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Handling of Fungal Infections in Patients with Chronic Immunosuppression Post Renal Transplant

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1. Introduction

Invasive fungal infections (IFI) are serious diagnostics-therapeutic problem in recipients of vascularised organs. The nature of the IFI is determined by the type of the transplanted organ. Invasive candidiasis mostly occurs in liver recipients and invasive aspergilosis – in lung recipients. The greatest risk of IFI is in recipients of simultaneous lung and heart and liver transplants. Morbidity for IFI in the first year after transplantation is estimated to be in recipients of heart and lungs 8.6%, liver 4.7%, pancreas and kidneys 4% and heart 3.4%.

Incidents of IFI among kidney recipients is estimated by differed sources to lay between 0.01 – 1.5%. Although, IFI occur rarely in kidney recipients in comparison to recipients of other organs, invasive fungal infections carries a high risk of graft loss and high mortality in this population of patients. Among recipients which have developed an IFI the risk of graft loss was determined in approx. 50% of patients, and mortality in this group was approx. 15%. Yearly survival of patients after an episode of invasive aspergilosis is 59%, for mycosis caused by mould fungi from species other then *Aspergillus sp.* 61%, invasive candidosis 66%, cryptococcosis 73%. There is definitely a greater risk for recipients of kidneys collected from cadavers, compared to living donors related to the recipient, respectively 16.5% and 7.3%. Additionally in cases of a deceased donor there is a high transfer risk for yeast-like fungi colonising in the urinary tract of a terminal state patient, in result of the breakdown of the defensive mechanism and contamination of preservative fluids, to the uninfected recipient.

Mortality is determined by the virulence of the microorganism, localisation of the infection, weakened inflammatory response of the macroorganism, frequent co-occurring of renal insufficiency and diabetes and other predisposing factors. Unspecific clinical symptoms, fast progression of the disease and, what appears to be particularly important, lack of a precisely set algorithms of diagnostic procedure, contribute to the fact that IFI in kidney recipients are a diagnostics challenge and has a questionable therapeutic result.

1.1 Factors for fungal infections

Factors for fungoid infections:
- impairment of cellular resistance
- congenital genetic defects in phagocythosis, complement system

- use of immunosuppressants, cytostatics, broad spectrum antibiotics, corticosteroids
- interruption of tissue continuity (insertion of a needle into a vessel, diagnostic puncture)
- inflammatory disorders and functional in the gastrointestinal tract (favourable factors for translocation of fungi from the intestinal lumen)
- catheterization of the urinary bladder
- parenteral feeding
- dialysis therapy
- surgical procedures, particularly in the abdominal cavity
- tracheotomy, intubation, endoscopy
- blood transfusion
- tuberculosis
- metabolic disorders
- endocrinological disorders
- pregnancy
- extreme age groups
- nutritional factors (intake of food containing moulds – strains used in food production or contaminants).

Patients with final renal insufficiency qualified for the transplant procedure, despite the main illness, often have also at least a few of the risk factors described above, are part of the population particularly predisposed to developed an IFI. There is a very high risk of IFI occurrence in recipients which had consecutive kidney transplant procedures, recipients of a simultaneous pancreas and kidney transplant and highly immunised recipients, requiring induction of the immunosuppression with anti-thymocyte globulin.

1.2 The remaining infection risk factors

The remaining infection risk factors differ depending on the period after the kidney transplant.

1.2.1 Early period

Early period – 1 month after kidney transplant. Mostly infections are caused by yeast-like fungi: *Candida sp., Trichosporon sp., Saccharomyces sp.* Risk factors in this period are related to the used surgical technique, transfer of the fungal infection form the infected organ or preservative fluid or fungal colonization form a period before the transplant. Infection with mould fungi of the *Aspergillus* genus are rare and are related to the prolonged operation time or organ transport.

1.2.2 Interim period

Interim period – from 2 to 6 months. Among etiological factors, yeast-like fungi responsible for infections in the early period are predominant, as well as mixed fungoid-bacterial infections. The basic risk factor in this time is a weakened immunological response due to:

- infections and/or reactivation of immunomodulation viruses: CMV, HIV, EBV, HBV, HCV and other
- site effects of transplanted kidney rejection therapy, with high doses of corticosteroids or other cytotoxic lymphocyte inhibitors.

1.2.3 Late period

Late period – over 6 months. Relatively often occurring infection in this period is the cryptococcosis, often in scattered form. Other etiological factors for IFI are yeast-like fungi, mould fungi of the *Aspergillus* genus and others. In this period the level of basic immunosuppression is remained on a low level, which means that in cases without complications the state of the recipients immunological system is close to the state of the general population's. IFI episodes are sporadic and caused by an individual course of the post-transplantation period or individual predispositions:

- complications in previous periods in graft function – treatment of acute rejection episodes
- exceeding of drug level limits of the basic immunosuppression protocol in systemic fluids
- reactivation of infections with immunomodulation viruses
- environmental factors.

1.3 Difficulties due to lack of an effective strategy for mycological diagnostics

Difficulties due to a lack of an effective strategy for mycological diagnostics and often sparse clinical symptoms are the reason that important data for diagnosis of invasive mycosis on kidney recipients are undervalued. Statistics on mortality due to disseminated mycosis do not contain, also for reasons outlined here, all cases. Lack of specific symptoms indicating an early period of an IFI, or transition of colonization into an active infection, lack of criteria allowing differentiation of infections and colonisations and in result lack of precisely described evidence for implementation of preventive – therapeutic treatment. Also important is to decide – does every positive mycological result, obtained by classic diagnostic methods, mean an infection and the necessity to implement antimycotic therapy, and does every colonising strain cause systemic infections, also which genotype and phenotype can benefit this process.

Additionally the treatment of a developed invasive fungal infection carries a high risk for patients due to frequent interactions between antimycotics and immunosuppressants. Taking this into account, it appears to be of outmost importance to develop a diagnostics algorithm minimising the risk of IFI in kidney recipients, based on an active surveillance of particularly predisposed recipients.

2. Invasive systemic mycosis: aspergilosis

Invasive aspergilosis (IA) is an acute infection with a mortality rate of almost 70%. Mostly it occurs in recipient of allogeneic bone marrow from an unrelated recipient – 10.3%. Among organ recipients lung recipients (Lung Transplant) are at highest risk, with morbidity at 8.4%. Among heart recipients (Heart Transplant) morbidity for IA is estimated to be 6.2%, liver (Liver Transplant) –1.7%, pancreas (Pancreas Transplant) –1.3%. Morbidity in kidney recipients (Kidney Transplant) applies for 0.7% of patient population.

An etiological factor in more then 90% of cases is *Aspergillus fumigatus*. Another species of clinical importance are: *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus terreus*. Different species participation is to be observed in external and middle ear aspergilosis, in most cases *Aspergillus flavus* and *Aspergillus niger* are responsible.

Aspergillus spores are now generally present in the environment. Etiology of *Aspergillus* infections are usually results of *Aspergillus* inhalation. The invasive form is the most severe

form of infection. In the initial stage, spores infect the lungs (pulmonary aspergilosis), next they get into cardiovascular vessels and to other locations eg.: sinuses (nasal sinus aspergilosis), eyes, skin, kidneys, bones, central nervous system (CNS aspergilosis).

The most common clinical manifestation is lung aspergilosis – 75% of all IA cases, *rhinosinusitis* (infection of the nasal mucosa and nasal sinuses) - 5 – 10%, disseminated multiorgan – 25%, IA with an affected central nervous system makes out 10 – 40% of all cases.

IA Diagnostic: an early diagnostic is critical for an effective therapy, also to avoid the unnecessary administration of a costly and toxic antimycotic therapy.

Traditional methods: as a „golden standard" in the diagnostic of invasive aspergilosis until now, remains the cultivation of a strain from the sample clinical material and identification fungi fimbriae by histopathology.

Appropriate test materials are bronchalalveolar washings in case of pulmonary aspergilosis and sinus washings in *rhinosinusitis* with *Aspergillus* etiology. The value of a mycological rest of sputum is restricted by its low sensitivity and specificity, difficulties in obtaining enough material (lack of a productive cough in the treated patient) or a frequent lack of morphologic elements of fungi and spore. In cases when it is not possible to sample material with invasive methods, morning sputum should be sampled a few times, on consecutive days, with securing of the material from contamination.

A difficult to diagnose form of aspergilosis is the disseminated multiorgan form. In this case the probability to cultivate an etiological factor from blood is infinitesimal compared to disseminated infections caused by other microorganisms (yeast-like fungi and bacteria). In case of invasive aspergilosis with the seizure of central nervous system, cerebrospinal fluid and serum become materials of questionable significance, the only valuable diagnostically material in this case is biopsy material sampled from the lesion. Diagnostic in this case should be based on precise analysis of all available tests: exact evaluation of the interview data, subject test results, biochemical tests results, assessment of the inflammation exponents, and results of imaging tests. As supportive tests can be used: detection of galactomannan antigen and genetic fungi material in cerebrospinal fluid and in serum.

Validity of the mycological test depends on the correct performance of every stage. Material sampling should be assisted by a microbiology specialist or a specially trained physician. It is necessary to take an appropriate number of samples and to sample material on the right microbiological medium for cultivation and direct microscopic preparation. Microscopic testing of clinical material and the correct evaluation are an immensely important stage of the diagnostic, which should be taken into account at sampling: sample material with a sterile smear dampened in a sterile 0.9% NaCl solution, transport medium should not be used for this purposes.

Histopathology diagnostic value is particularly important due to a relatively short time of waiting for results, high sensitivity and possibility to obtain positive results in an ongoing antimycotic therapy and is based on detection of fungal fimbriae. This method is restricted by lack of microorganism identification possibility and antimycotics sensitivity assessment, which can be preformed only on the basis of mycological cultivation.

Microscopic tests or cultivation are a limited possibility to retain testing material, only invasive methods and is connected to long results awaiting period.
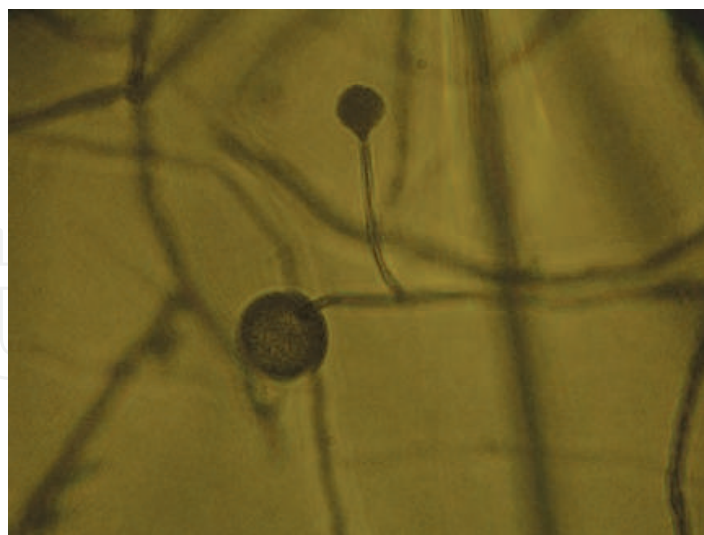
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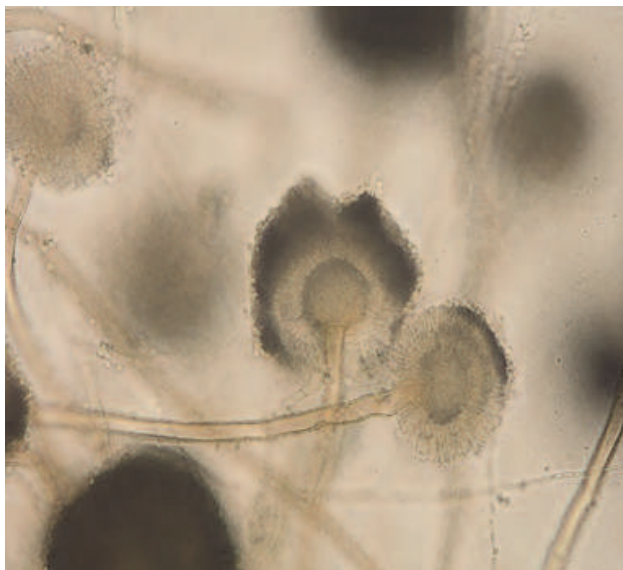
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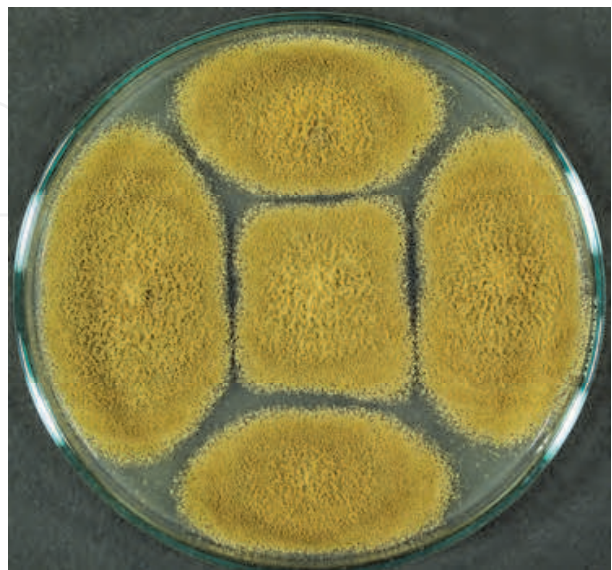
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Fig. 1. *Aspergillus fumigatus:* **1** Microscopic slide (x400) Microscopic tests of expectoration or BAL, **2** Colonies on Sabouraud Agar. **3** *Mucor spp.* Microscopic sidle (x400).
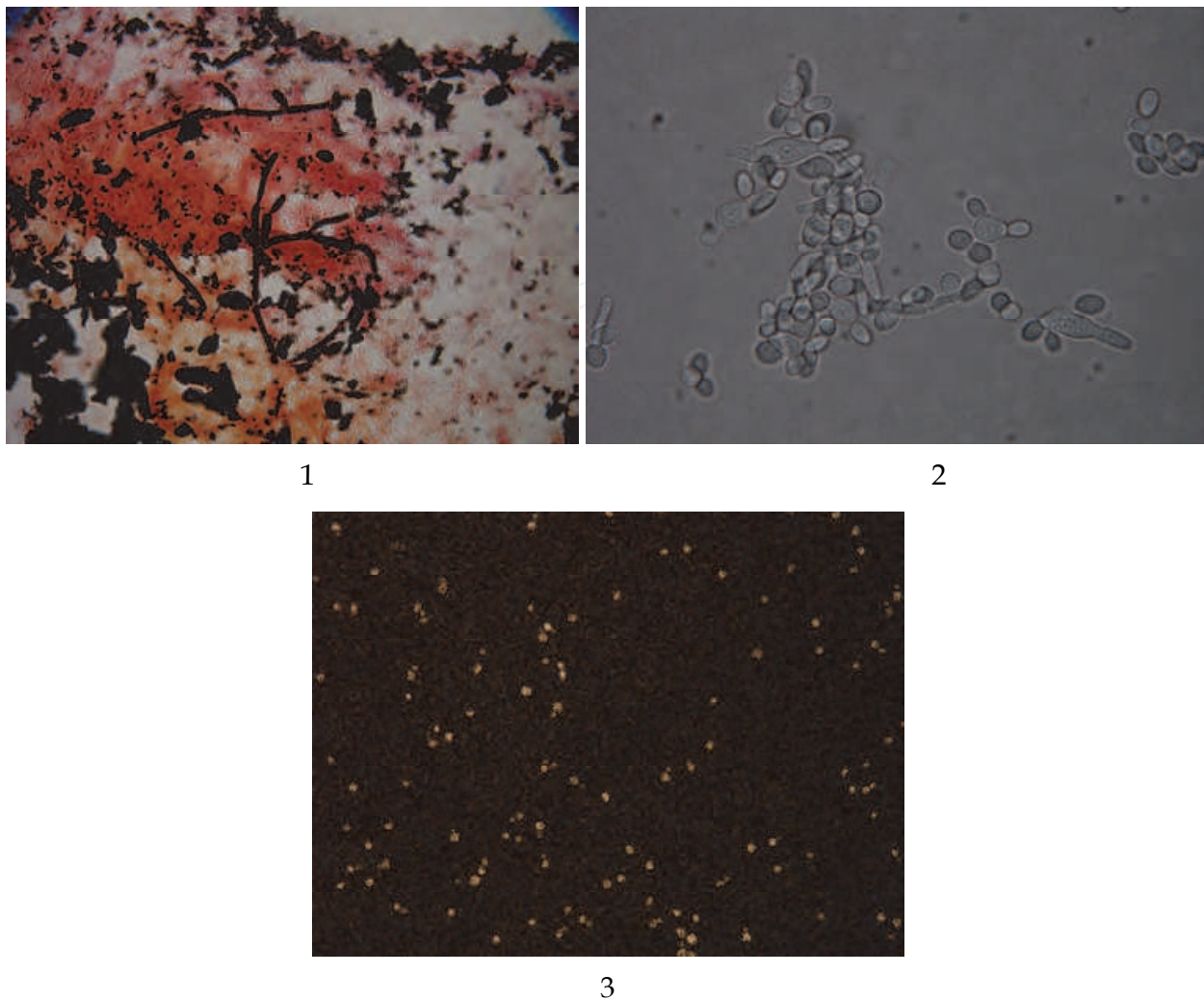
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Fig. 2. **1** Invasive form of *Candida albicans* in blood (x1000)**, 2** *Trichosporon asahii*, microscopic slide (x400), **3** *Cryptococcus neoformans*. India Ink preparation (x400)

3. Infection with mould fungi other than Aspergillus supp. (non-Aspergillus mould infections): zygomycosis

Zygomycosis – is an invasive infection frequently with *Mucor spp., Rhizopus spp., Rhizomucor spp., Absidia spp.* fungi of the *Zygomycota* division. It has been noted in the past years that the number of zygomycosis in the general number of infections with mould fungi in a general population of recipients from 4% to 25% in the period between 2001 and 2003 has increased.

Mostly the infection will have a *sinusitis* form with a tendency to quickly spread to the central nervous system – *rhinocerebral* form. Additionally the following forms can be observed: pulmonary, cutaneous and subcutaneous tissue infection, renal, gastrointestinal tract and disseminated form, often diagnosed *post-mortem.*

Most important risk factors of this type of infection are: persisting acidosis caused by a badly monitored diabetes or renal insufficiency and immunosuppressive therapy, particularly in connection with chronic nasal sinus infection. In the organ recipients population as the most important risk factor was perceived a previous exposition to voriconazole therapy of a previous infection with yeast-like fungi or *Aspergillus spp.* Often

those are „infections with breaking-point" with strains belonging to species, which do not fall in the spectrum of this drug.

Cases of zygomycosis in kidney recipients population in industrialised countries are rarely documented, but in developing countries: India, Pakistan, and Iran – they are quite common. Also the trend of „transplant tourism" observed in the last years – travelling to „third world" countries for transplants due to economic reasons and greater availability of organs, while trivialising a great risk of complications.

Rejections of transplanted organs and infection complications are serious problems, which European doctors providing post-transplant care for patient in this group may have to face. Aspergilosis with mucormycosis are most common infection complications in patients who have practised this kind of „tourism".

The death rate in this group is estimated at 59%, and further 82% suffer from graft loss, or from a disseminated form of infection with an infection of the central nervous system. The diagnostic procedure for zygomycosis is norm for all mould fungi and has been described in the chapter in aspergilosis diagnostics.

4. Invasive yest-like fungi

4.1 Invasive candidiasis (IC)

The most important exponent of the invasive candidosis is candidemia – fourth in frequency etiological factor of in hospital blood infection (BSI): 6-10% of the total positive blood culture in hospitalised patients. In patients after vascularised organ transplantation, IC makes out 53% of all IFI.

Infections with yeast-like fungi occur in 5% of kidney recipients with a death rate between 30 - 70%. Most common forms of candidosis among kidney recipients are mucosa-cutaneous and oesophagus candidosis. Heavy complications endangering the survival of the transplant are urinary tract infections: *cystitis*, *pielonephritis* and *ureteral obstruction by Candida elements*.

In an early period it is often conditioned by a colonisation of yeast-like fungi in the insufficient kidney in a period before the transplant and presence of urinary catheters in a post operational period, the presence of which connected with a high level of immunosuppression makes the spreading of the colonisation to the membrane and an invasive infection easier. Also a very important mechanism of developing a urinary tract infection is the pathogen translocation form the intestinal lumen. In this case it is an endeogenic infection, but with the wrong medical care and hospital hygiene it can cause spreading of the yeast-like fungi from the exogenous source.

Urinary tract infections with occurrence of additional factors can lead to candidemia, spreading of the infection and vascular complications on the transplanted kidney. Invasion of yeast-like fungi to the vascular endothelium can lead to *arteritis* or aneurysms.

Complications can lead to graft loss or patients death. Other dangerous fungal infections are suprainfections of fluid vessels forming around the transplanted kidney, peritoneum infection and catheter fungemia.

The most important factor in invasive candidosis remains the species *Candida albicans but* there is an observed growing participation of non – albicans *Candida spp*. to which belong: *C. glabrata, C. tropicalis, C. parapsilosis*. This species are characterised by being more drug resistant compared to *C. albicans*. The species *C. glabrata* shows a natural lower sensitivity to Fluconazole, and *C. parapsilosis* – to echinocandin.

One of the reasons for a larger participation of species of non – albicans *Candida* spp. possibly can be related to the wide use of prophylactic antifungal drugs. *C. parapsilosis* is characterised by a larger adhesion ability and the ability to produce a biofilm on the surface of biopolymers, including vascular catheters, which often is related to spreading of microorganisms colonised in the catheter into the system.

Widely used fluconazole prophylactic can also be one of the reasons for a larger participation of the *Trichosporon* species as a etiological factor for IFI. *Trichosporon* spp. is not the most important etiological factor for fungal infections in immunocompetent patients.

It can be a reason for an invasive mycoses in patients after vascularised organ transplant, with a high death rate. *Trichosporon* spp. is a yeast-like fungi generally occurring in nature.

Trichosporon asahii mostly is connected with surface infections such as white piedra, skin and nails mycoses in immunocompetent patients. Among the species of *Trichosporon T. asahii* is the main reason for deep invasive infections in patients treated with immunosuppressants.

The most important factor predisposing for this kind of infections are renal insufficiency, phagocytolic disability and neutropenia. Mortality in result of infections with *Trichosporon* fungi is estimated to be 80-100%, which can be related to the late diagnosis of the etiologic factor, wrong treatment and lack of correlation between the efficacy of treatment and sensitivity of the strain *in vitro*.

Additionally one of the characteristics of this species, which makes the prognosis of the course of the infection pessimistic is the variable sensitivity to amphotericin B. It can enable the strain *Trichosporon asahii* to produce biofilm.

4.2 Diagnostics of yeast-like infections

Clinical symptoms of IFI in kidney recipients are sparse and lack specificity, which makes a quick diagnosis difficult and effects efficacy of treatment and the predictions.

Classic mycological diagnostic methods: microscopic tests, culture of clinical material, identification of biochemical strain of pathogen, testing of drug resistance remain standard diagnostics. Those methods are without doubt very important in most cases and allow to implement a target treatment including a minimal inhibitory concentration of specific antifungal drugs to the etiological factor IFI.

It has to be stressed that sensitivity and specificity of the classic mycological diagnostic method are insufficient to be used as a single element for the confirmation of IFI diagnostic.

An additional problem is the often occurring mucocutaneous colonization, which causes result interpretation difficulties – not every positive result for the mycological culture allows to implement an antifungal therapy and not every negative result of the culture excludes an invasive infection.

Sparse symptoms of a systemic infection and the difficulties in a quick mycological diagnosis are reasons for delays in implementing of an appropriate antifungal therapy, which can result in the loss of the graft and even the death of the patient.

The problem to identify the participation of the infection appears interesting, also in those caused by fungi in organ rejection.

The „golden standard" in diagnostic of invasive candidosis in patients in high risk groups remains blood cultivation, which has serious restrictions. The most important are:

- low sensitivity: approx. 50% of results in patients with a confirmed or possible IC can be a false negative
- long result waiting time: in most case the waiting time is longer then 2 up to 3 days, in patients receiving antifungal drugs even 14 days.

Developed are also other criteria allowing a rather quick identification, if the active monitoring strategy of the patients state has been adjusted to the mycological diagnostic. One of this criteria is determining if the yeast-like fungi in culture of clinical material is received from one or more parts of the body.

Diagnostic of the invasive candidosis leading to the implementation of the appropriate antifungal treatment consists of clinical, radiological and microbiological tests results.

4.3 Cryptococcosis

To the *Cryptococcus* belong 37 species. The most important pathogenic species for humans is *Cryptococcus neoformans* widely spread in nature globally.

Infection vectors are birds, mostly pigeons, infection sources are also soil particles containing bird faeces contaminated with the fungi, and the most common infection mechanism is inhalation.

A specific characteristic of the yeast-like fungi from the *Cryptococcus* species, used in diagnostic routine is a lack of a polysaccharide capsule with an antiphagocytic function.

Cryptococcosis is an opportunistic infection in patients with immunological defects, it is known as an indicative illness for AIDS. The primary infection localisation are the lower airway, from which it quickly spreads to the lungs and the CNS.

The most dangerous clinical form of cryptococcosis is menigoencephalitis, other clinical forms: chronic or acute pulmonary form or disseminated in relation to the CNS, skin and other tissue and organs. After the pulmonary stage and spreading to the CNS, in the blood serum and PMR rapidly appear dissolving capsule antigens, possible to detect with enzyme immunoassay methods.

The cryptococcosis diagnostic is based on:

- microscopic determination of yeast cells with a polysaccharide capsule in cerebrospinal fluid
- microbiological cultivation of *C. neoformans* from blood or cerebrospinal fluid. The waiting time for results of this test is longer then in case of diagnostic of fungal infections with *Candida* due to a longer incubation time and can take even up to 7 days.

Detection of capsule antigens *Cryptococcus spp.* in serum or cerebrospinal fluid. Sensitivity of the method increases the parallel marking of antigens in serum and PMR.

Diagnosis of cryptococcosis can be determined only based on joined results of clinical, radiological and microbiological tests, (microbiology, histology, serology), each of those elements should be carefully considered.

4.4 Serological diagnostic

The current use of serological diagnostic is based on the detection of specific fungi species – mannoproteins, which have the role pf antigen markers. In the period of sustained systemic fungal infection, those antigens occur in the blood stream temporarily, are easily eliminated by the formed immunological complexes and by way of endocytosis through the Kupffer cells in the liver. Regular monitoring of their presence in systemic fluids has a great practical value in high risk patients.

In diagnostics of invasive aspergilosis (IA) detection of the galactomannan (GM) antigen in the blood stream is valuable (GM). GM is a polysaccharide of the vascular wall of mould fungi belonging to the *Aspergillus* species (*A. fumigatus, A. flavus, A. niger, A.terreus* and other).

A positive test result for *Aspergillus* is one of the microbiological criteria recommended by the *European Organization for Research and Treatment of Cancer* and the *Mycosis Study Group (EORTC/MSG)* to determine IA, despite negative results in classic mycological tests in patients with haematological neoplasm.

Using of similar criteria seams to be justified for diagnosis of IA in vascularised organ recipients, including kidneys. For the purpose of maximising test sensitivity it should be performed at least once a week for patients in the high risk group.

For al patients with a positive result it is recommended to repeat the test with anew blood sample.

According to the *EORTC/MSG* criteria two consecutive positive results are necessary to classify the test as a real positive.

GM detection sensitivity depends on the infection localisation. In the localised invasive pulmonary aspergilosis the sensitivity of the method is significantly lower compared to the disseminated form. Monitoring of GM levels can be useful in infection course prognosis – a drop of the levels can be connected to good prognosis.

IC serodiagnostic (invasive candidosis) is based on the detection of mannan antigen circulating in serum, which is the main component of the cellular wall of a *Candida species fungi*. Mannan is a highly immunogenic polysaccharide antigen with immunomodular characteristics. It allows to use in IC diagnostics parallel monitoring of circulating mannan and titre of antimannan antibodies.

Parallel detection of both markers allowed to increase the sensitivity to 80% and to 93% the specificity of the method. Detection of circulating *Candida spp.* antigen in serum or plasma should enhance IC diagnostic in high risk patients regarding to sensitivity and detection time of infection development. Among *Candida* antigens, mannan which is the main component oft he cellular wall of yeast-like fungi from *Candida* species, appears to be one of the most important biomarkers in the diagnostic of invasive candidosis.

Regular testing of IC high risk patients, connecting the detection of mannan antigen is helpful in diagnostic of invasive candidosis.

The usefulness of testing levels of antibodies in patients treated with immunosuppressants is controversial, but in the period of graft stability in patients without additional problems can be helpful, as long as parallel marking of mannan antigen is performed.

It should be kept in mind that the highest sensitivity is proved in IC diagnostic for *C.albicans, C.glabrata, C.tropicalis* etiology. The lowest sensitivity of mannan detection and mannan antibodies, 40-50% applies for infections with *C.krusei, C.parapsilosis, C.quilliermondii* etiology.

Beta-glucan detection methods in serum (Fungitell-Associates of Cape Cod) proposed as marker in IC diagnostic has many gaps, a positive result does not allow to determine a fungal etiological factor, allows only to differentiate between a fungal and bacterial infection. Additionally the test has a low specificity and a high cost of marking and expensive equipment means that it is rarely used in routine diagnostic.

4.5 Molecular biology methods

Development of molecular techniques, which took place in past years allowed to introduce routine microbiological diagnostic to the tests based on detection of nucleic acids of microorganisms.

In mycological diagnostic genetic methods are used for microorganism detection in material sampled from patients, as well as identification of specific species in this material. It has to

be underlines that they should be used as complimentary to the classic tests protocol or as a confirmation of its results.

Molecular methods should not be used instead of classic diagnostic techniques.

Their application with great success serve in diagnostic of infections difficult to detect with conventional cultivation methods.

Most tests are restricted to detecting of frequently occurring and consequently the most important clinically species, which are factors in general infection such as *Aspergillus fumigatus* or *Candida albicans*.

Although lately molecular identification tests are introduced for a wider group of fungal pathogens.

They have been developed for detection of some of the species *Aspergillus*: *A. flavus*, *A. niger*, *A. versicolor*, *A. terreus* and *A. nidulans* as well as etiological factors for zygomycosis isolated from clinical materials sampled from patients with a general infection.

In case of detection of ethological infections with yeast-like fungi, the newest application of molecular tests are for pathogens from the non-albicans group *Candida spp.*: *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, species with an undeniable clinical expansion in recent years.

Detection methods in use are usually based on techniques which are modifications of the standard reaction PCR: real- time PCR, nested PCR, multiplex PCR to detect the ribosome gens RNA: 18s rRNA, 28s rRNA or regions ITS1 and ITS2.

Real-time reaction PCR is characterised by a fast analysis and does not require an electrophoretic test of products.

The reaction is based on the standard PCR method, allows a fast identification and quantitative assessment of the pathogen in a clinical sample. Nested PCR is a modified standard reaction in which two pairs of starters are being used: outer and inner , with different melting temperatures. The product received with outer starters becomes a live matrix for reaction with inner starters. The product received in the first stage – DNA fragment with a sequence specific for microorganisms in the second reaction stage is classified to the searched for species.

Multiplex PCR method differs from the classic method only slightly.

The reaction is performed in the same way, but with a use of more then one pair of starters with a similar melting temperature, which allows to detect more then one pathogen present in the clinical sample.

The choice of material for genetic testing is based on the localization of the infection.

In case of general infections, it is recommended to perform full blood tests on the patient, from which the DNA will be extracted in search of the pathogen.

Currently more often, particularly in mycosis diagnostics, applications are being used which do not allow for diagnostic in Rother clinical material:
- tissue biopsy,
- cerebrospinal fluid and Rother systemic fluids,
- bronchial washings (BAL),
- expectoration,
- tracheal aspiration.

Methods based on molecular techniques are characterised by higher sensitivity and specificity then classic cultivation.

It is estimated that real-time PCR sensitivity for *Candida* has 81-100% sensitivity and specificity from 97 to 86%.

While using the nested PCR method for *Candida* the reaction sensitivity can achieve 86-79%, and specificity 54%. Sensitivity in multiplex PCR is 98% with specificity of 88%.

Specificity and sensitivity in real-time PCR for *Aspergillus fumigatus* oscillates around 100% and depends on the amount of material sampled for testing.

Despite a sorter time needed for the performance of diagnostics, and in result of the waiting time for results, molecular methods hale some restrictions.

The first is the cost of testing, surely higher then in case of classic methods.

Another is a lack of possibility to determine sensitivity spectrum for antimycotics.

It is necessary to perform his test with the classic method.

It should be stressed that the possibilities of use for molecular testing is restricted to detection and identification of pathogens most frequently occurring in clinical material with the exception of the rare ones, which often cause serious infections.

5. Diagnostics algorithm for general fungal infection risk

5.1 Diagnostics algorithm for general fungal infection risk minimization in patients after kidney allotransplantation

In result of many years of observation of a 1301 kidney recipients from cadavers and/or a related donor (including 213 with diagnosed diabetes) a scheme of diagnostic procedure Has been developed, for timely diagnostic of fungal infection, differentiation from colonization with opportunistic fungi strains.

Influence of the fungal infections on an early and late function of the transplant has been analyzed and an algorithm has been developed for diagnostic procedure minimizing the risk general fungal infections in patients after a kidney allotransplantation.

For most patients a 3-drug immunosuppressive protocol has been used: Cyclosporine A (CsA) or tacrolimus (FK) with glucocorticosteroids (GS), with antiproliferative preparations: mycophenolate mofetil (MMF) or azathioprine (AZA), some of the patients received an immunosuppression induction with biological agents (ATG, OKT3).

5.2 An algorithm for an effective diagnostic procedure minimizing the risk of general fungal infections in kidney recipients

An algorithm for an effective diagnostic procedure minimizing the risk of general fungal infections in kidney recipients should include:
- strategy for a quick diagnostic of systemic mycosis in case of justified suspicion of such infection
- tactic of routine diagnostic minimizing the risk of developing a fungal infection in the general population of kidney recipients
- strict criteria for choosing of recipients from the high risk group for fungal
- programs for active monitoring of recipients from the high risk group.

5.3 Strategy for a quick diagnostic of systemic mycosis in case of justified suspicion

The diagnostic procedure should be based on all available testing methods: clinical, radiological, histological, classic microbiology diagnostic, serological methods, and molecular biology. It should be underlined that every diagnostic method has serious restrictions, the decision about the necessary implementation of a antifungal therapy should be based on the interpretation of a number of tests. The basic and crucial element of each of the schemes outlined above remains cultivation of etiological factor IFI from materials sampled from the patient: blood and other systemic fluids, biopsy materials, materials from lower airways, urine, smears from the oral cavity, throat, rectum and other parts, faeces and other materials sampled from the patient, regarding to the clinical situation at hand.

Microbiological criteria for diagnosis of general mycosis are:
- positive results for blood cultures and other physiologically sterile body cavities
- determination of fungi presence in the direct preparation from the tested material
- identification of antigens circulating by serological methods
- identification of genetic fungal material in clinical samples from usually sterile parts
- identification of specific antibodies
- determination of specific fungal enzymes in urine or other systemic fluids
- determination of morphological elements of the fungi in tissues sampled by biopsy.

Differentiation of colonization from infection:
- simultaneous cultivation of strains of the same species from different sampling locations (at least three)
- multiple cultivation of strains of the same species from one sampling location
- simultaneous occurrence of fungal antigens in serum or other naturally sterile systemic fluids
- significant (at least 4 times) of the antibodies titre in serum
- occurrence of hydrolytic enzymes specific for a species in systemic fluids.

The proposal of a procedure in cases of suspected invasive fungal infections scheme is presented in figure 3.
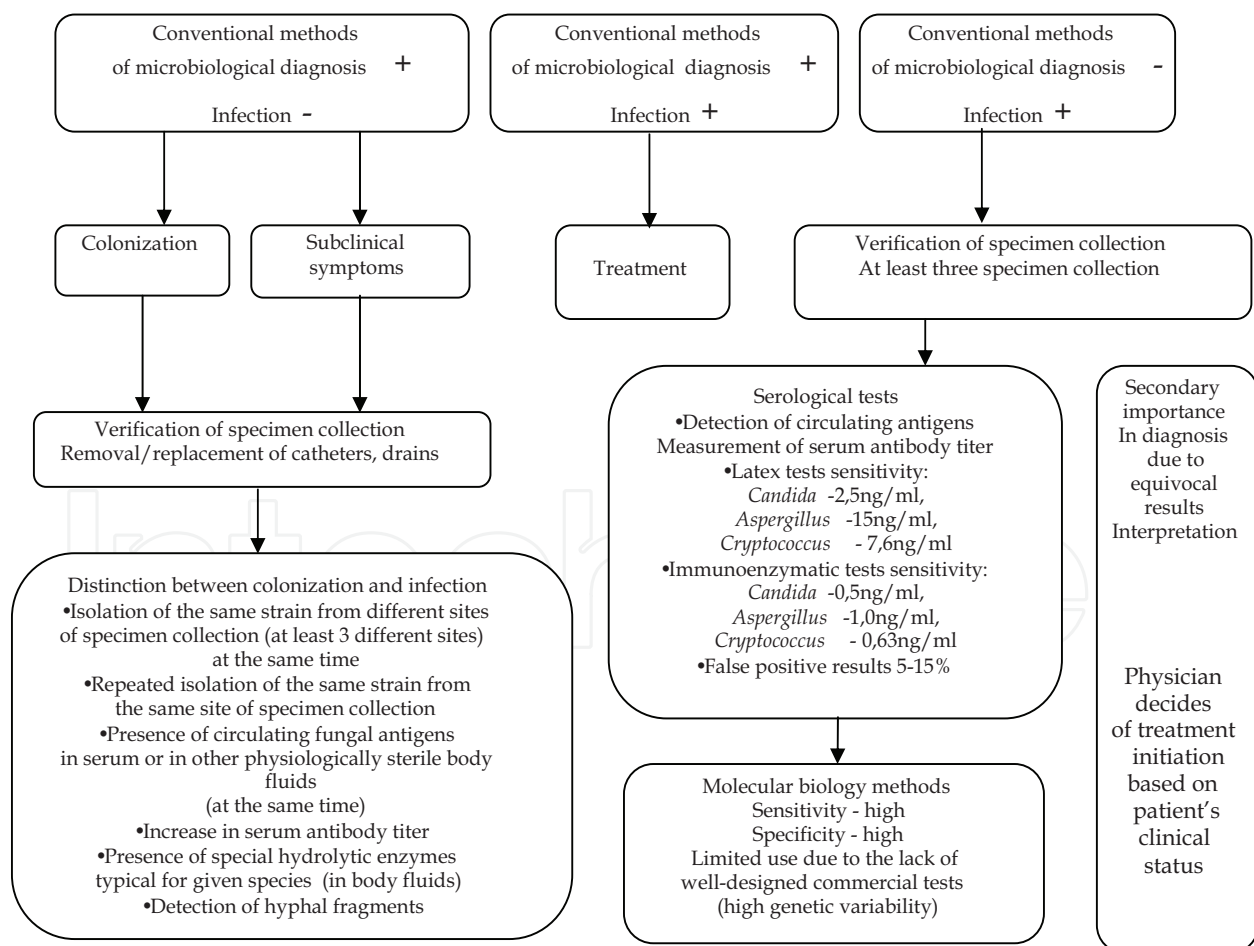

Fig. 3. Diagnostic algorithm for prevention of invasive fungal infection in solid organ transplant recipients.

5.4 Routine diagnostic tactic for minimizing risk of fungal infection in kidney recipients without risk factors disorders

Kidney recipients without additional risk factors should be monitored for a surviving skin, mucosa, urinogenital tract colonization with a classic microbiological method. Proposed is a regular cultivations for fungi in urine, oral cavity smears and qualitative diagnosis of yeast-like fungi present in the gastrointestinal tract.

Frequency of cultivations depends on the period after the Tx. In an early period it is justified to sample materials for microbiological testing not less then once every 5-7 days. In the second period once every 2-3 weeks. In the third – once every 1-2 months.

5.5 Strategy of diagnostics-prophylactic procedure minimizing the risk of a systemic fungal infection in kidney recipients with a specific predisposition

A more aggressive scheme should be applied in cases of additional risk factors for a general fungal infection.

In the early period after Tx, presence factors determined before Tx are very important:
- skin and mucosa colonization by yeast-like or mould fungi
- immunosuppression induction with biological agents in highly immunized patients
- therapy with broad-spectrum antibacterial agents
- blood transfusions
- a particularly important risk is a possibility of colonizing factor or causing a fungal infection in donor transmission from donor-recipient, especially in a diseased donor . To exclude such risk factors it is necessary to perform blood and urine cultivation for fungi
- contamination of preservative fluids with fungi
- risk factors occurring in the early period after Tx:
- skin and mucosa colonization with strains of yeast-like fungi
- bacterial infections
- another kidney transplantation
- acute insufficiency of the transplanted kidney
- repeated surgery due to early postoperative complications
- prolonged transplantation time
- treatment of acute transplant rejection with high doses of steroids or biological agents
- elevated levels of the basic immunosuppressive scheme

Risk factors in the second period after Tx:
- skin or mucosa colonization with yeast-like fungi strains
- treatment of acute transplant rejection with high doses of steroids or biological agents
- infection or reactivation of infections with immunomodulatory viruses: CMV, HHV, EBV
- leukopenia, hypogammaglobulinemia
- repeated bacterial infection
- long-term antibiotics therapy

Risk factors in the third period after Tx:
- skin or mucosa colonization with yeast-like fungi strains
- treatment of acute transplant rejection with high doses of steroids or biological agents
- infection or reactivation of infections with immunomodulatory viruses: CMV, HHV, EBV

- leukopenia, hypogammaglobulinemia
- repeated bacterial infection
- long-term antibiotics therapy
- chronic nephropathy of the transplanted kidney
- treatment of neoplastic diseases.

With the co-occurrence of two or more risk factors it a broader routine procedure, based on classic mycological diagnostic methods needs to be considered.

Together with performing cultivation of clinical samples for fungi on a regular basis, we recommend regular, weekly mannan, galactomannan and glucuronoxylomannan antigen marking, in serum with immunoenzyme method.

5.6 Therapeutic-prophylactic procedure - conclusions

Implementation of a therapeutic procedure, as far as it is possible, should each time be procedure by the identification of the etiological factor of the fungi infection species, taking into account natural resistance or a naturally lowered sensitivity to antimycotic substances.

In 7744 microbiological test of material sampled from kidney recipients, positive cultivation for fungi were received in 475 samples (6.13%). Fungi strains causing infections in his group of recipients belong mostly to the *non-albicans Candida spp.*, in which a large group are strains with a diversified sensitivity to antimycotics. *Candida albicans* was isolated only in 38.1%, where recently we domination of his species in vascularized organs recipients has been observed.

The participation of species which have replaced *C. albicans* is concerning, selected by an irrational treatment of prophylactic. Those are the strains with a restricted sensitivity to the available antimycotics, mostly from the azole group, resistant to flukonazole, namely: *C. glabrata, C. krusei, C. kefyr, C. inconspicua, Trichosporon asahi.* It has been proven that species with a natural resistance or natural sensitivity to flukonazole – *C. glabrata* and *C.krusei* make up for 40%. The acquired resistance to flukonazole has been observed in 5.27% of strains. Clinical material from which the fungi hale been cultivated, were sampled mostly from the urinary and respiratory tract, then from drainage and postoperative wounds and blood. Fungemia were caused only by strains belonging to the *non-albicans Candida spp.*

From literature and our own data appears that the popular use of flukonazole in antifungal prophylactic caused a negative selection of resistant strains. In this period it has also an increased rate of deaths due to general fungal infections with the genus *Aspergillus* has been observed.

Antifungal infection prophylactic can not be used commonly, but only in clinically justified cases, and prophylactic should be even given up, on behalf of active monitoring of recipients and progressive therapy. In this cases an implementation of drugs other then flukonazole or amphotericin B, Itrakonazole, Posaconazole should be considered. In prophylactic the toxicity of the preparations and resulting growing resistance should be considered.

Empirical therapy. In cases of suspected invasive fungal infection based on clinical evidence the appropriate drug would be amphotericin B or its liposomal forms and Itraconazole and new azoles – Voriconazole, Posaconazole as well as caspofungin. Flukonazole can be used only in large doses only in wards, where natural species resistant to flukonazole occur rarely and no prophylactic product has been used.

Treatment of diagnosed mycosis. In confirmed cases of systemic mycosis, the drug used should take into account the sensitivity to the drug of the isolated strain with a marked MIC (minimal inhibitory concentration). The dose should be calculated for each patient individually based on the MIC value. In severe cases it is possible to use a joined treatment. The length of the treatment depends on the clinical state of the patient, it usually takes a few weeks. Postoperative complications requiring a new operation bear a high risk of a fungal infection.

Due to this it appears justified to begin antimycotic treatment from the start of a repeated surgery and to perform an earlier decontamination of the gastrointestinal tract from fungal flora.

Relatively new group of antimycotic drugs are echinocandins. To this group belong: caspofungin, anidulafungin, micafungin. The mechanism of echinocandins is based on the inhibition of the polysaccharide glucan synthesis (1-3β-D-glucan), which is one of the main components of the fungi cellular wall.

Diminishing the concentration of the glucan in the cellular wall leads to changes in the permeability of the membrane and its osmotic instability, which eventually leads to cell lysis. Echinocandins can be only administered intravenously due to very low bioavailability in oral administration.

In adult patients the pharmacodynamic of those drugs is similar – all of them are used intravenously in one daily dose. They are eliminated from the organism by way of a non-enzymatic decomposition All echinocandins do not require dose modifications in case of renal insufficiency and renal replacement therapy.

Caspofungin and micafungin are metabolized in a small way in the liver, but without the cytochrome P450 superfamily enzymes. Caspofungin is the only echinocandins which requires a diminished dose in cases of moderate or severe liver insufficiency.

Echinocandins due to their mechanism are effective for fungi of the *Candida* and *Aspergillus* species similar to classic and lipid forms of amphotericin B. In a multiple site, randomised double blind a similar efficacy of caspofungin therapy with liposomal amphotericin B in treatment of fungal infection of the oesophagus in adults has been confirmed. Caspofungin has been better tolerated by the patients.

According to the IDSA 2009 guideline echinocandins are recommended as first choice drugs in fungemia treatment, particularly caused by *C. glabrata* strains in patients with neutropenia and previously treated with antimycotics from the azoles group. In the population of patients without neutropenia echinocandins are recommended as alternative therapy. Particularly justified is the use of echinocandins in case of fungemia due to their unique ability to penetrate the biofilm environment and to inhibit outer-cellular matrix composition production. This characteristics of echinocandins can have great significance in the inhibition of pathomechanisms in fungemia

Echinocandins also find use in therapy of infections with mould fungi treatment, mostly those belonging to the *Aspergillus* species, though it is restricted due to fungistatic effect of echinocandins on this type of microorganisms.

Caspofungin is recommended in treatment of invasive cadidosis and invasive aspergillosis in patients who hale previously not responded to therapy or intolerant to other antifungal drugs.

Anidulafungin approved by the FDA for treatment of esophagus candidosis, candidemia and other complications of infections with *Candida spp*. It Has been proved in clinical studies

that anidulafungin has higher efficacy compared to flukonazole in patients with invasive candidosis and fungemia.

Micafungin is recommended in treatment of invasive cadidosis, for treatment of esophagus candidosis, prophylactic of infections caused by *Candida spp.* in patients with allogenic blood stem cells transplants. An important advantage of micafungin is its small potential for drug interactions. This applies for immunosuppressants: cyclosporine A, tacrolimus, mycophenolate mofetil. With a simultaneous treatment with sirolimus, Itraconazole or nifedipine with micafungin the patent should be monitored for toxic effects and if necessary the dose should be decreased. Lately reports of clinical efficacy of micafungin in treatment of infections caused by appear more frequently *non-albicans Candida species* and *Aspergillus spp.* In patients with immunological insufficiency or without.

6. Conflict of interest

The authors declare that there is no conflict of interest.

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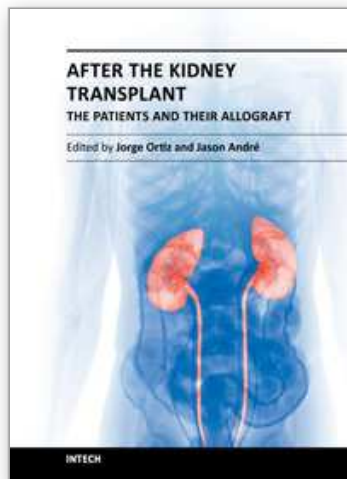
After the Kidney Transplant - The Patients and Their Allograft

Edited by Prof. Jorge Ortiz

There are many obstacles in kidney transplantation. For the transplant team, there is the balance between immunosuppression to aid in the recipientâ€™s tolerance of the allograft and the infection risk of a suppressed immune system. These potential long term complications of kidney transplantation are relatively well known, but there are many other complications that patients and families do not consider when preparing themselves for a kidney transplant. Although the benefits of attempting a kidney transplant far outweigh downfalls of the long term sequelae, kidney transplantation is by no means a benign procedure. It is the hope of these authors that the reader will leave with a sense of understanding towards the kidney recipients.

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