

## 3. Complications of prolonged immunosuppression post-transplant

As a result of the success of effective immunosuppression, many more transplant recipients live now longer after transplant compared to decades ago and have time to manifest the long term effects of chronic immunosuppression. This has become increasingly more evident with the longer follow-up of successful transplant recipients. Indeed, after achieving excellent survival rates across organs, a constant focus of research and current clinical trials are now concentrating on how to reduce or prevent or antagonize the burden of chronic immunosuppression. It is becoming increasingly clear that if an effective control of rejection on the one hand protects the graft function and prolongs patient survival, at the same time the patient is exposed to the risk of complications of prolonged immunosuppression and also to new post-transplant disease, even in presence of excellent graft function. These complications result from either persistently low immune defenses as a result of immunosuppressive therapy (infections and malignancies) or as a result of side effects of immunosuppressive drugs, which affect virtually every organ system (renal function impairment, diabetes, cardiovascular disease and others, see below).

### 3.1. Infections

The most obvious consequence of a decreased immune defense is the increased risk of infection. Indeed, infectious complications are among the most common causes of morbidity and mortality after transplantation. Improved immunosuppressive *regimens*, while reducing the incidence of allograft rejection, have increased the susceptibility to opportunistic infections. In addition, other factors including malnutrition, co-morbidities associated with end stage renal or liver disease and alterations of the muco-cutaneous barriers following the transplant procedure contribute to increase the risk of infections post-transplant. Post-transplant infections have been classified in 1. peri-operative infections (during the first month post-



transplant, usually nosocomial infections or donor-derived), 2. early post-transplant infections (within the first 6 months, usually due to reactivation of latent infections, mostly viral) and 3. late infections (occurring usually after 6 months from transplant, mainly community acquired infections) [14]. Strategies to prevent infections post-transplant are based on either universal prophylaxis (administration of antimicrobial therapy to all patients at risk of infection for a limited period, usually 3 to 6 months post-transplant) or pre-emptive therapy (monitoring patients at established intervals for early detection and treatment of infection). A large number of viruses, bacteria and fungi can cause significant infections post-transplant. Here we discuss the most common viral, bacterial and fungal infections post-transplant.

### 3.2. Viral infections

The most common viral infections post-transplant are caused by viruses listed in Table 2.

Adenovirus
Cytomegalovirus (CMV)
Epstein-Barr virus (EBV)
Herpes simplex (HSV)
Influenza-Parainfluenza
Polyoma (BK)
Rotavirus
Varicella-zoster virus

**Table 2.** Viral infections post-transplant

Cytomegalovirus and Epstein-Barr virus, among others, are causing significant morbidity post-transplant and will be discussed here.

#### 3.2.1. Cytomegalovirus [15,16]

The incidence of CMV infection post-transplant (ie detection of active viral replication in the recipient) ranges from 25 to 50%, depending on the organ [17] The incidence of CMV disease (ie organ damage by CMV infection) is lower, reported between 3 and 14% [18]. The main risk factors for CMV infection and disease include serology mismatch (donor CMV IgG positive, recipient CMV IgG negative), degree of immunosuppression, use of antilymphocyte antibodies for the treatment of rejection and the type of graft (more common in lung and intestinal transplant, likely related to the heavy immunosuppression *regimens* used in these recipients). Manifestations of CMV disease vary from flu-like symptoms to invasive organ disease. Most commonly affected are the gastrointestinal tract (ulcers), the lungs (pneumonitis) and the liver (hepatitis). The morbidity associated with CMV post-transplant is not only related to its direct effects (see above) but also to its indirect effects, including increased risk of rejection, of other infections and of EBV-related lymphoproliferative disorders [19] (see below). Prophylaxis of



CMV is usually with either intravenous ganciclovir or with oral valganciclovir, an oral prodrug of ganciclovir with equivalent drug exposure [18]. Standard treatment of invasive disease usually requires intravenous ganciclovir for 2-3 weeks, often extended for a longer period to treat severe disease. Foscarnet and cidofovir are alternative agents active on CMV but are rarely used because of their toxicity

### 3.2.2. Epstein-Barr virus [20, 21]

EBV is a DNA virus associated with the common, usually self-limited infectious mononucleosis affecting young immunocompetent subjects. In the transplant recipient EBV infection may cause significant morbidity and mortality related to the development of post-transplant lymphoproliferative disorders (PTLD). EBV transforms and immortalizes B cell, which proliferate uncontrolled when the surveillance of EBV immunocompetent T cells is lacking secondary to immunosuppression. EBV infection post-transplant occurs either as primary infection, especially in children, or as reactivation. Risk factors for PTLD include primary EBV infection in a seronegative transplant recipient, the net state of immunosuppression (especially the use of antilymphocyte antibodies) and prior CMV infection. Quantitative EBV viral load assays are used for surveillance, diagnosis and disease monitoring. Non-PTLD manifestations of EBV disease post-transplant vary from mononucleosis-like viral syndrome to organ involvement (lungs, liver, gastrointestinal tract, bone marrow). PTLD presents a wide spectrum of histology and clinical presentations, from benign self-limited lymphoproliferation to aggressive disseminated lymphoma [22]. These lymphoproliferation are commonly extranodal and the transplanted organ may be involved as well. Outside the allograft, typical sites of involvement include the liver, gastrointestinal tract, skin and central nervous system.

The incidence of PTLD varies across organs from 1-5 % in kidney and liver transplant to as high as 15-20% in intestinal transplant recipients [23]. Based on morphologic, immunophenotypic, and molecular criteria, PTLD are classified into 4 pathologic categories: early lesions, polymorphic, monomorphic, and classical Hodgkin lymphoma. They present with a wide spectrum of pathologic and clinical manifestations ranging from benign lymphoid hyperplasia to aggressive lymphomas. Given the pathologic and clinical heterogeneity of PTLD, treatment is often individualized. Although there is no generally accepted protocol, treatment includes reduction or discontinuation of immunosuppression and a combination of rituximab (a chimeric anti-CD20 monoclonal antibody), chemotherapy, antiviral therapy and surgical resection depending on the aggressivity (review in [20] and [24]). New strategies are being tried such as adoptive immunotherapy [25].

### 3.3. Bacterial infections

The most common bacterial infections post-transplant are listed in Table 3.

The majority of bacterial infections early post-transplant (first month) are hospital acquired and are usually characterized by a high incidence of multidrug-resistance (review in [26]). Opportunistic bacterial infections, usually occurring between 2 and 6 months post-transplant, are caused by *Listeria monocytogenes* and *Nocardia* spp. Six months after transplant or later,

when immunosuppression is generally lowered, community-acquired bacterial infections are the most common, especially urinary tract infections by *E. coli* and *S. pneumonia pneumonia*.

<i>Gram negative</i>
Bacteroides and other anaerobes
Enteric bacteria
Pseudomonas
<i>Gram positive</i>
Staphylococcus spp
Streptococcus spp
Enterococcus spp (incl VRE)

**Table 3.** Common bacteria of post-transplant infections

Common bacterial infections post-transplant affect the urinary tract, the respiratory tract, the surgical wound and the bloodstream. The incidence of bacterial urinary tract infections ranges between 4.4% in non renal transplant recipients and 7% in renal transplant recipients, most commonly secondary to *E. coli* [27]. In one study the need for immediate post-op dialysis was risk factor for bacterial urinary tract infection in kidney transplant recipients, whereas age and diabetes were main risk factors in non renal transplant recipients [27].

Skin and wound infections, although not life-threatening, are common after solid organ transplantation. One study reported an incidence up to 45 % in kidney-pancreas recipients [28]. Most common isolates are *S. aureus*, but also enteric gram negative bacteria in abdominal organ recipients. The incidence of pneumonia also varies between organs from 7.3% within the first year after kidney transplant [29] to 22% after liver transplant [30] to 36% in lung transplant recipients [31] and is associated with prolonged intensive care stay and hospital stay. The source of bacterial bloodstream infections after transplant, in addition to intravenous catheters, include the respiratory tract, the urinary tract and the abdomen. However, often the source of bacteremia is not identified. Both Gram negative and Gram positive bacteria are isolated but in recent years methicillin-resistant staphylococci and vancomycin-resistant enterococci have become more common [32]. The presence of polymicrobial infection, the early onset of bacteremia after transplantation, the association with pneumonia, liver failure or kidney failure increase the mortality risk associated with bacteremia, reported up to 25% in lung transplant recipients [33].

### 3.4. Fungal infections

Fungal infections post-transplant cause significant morbidity and increase the mortality risk (review in [34]). *Candida spp.* and *Aspergillus spp.* are the most common causes of invasive fungal infections after transplant.

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Aspergillus spp.

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Candida spp

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Cryptococcus spp

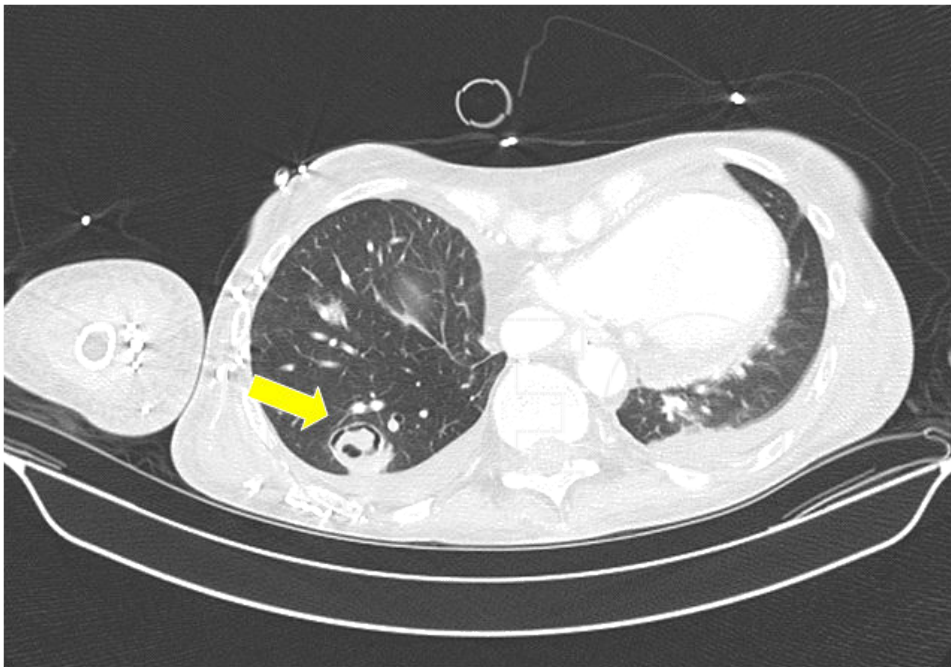
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Histoplasma capsulatum

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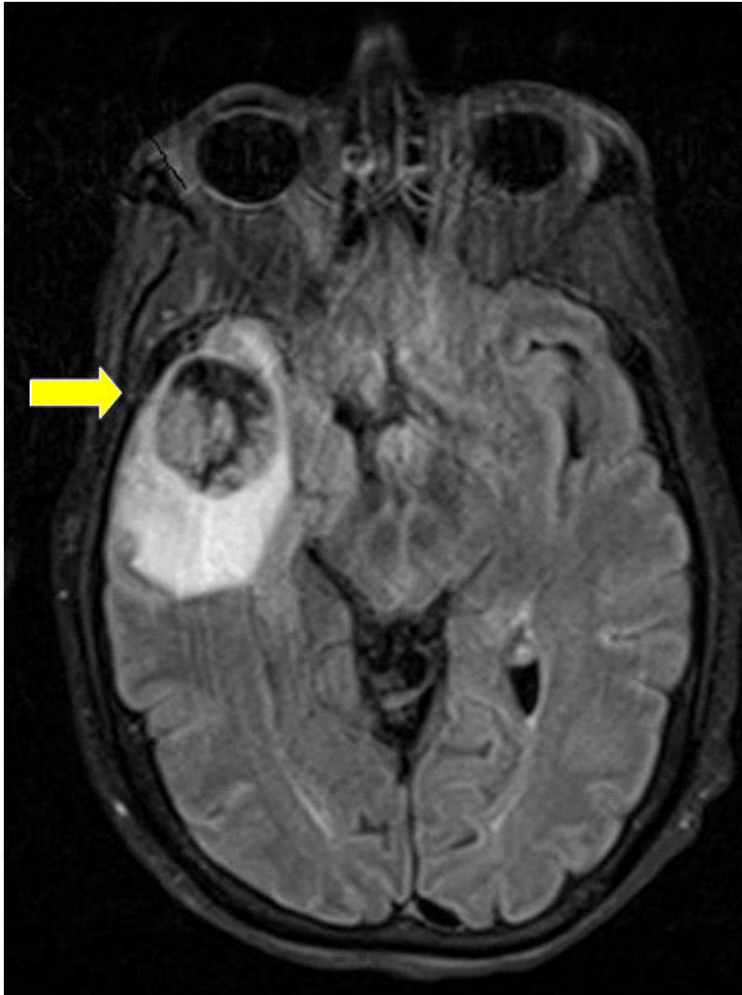
**Table 4.** Most common fungal infections post transplant

The incidence of invasive fungal infections varies across organs between 7 and 14% in pancreas transplant, 5-42% in liver transplant, 15 to 35% in lung transplant and 40-59% in intestinal transplant [35]. There are multiple risk factors related to the net state of immunosuppression (high doses of corticosteroids, use of antibody induction) and peri-operative factors such as prolonged complex operations including re-transplantation and renal dysfunction [36]. In addition, concomitant viral infections (ie CMV) exert immunomodulatory and immunosuppressive effects that increase the risk of fungal infections [37]. The clinical manifestations of invasive Candida infection vary across organs and include wound, intra-abdominal (peritonitis), thoracic (tracheobronchitis, pneumonitis) and bloodstream infection. The majority of cases of invasive Aspergillosis involve the lungs with single or multiple nodular infiltrate that may become cavitory lesions (Figure 1)



**Figure 1.** Pulmonary aspergillosis

The second and most invalidating site of invasive aspergillosis is intracranial, causing mental status alterations, seizures and focal neurologic deficits secondary to brain abscesses that most commonly involve the fronto-parietal lobes. The angiotropic character of *Aspergillus* infection tends to cause vascular invasion resulting in intracranial infarcts or hemorrhagic lesions (Figure 2)



**Figure 2.** Intracranial aspergilloma

The overall 3 month mortality risk from invasive fungal infections after transplant across organs has been reported up to of 29% [34] and key strategies remain prophylaxis of high risk recipients and early diagnosis and prompt treatment.

### 3.5. Malignancies

In addition to increased risk of infections, chronic suppression of the immune defences is associated with increased risk of malignancies. The incidence has been reported 3- to 5-fold higher in transplant recipients than in the general population and increases with the length of follow up. It has also been reported that after 25 years of immunosuppression, about half of the recipients are at risk of developing some kind of tumor [38]. Indeed, in renal transplant recipients, cancer is the third most common cause of death after cardiovascular accidents and infections [39].

Squamous and basal cell carcinomas of the skin are the most common *de-novo* malignancies, accounting for almost half of cancers post-transplant, although recently melanoma has been re-emphasized as also having an increased frequency following transplantation. The ratio of squamous and basal cell carcinoma is 4:1, the opposite of what is found in the immunocompetent population. Squamous cell carcinoma is more aggressive in transplant recipients compared to the general population, tends to recur and occasionally to metastasize [40]. Risk factors for skin cancer post-transplant, in addition to prolonged immunosuppression, are age, skin type and exposure to ultraviolet radiation. Therefore, attempts are made to reduce the dose of maintenance immunosuppression after developing skin cancer and to reduce skin exposure and to seek dermatology yearly survey in order to reduce the risk of recurrence. In addition, clinical data have shown beneficial effects of the use of mTORinhibition (sirolimus, see above) in preventing cancer development in transplant recipients. It is likely that these effects are the result of sirolimus' antitumor and antiangiogenic properties [41]. The second most common group of malignancies after transplant are lymphoproliferative disorders (PTLD), usually Epstein-Barr Virus-induced (see above, review in [42]). Excluding skin cancer and lymphoproliferative disorders, the incidence of *de-novo* malignancies (gastrointestinal, pulmonary and others) has been reported between 0.7% and 5.6% at 5 years [43]. A more recent study reported that the risk of *de-novo* malignancies after liver transplantation is 2-3 times higher than the general population [44].

### 3.6. Renal dysfunction

The effects of immunosuppressive drugs extend far beyond lowering the immune defense of transplant recipients and have an impact on virtually every organ system. Among them, renal dysfunction is a common and significant complication of solid organ transplantation. Long-term use of calcineurin inhibitors as part of immunosuppressive *regimens* is considered to be a major contributing factor in the development of chronic kidney disease (CKD). Renal failure post-transplant is associated with a 4-fold increase in mortality risk [45]. The incidence of renal dysfunction varies across organs depending on the length and level of calcineurin-inhibitors based immunosuppression. The mechanism of nephrotoxicity, like in hypertension, is thought to be related to alterations of the vascular tone of the endothelium at the level of the afferent arteriole [46]. However, CNI cause both acute and chronic nephrotoxicity. Acute nephrotoxicity involves afferent arteriolar vasoconstriction and reduced renal plasma flow, and is predictably associated with high trough levels. In contrast, chronic CNI-induced nephrotoxicity is not predicted by individual trough levels, and is characterized by potentially irrever-

sible structural changes including arteriopathy, tubulointerstitial fibrosis and, eventually, glomerulosclerosis. Among other factors implicated in renal dysfunction post-transplant are hypertension, diabetes, pre-transplant renal function impairment and post-transplant acute kidney injury [47]. Several strategies have been proposed in attempt to reduce the risk of nephrotoxicity post-transplant, including CNI reduction or avoidance (review in [48]) and conversion to mycophenolate mofetil-based [49] or sirolimus-based [50] immunosuppression *regimens*. Although there is no general consensus on the optimal combination of immunosuppressive agents for maintenance of graft function while minimizing nephrotoxicity, it has become increasingly evident that immunosuppression *regimens* may need to be individualized based on patient- and organ-specific factors (see below).

### 3.7. Cardiovascular disease

Post-transplant, several preexisting risk factors like hypertension, dyslipidemia and hyperglycemia usually get exacerbated resulting in accelerated atherosclerosis causing significant cardiovascular disease post-transplant, including ischemic heart attack, congestive heart failure, cerebrovascular accidents and peripheral vascular disease. Indeed, cardiovascular disease is the most common cause of death in transplant patients, with a 2.5-fold greater risk of cardiovascular mortality and threefold greater risk of ischemic events compared to the general population [51]. Prevention strategies to limit the impact of cardiovascular disease after transplant include lifestyle modifications, correction of modifiable risk factors (hypertension, diabetes mellitus and dyslipidemia, see below) and tailoring of immunosuppression [52].

### 3.8. Hypertension (review in [53])

*De-novo* hypertension post-transplant or the acceleration of hypertension (>140/90 mmHg) is common after solid organ transplantation, affecting up to 50%-75% of patients within the first weeks to months [54] and can pose a significant hazard both early and late after transplant. Both calcineurin inhibitors cyclosporine and tacrolimus have been associated with development or worsening hypertension post-transplant [55]. Since CNI based immunosuppression *regimens* are very common in virtually every transplant program, is it no surprise that hypertension remains a major cardiovascular risk factor in organ transplant recipients. CNIs are known to increase sympathetic tone, vasoconstriction and to cause sodium dependent volume expansion [56]. Studies have demonstrated the beneficial effect of lowering blood pressure post-transplant and the association of controlled blood pressure with prolonged patient and graft survival [57].

### 3.9. Diabetes mellitus

New-onset diabetes after transplantation (NODAT) refers to the occurrence of diabetes in previously non-diabetic persons after organ transplantation. The incidence of NODAT vary by organ transplanted and post-transplant interval. The estimated rates at 12 months post-transplant are 20-50% for kidney transplants, 9-21% for liver transplants, and approximately 20% for lung transplants [58]. However, a meta-analysis of 56 studies across all organs reported a 13.5% incidence of NODAT when the diagnosis was made using current guidelines. In



previous studies using different criteria for the diagnosis of diabetes post-transplant, including transient peri-operative hyperglycemia, the reported incidence was higher up to 21% in renal transplant recipients [59]. The risk factors for NODAT are the same as in the general population with the added effect of immunosuppressive medications, namely corticosteroids, calcineurin inhibitors and sirolimus. Among calcineurin inhibitors, tacrolimus was found to be more diabetogenic than cyclosporine [60]. However, both calcineurin inhibitors and steroids play a major role. Both CNIs have been associated with decreased insulin sensitivity and reduced insulin release. The reduced insulin release might result from CNI induced damage to pancreatic beta cells. Comparing the CNIs, most studies show higher rates of post-transplant diabetes mellitus with tacrolimus use compared to cyclosporine [61]. Other risk factors are pretx diabetes and obesity. Both pre-existing diabetes and NODAT are important cardiovascular risk factors, with a 2–5 times increased risk of cardiovascular disease, compared with transplant recipients without diabetes. In addition, new-onset diabetes is also an independent risk factor of graft failure and graft loss in kidney transplantation [62]. Monitoring of HbA1C is not recommended before three months following transplantation because the test may not be valid until new hemoglobin has been synthesized and glycosylated for the appropriate period in the diabetogenic post-transplant setting [63]. The management of post transplant diabetes follows the principles of treatment in non transplant populations but in addition it often requires adjustments in the immunosuppression *regimens*. Although these adjustments should be weighed against the risk of allograft rejection, options include reduction or weaning of corticosteroids and switching maintenance immunosuppressive drugs to less diabetogenic agents.

### 3.10. Dyslipidemia

The prevalence of dyslipidemia after transplantation has been reported up to 60-70% [64].

The mTOR inhibitors (sirolimus and everolimus, see above) have been associated with increased risk of dyslipidemia [65]. As hypercholesterolemia has been associated with increased prevalence of cardiovascular diseases, blood cholesterol levels should be maintained in the range recommended by practice guidelines, especially in transplant recipients receiving mTOR inhibitors.

## 4. Strategies to reduce the burden of immunosuppression and future directions

Strategies to limit the impact of chronic immunosuppression include protocols of drug minimization towards individualization of organ-specific immunosuppression *regimens*, development of new non-nephrotoxic agents and trials of tolerance induction. Drug minimization *regimens* are being explored in select patient populations to improve the safety of current immunosuppression protocols while preserving their efficacy. This strategy is based on the concept that, over time, the risk of rejection decreases and, at the same time, the cumulative risk for toxicity increases. Studies have concentrated on corticosteroids minimization and



calcineurin-inhibitors minimization (review in [66]). Careful patient selection and close monitoring of graft function are mandatory steps for a successful conduct of a drug minimization attempt in order to avoid rejection and graft loss.

At present we still lack reliable methods to identify transplant recipients who can be weaned of immunosuppression, although a number of candidate assays have been proposed to identify operationally tolerant patients. Among them, transcriptional profiling with either microarray or real-time PCR is currently a promising approach [67]. Peripheral and intra-graft expression markers of immune activation are used as tools to guide patient selection and monitor the progress of drug minimization trials [68]. In renal transplantation, non-invasive urine biomarkers have been described by measuring mRNA of inflammatory cytokines [69]. In addition, studies on urine proteomics allowed to identify different causes of graft dysfunction [70]. These non-invasive tools with or without protocol allograft biopsy offer the opportunity to monitor patients enrolled in trials of drug minimization.

In recent years, advances in immunosuppression that target specific pathways of the alloimmune response have been developed (review in [71]). In particular, new medications targeting the processes related to ischemia-reperfusion injury are currently under evaluation [72]. The ischemic insult to the allograft associated with the procurement and implantation processes contributes to trigger the immune activation of the recipient via the release of immunologically active substances known as damage-associated molecular patterns (DAMPs) [73]. In addition, new agents are being developed acting on the cellular and humoral mechanisms of the adaptive immune response. These include antibodies and fusion proteins interfering with T-cell-mediated activation via LFA-1/ICAM-1, CD2/LFA-3, CD40/CD154, and CD28/B7.1 and B7.2 interactions [74]. Furthermore, intracellular targets involved in T- and B-cell activation pathways are being evaluated, including protein kinase C inhibitors, Janus-associated kinase (JAK) inhibitors, and proteasome inhibitors. Several new medications demonstrate promise in inhibiting donor-directed humoral immunity by targeting B-cell-activating factor (BAFF) and complement activation pathways. Finally, other strategies are targeting the “memory” component of the T-cell repertoire [75] or the regulatory component [76].

Currently, transplant recipients are bound to lifelong immunosuppression. However, there have been reports of rare instances of “tolerance”, defined as the maintenance of allograft function without immunosuppression. Although several definitions of tolerance have been proposed (“complete tolerance”, “prope” tolerance, “operational” tolerance and others) and consensus is still lacking on the underlying mechanisms involved in tolerance, indeed there are patients who either intentionally or accidentally fail to reject the allograft and maintain allograft function while under minimal or no immunosuppression. As an example, in 1993 a series was reported of 11 liver transplant recipients maintaining normal liver function following the discontinuation of all immunosuppressive drugs as a consequence of either noncompliance or lymphoproliferative disorders [77]. Unfortunately, due to the heterogeneity of the human immune response, it has been so far prohibitively difficult to replicate these results on a larger number of patients and to establish tolerance in the clinical setting. The individualization of immunosuppression, identification of biomarkers of tolerance and of rejection and real-time monitoring of post-transplant immune responses may facilitate

induction of lasting tolerance in humans [78,79] The advancement of many high-throughput 'omic techniques such as genomics, proteomics and metabolomics has allowed to identify potential mechanisms of specific graft injury and to develop novel biomarkers for acute rejection, chronic rejection and operational tolerance [80,81]. Finally, the pharmacogenomics of organ transplantation has emerged recently as a complement to the immunogenetic information that has accumulated over the past decade [82]. Polymorphism studies focus on genes that interact across the group of immunosuppressive drugs (cyclosporin, tacrolimus, sirolimus and corticosteroids) such as CYP3A5, ABCB1, IMPDH1 and IMPDH2, and cytokines and growth factors. Although not routinely used in the clinic, it is expected that in the near future clinical pharmacogenomics techniques will become additional tools in the management of organ transplant patients.

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