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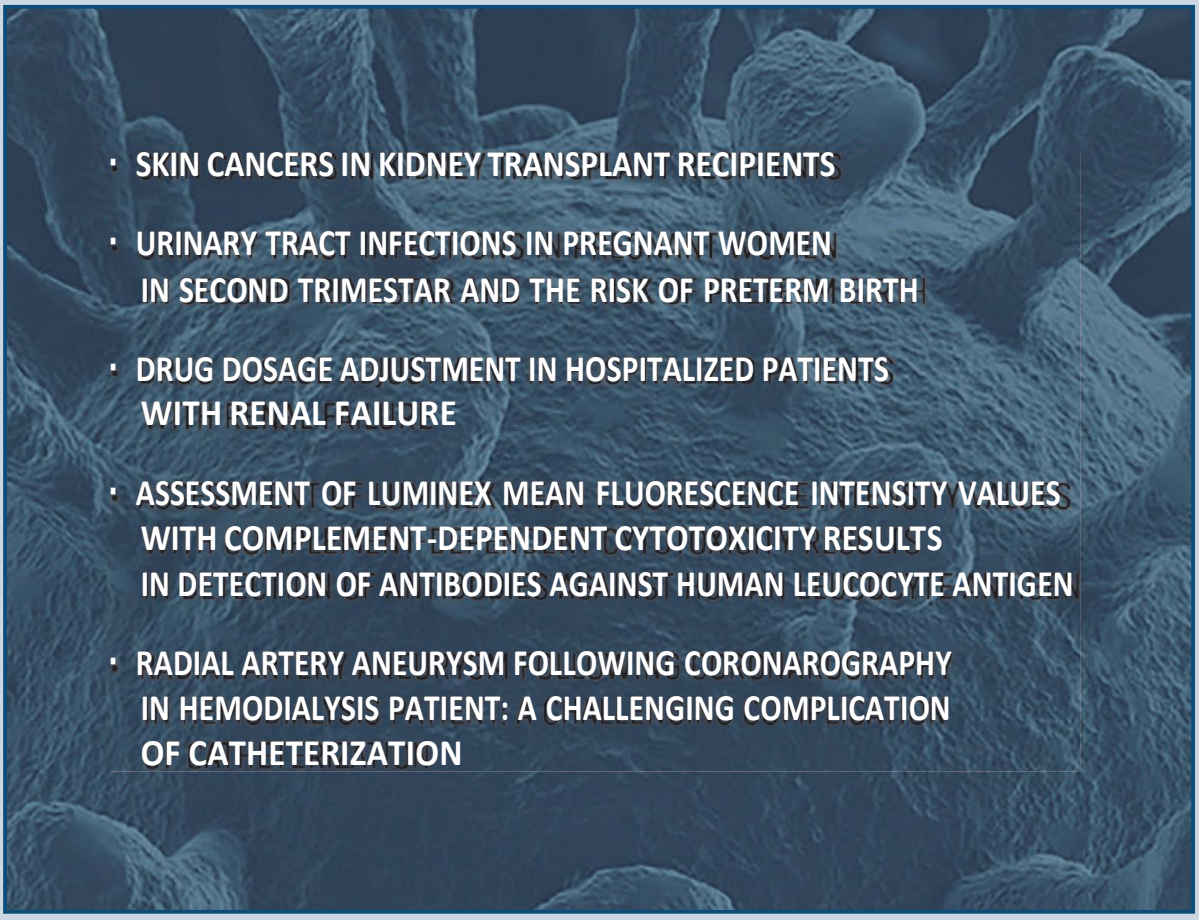

- SKIN CANCERS IN KIDNEY TRANSPLANT RECIPIENTS
- URINARY TRACT INFECTIONS IN PREGNANT WOMEN IN SECOND TRIMESTAR AND THE RISK OF PRETERM BIRTH
- DRUG DOSAGE ADJUSTMENT IN HOSPITALIZED PATIENTS WITH RENAL FAILURE
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Review

Skin Cancers in Kidney Transplant Recipients

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Abstract

Immunosuppressive therapy exposes kidney transplant recipients (KTRs) to increased risk of infections, cardiovascular diseases and cancers. Nonmelanoma skin cancer is the most common malignancy after kidney transplantation with the squamous cell carcinoma (SCC) being the most prevalent type. Increased incidence of cancer is associated with duration and degree of immunosuppressive therapy. It affects mechanisms of DNA repair which leads to altered DNA and carcinogenesis. Also, UV radiation, causing genetic mutations, plays a major role in DNA impairment. Increased risk for viral infections can lead to viral oncogenesis, especially of Human papillomavirus (HPV). The immune system cannot control the HPV infection which makes it persistent and leads to cancer. About 80% of SCC are linked with HPV. The Merkel cell polyomavirus is connected with development of Merkel cell carcinoma, herpes virus type 8 is associated with Kaposi sarcoma- all more common in renal transplant patients than in the general population. To minimize the risk of carcinogenesis but at the same time prevent graft rejection, it is advisable to reduce the dose of immunosuppression therapy. Moreover, switching from calcineurin inhibitor to mTOR inhibitor has promising effects on lowering skin cancer risk. It is recommended to do a dermatological screening before transplantation. Regular posttransplant dermatology visits and sun-protective behaviour are by now the only effective way to detect cancer at an early stage.

Keywords: skin cancer, kidney transplant, immunosuppression, oncogenic virus

Introduction

Kidney transplantation is the most frequent solid organ transplantation worldwide with total of 90,306 transplantations in 2018 [1]. To minimize the risk of graft rejection, patients need to take lifelong immunosuppressive therapy. However, this makes patients susceptible to infections, cardiovascular diseases and malignancies, especially skin cancer [2]. Skin cancer is the most common malignancy after kidney transplantation [3]. Kidney transplant recipients (KTRs) are at a higher risk of developing cancer than the immunocompetent population and it is also known that cancers in kidney-transplanted population are more aggressive [4]. The most common type of skin cancer in transplanted population is squamous cell carcinoma, whereas in general population leads the basal cell carcinoma [5]. Cancer is the second most common cause of death in KTRs after cardiovascular disease [6].

Histopathological examination is essential for diagnosis and therapeutic approach. This review deals with the development of skin cancers in patients after kidney transplantation, pathogenesis, immunosuppression and possible prevention strategies based on available literature (ClinicalKey, PubMed and UpToDate).

Incidence and risk factors

Skin cancer is the most frequent posttransplant malignancy and nonmelanoma skin cancer (NMSC) represents 95% of posttransplant skin cancer [7]. The incidence of skin cancer in organ transplant recipients in the United States of America has been reported at 1427 per 100,000 person-years [8].

Incidence of skin cancer in KTR increases with time posttransplant due to long-term immunosuppressive therapy [8]. While for basal cell carcinoma (BCC) risk increases linearly, for squamous cell carcinoma (SCC) risk increases exponentially with posttransplant survival time [9]. In the transplant recipients the overall ratio of BCC: SCC is 1:4 [10]. In non-transplant population younger than 30 years BCC:SCC ratio is 3.5:1, while in 60 years and older reaches 2:1 [11]. In Oxford Transplant Centre, the cumulative incidence of skin cancer is 61% 20 years after kidney transplantation [12]. For BCC in KTRs, the cumulative incidence is 14% after 20 years [13]. SCC starts to develop about 3 to 5 years posttransplantation [14]. The time that passed from kidney transplantation to diagnosis of the first BCC is 11.1±6.3 years. (15) Study

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by Harwood *et al.* reported that BCC usually occurs in male organ transplant recipients (OTRs) at younger age [16]. Opposite of that, study by *Mertz et al.* finds BCC predominately in female KTRs, in ratio of 3.3:1 and in earlier age than in males, what may be connected with higher UV exposure [15].
In the general population basal cell carcinoma is the most common type of NMSC and SCC is the second most common type of NMSC. In the transplant population SCC takes the first place [2]. Organ transplantation increases the risk of SCC by 65-200 times and BCC by 10-15 times [17]. SCC occurs at least 25 times more frequently than in the general population. SCCs are found to be more aggressive in transplant recipients, with higher recurrence rates and metastasis potential [18].
A study from the Transplant Skin Cancer Network from 26 centers with more than 10,000 patients identified the following statistically significant risk factors for posttransplant skin cancer (Table 1) [8].

Table 1. Significant Risk Factors for Development of Skin Cancer in Organ Transplant Patients (including: Heart, Lung, Kidney, Liver)

Pretransplant skin cancer
Male sex
White race
Age at transplantation 50 years or older

The incidence of melanoma in solid organ posttransplant patients is still in doubt. While some studies demonstrated 2.1 fold to 8 fold higher risk compared with the general population, others did not find increased risk [20]. In KTRs one large study found a 3.6 fold increased risk for melanoma [21].
Other risk factors for development of skin cancer in KTRs are UV radiation, intensity of immunosuppression and use of calcineurin inhibitors (CNIs) versus mammalian target of rapamycin inhibitor (mTORi) [22].

Pathogenesis

The pathogenesis of NMSC in KTRs is multifactorial. Increased incidence of cancer, especially of NMSC in KTRs is directly associated with duration and degree of immunosuppressive therapy [23]. The goal in KTRs is to maintain low kidney rejection rates, while not raising malignancy rates. Immunosuppressive therapy has a negative effect on immune surveillance. It affects deoxyribonucleic acid (DNA) repair mechanisms resulting in altered DNA and carcinogenesis. Immunosuppressive treatment also inhibits Langerhans cells [24].
Innate and adaptive immunity play a major role in cancer development. Natural killer (NK) cells are found to have a protective role in the control of tumor growth and dissemination, as well as tumor specific T cells [25]. T regulatory cells (Treg) Foxp3+ participate in appearance and proliferation of SCC, therefore we can conclude there is a correlation between the number of cells-NK and Treg Foxp3+ and the risk of SCC development [26]. NK lymphopenia and CD4 lymphocytopenia are risk factors for SCC in KTRs [27].
Calcineurin inhibitors (CNIs) are immunosuppressive agents used after kidney transplantation, some of which are cyclosporine and tacrolimus. They selectively inhibit calcineurin, thus suppressing the T cell activation [28]. CNIs might induce carcinogenesis in several ways such as increasing the production of TGF β, increasing the expression of VEGF and also by inhibiting apoptosis and DNA repair in immunocompromised host [20].
Antimetabolic agents are also used in immunosuppression therapy. They interfere with the nucleic acids' synthesis and inhibit T and B cell proliferation [29]. Mycophenolate mofetil (MMF) or mycophenolate sodium (MFS) and azathioprine belong to this group. Azathioprine may also be carcinogenic and its carcinogenic potential is probably associated with impaired DNA mismatch repair and microsatellite instability [30, 31]. Azathioprine is also associated with a selective UV-A photosensitivity generating chronic oxidative stress [32]. MMF has better efficacy and less possibility of developing skin cancer, although it has carcinogenic potential [33]. According to Sarah Yusuf *et al.* [33] MMF is more linked to the development of basal cell carcinoma than squamous cell carcinoma. Also, the subtypes of these tumors are not aggressive forms.
While KTRs on CNI and azathioprine are more likely to develop malignancy, KTRs treated with mTORi have significantly lower risk of NMSC and malignancies in general [34]. Conversing patient to mTORi, in a period of a year, leads to significant functional improvement of antitumor T cells [26].
UV radiation causes genetic mutations and a decrease in the density of epidermal Langerhans cells leading to local immunodeficiency [35]. UVB is more likely to induce skin cancer than UVA, at least in animal models [36]. Melanocytes are responsible for protecting DNA damage by melanin synthesis. Because of low pigmentation capacity, there is more chance for skin cancer development in white Caucasians [36]. For example, in African American kidney transplant recipients, lower incidence is found compared with white Americans [22]. Characteristic type of mutation is found in SCC in p53 tumor suppressor gene causing uncontrollable cell proliferation [22]. The mentioned mutation is a result of cytosine to thiamine transitions through the formation of cyclobutane dimers [31]. In BCC, pathogenic pathway are mutations in Hedgehog pathway related genes, like PTCH1 [36]. In the healthy population, the immune system would recognize and eliminate precancer, but in KTRs the immune system is under suppression causing cancer advancement.
KTRs due to long-term immunosuppressive therapy are at increased risk for viral infections, either new or reactivating latent which can lead to viral oncogenesis. NMSC in KTRs is connected with human papilloma-

virus infection, while their immune system is not able to eradicate an infection [37]. In KTRs with SCC greater quantities of HPV DNA are found [7]. Carcinogenesis of HPV is provided by its oncoproteins E6 and E7 that bind to p53 and pRB tumor suppressor proteins included in the regulation of cell cycle.
Most of the studies have shown that reducing or stopping immunosuppression can lead to regression of certain virus-associated cancers and skin cancers [38]. On the other hand, minimization of immunosuppressive therapy could lead to transplant rejection.
There is a correlation between a history of pretransplant skin cancer and risk of developing subsequent skin cancer in KTRs [3].
Smoking does not significantly influence the risk of skin cancer in KTRs [39]. Older age at transplantation increases the risk for NMSC development [40].

What is the role of DNA methylation of T cells?

Immunosuppressive therapy suppresses the activity of T cells, which have an important role in anti-tumor immune surveillance (CD8+), but can also provide an immune-tolerant environment for the tumor (CD4+). (41) DNA methylation is an epigenetic mechanism of regulation of cellular function. Peters FS *et al.* [41] researched differentially methylated regions (DMRs) in T cells involved in *de novo* posttransplant squamous cell carcinoma development. Analysing the samples collected before transplantation, they compared patients who developed SCC after transplantation to patients without SCC.
They found 16 regions significantly differentially methylated between the patients who developed SCC and those who did not, which may demonstrate that T cells of patients with posttransplant SCC have different DNA methylation profiles compared to the T cells of kidney transplant patients without SCC [41]. This gives the potential of studying DNA methylation of the T cells to possibly identify patients who are at risk for posttransplant SCC.
In another study, Peters FS *et al.* [42] found higher methylation of *SERPINB9* region in circulating T cells in patients with posttransplant cutaneous squamous cell carcinoma. *SERPINB9* gene encodes serpinB9 protein, which is a serine protease inhibitor that inhibits granzyme B, a serine protease found in granules of natural killer cells (NK cells) and cytotoxic T cells (CD8+). Granzyme B protein is necessary for target cell apoptosis in cell-mediated immune response. SerpinB9 is expressed by the cytotoxic T cells that protect themselves from granzyme B activity. It makes T cells stronger and more potent cancer cell killers, so the lower expression of serpinB9 has a potential role as a risk factor for skin cancer in patients with kidney transplant [42].

Types of skin malignancies in renal transplant patients

Nonmelanoma skin cancer (NMSC) represents 95% of posttransplant skin cancer. Risk factors contributing to the development of NMSCs in organ transplant recipients (OTRs) include a past medical history of any previous skin cancer, a personal history of significant sun exposure and a fair skin complexion or phototype. Further, greater immunosuppressive medication levels lead to an increased risk of NMSCs. Among immunosuppressants, specific older agents such as azathioprine and cyclosporine may increase the risk of developing NMSCs in contrast to newer agents such as sirolimus [43]. The most common types are BCC and SCC. Patients with squamous cell carcinoma present with erythematous, keratotic plaques with or without ulcer. SCC can occur on any part of the body, but it usually appears on sun-exposed sites such as face, which is the most common localization in older patients [31]. In organ transplant recipients (OTRs) SCC can be often found on hands ("transplant hands") and scalp ("transplant scalp"). SCC in younger OTRs is mainly located on the chest [44]. SCCs metastasize to the lymph nodes and can be aggressive. The risk of metastases in SCC in the general population is 0.5 to 5%. The risk increases to 8% for KTRs [19].
BCC looks like pink pearly papules, sometimes also presenting with ulcer. The most frequent location for this lesions is on the nose [45]. Moreover, BCC is best known for being locally aggressive which causes destruction.
Melanoma is an immunogenic cutaneous malignancy with higher risk of metastases, and its incidence continues to increase. It is important to know that the correlation between a risk of developing melanoma and an organ transplantation is less clear than that for NMSC [23]. There are several types of melanoma including: superficial spreading, lentigo maligna, nodular, and acral lentiginous. The 'ABCDE' acronym stands for a few changes that might indicate a melanoma (asymmetry, border irregularity, color variation, diameter more than 6 mm and evolution). Risk factors for developing melanoma, especially in renal transplant patients, include older age, male sex, recipient white race, less than four human leukocyte antigens (HLA) mismatches, living donors, and sirolimus and cyclosporine therapy. Importance of immunity role in melanoma pathogenesis is shown in the case report of a renal transplant patient who developed metastatic melanoma from a kidney donor [46].
Merkel cell carcinoma (MCC) is a rare and highly aggressive neuroendocrine tumor of the skin. MCC is thought to originate from the nerve-associated Merkel cell touch receptors, which are in the basal cells layer at the deepest portion of the epidermis. MCC tends to develop 7 to 8 years posttransplant. Reduction of immuno-

suppression may be recommended in OTRs who develop MCC [7]. Lesions are usually on the head and neck, presenting as dome-shaped pinkish-red to bluish-brown papules or nodules and are frequently ulcerated [23].

Can viruses lead to skin cancer in renal transplant recipients?

As we know, Human papillomavirus (HPV) is double-stranded DNA virus that belongs to Papillomaviridae family. There are more than 100 types of HPV [47]. Out of these, there are low-risk (6 and 11) and high-risk types [16,18,31,33,35,39,45,51,52,56,58,59,68,73,82] depending on their oncogenic potential. HPV selectively affects cutaneous and also mucosal membranes in different sites of the body [48]. Clinical manifestations of low-malignant potential types include cutaneous and anogenital warts. On the other hand, high-malignant potential types cause penile, vulvular and vaginal squamous cell carcinoma [48].
HPV is considered to be very common among renal transplant recipients because of the immunosuppression and low cell-mediated immunity (CMI). Because of the ineffective cell-mediated immunity (CMI), transplant recipients cannot control HPV infection. This can lead to persistent infection and increase the possibility of getting cancer [48]. Furthermore, transplant recipients get HPV associated cancer at an earlier age. Even 80% of squamous cell carcinomas are associated with HPV [49]. On the contrary, there is only 40% of SCC linked to HPV in immunocompetent individuals. When it comes to carcinogenesis, HPV of the beta genus (HPV-β) is included in the pathogenesis of posttransplant SCCs. The expression of E6 and E7 HPV oncogenes is necessary for malignant conversion [50].
Prevention includes three prophylactic HPV vaccines. First is Cervarix, a bivalent vaccine that targets types 16 and 18. Secondly Gardasil, a quadrivalent vaccine, targeting types 6, 11, 16 and 18 and lastly, Gardasil 9 which covers the same types as in the quadrivalent vaccine and 5 additional types (high-risk HPV types 31,33,45,52,28) [48].
The Merkel cell polyomavirus (MCPyV) was discovered in 2008 in Pittsburgh, Pennsylvania. It is small, circular, nonenveloped, double-stranded DNA virus that integrates into the tumor's genome [51]. This virus is responsible for development of Merkel cell carcinoma, highly aggressive and relatively rare skin cancer. The viral genome is divided into 3 main regions: non-coding regulatory region (NCRR), early coding region and the late coding region. The early region encodes the small T (sT) antigen and the large T (LT) antigen [52]. LT antigen plays a very important role in carcinogenesis. It inhibits retinoblastoma tumor suppressor genes, promotes cell division and is expressed in all of the tumor cells. MCPyV is also a part of the human skin microbiome [53]. Many people were exposed to MCPyV and did ddnot get MCC which only means that immunosuppression contributes to viral integration, mutagenesis and carcinogenesis.
Kaposi sarcoma (KS) is a vascular tumor caused by herpesvirus type 8 (HHV8) causing red or purple patches of abnormal tissue to grow under the skin. Although KS gained public attention as an AIDS-defining malignancy, it is also common among renal transplant patients [54]. The incidence is 400-500 times higher in renal transplant recipients than non-immunosuppressed population and it is responsible for approximately 3-5% of transplant malignancies [55]. The highest incidence is in the first year after transplantation, often presenting in an early period after transplantation (2-24 months). Specific locations for KS to disseminate are the trachea, lungs and gastrointestinal tract [56].

Management and treatment

The management of NMSCs in solid organ transplant recipients (OTRs) presents a variety of clinical challenges for physicians. Early skin biopsy and treatment of premalignant and malignant lesions are essential for treating these patients successfully [43]. Treatment for NMSCs includes topical therapies (5-fluorouracil 5% cream and imiquimod), photodynamic therapy (PDT) and surgical management. For metastatic SCC, medical, surgical, and radiation therapies should be combined. For patients who are not candidates for an operation, have lesions that cannot be completely excised or have perineural invasion, radiotherapeutical approach should be considered [57]. In addition, for metastatic BCC in OTRs, there is a Food and Drug Administration-approved medication, vismodegib. Vismodegib is a smoothened inhibitor in the sonic hedgehog pathway. Melanoma and MCC should be managed surgically with wide local excision with consideration of a sentinel lymph node biopsy based on the severity of the tumor. In life-threatening melanoma, immunosuppression changes and/or reduction should also be considered [23].
The mammalian target of rapamycin (mTOR) is a regulator of cell growth and survival, and it is often dysregulated in tumors. Switching from a calcineurin inhibitor to mTOR inhibitor, such as sirolimus can have promising effects on lowering skin cancer risk [58]. In KTRs on sirolimus there was a 56% reduction in the risk of NMSC [59]. Sirolimus and everolimus inhibit T and B cell activation by cytokines which prevents proliferation of the cell cycle. On the other hand, calcineurin inhibitors block the production of cytokines [60]. mTOR inhibitors have a potential effect on reducing the posttransplant malignancies associated with viruses [61]. The use of sirolimus can lower the risk of recurrent nonmelanoma skin cancer and bring to complete remission of Kaposi sarcoma [62]. A five-year randomized trial showed no difference in kidney rejection or mortality between sirolimus-based and calcineurin

inhibitors-based KTRs [63]. If needed, combining an mTOR inhibitor with reduced dose of calcineurin inhibitor could provide more sufficient immunosuppression and at the same time, reduce the risk of nephrotoxicity linked with calcineurin inhibitors [62].
Although mTOR inhibitors have certain side-effects (hypertension, hyperlipidaemia, pulmonary toxicity, delayed wound healing, leukopenia, thrombocytopenia and anemia) they should be considered as a secondary prevention of skin cancer development.

Education is prevention

Before transplantation a dermatological screening is recommended. All suspicious lesions should be excised and pathologically examined. Viral warts should be treated [64].
For transplant patients it is crucial to be conscious and sun-smart. The incidence of skin cancers may be greatly reduced by following some preventative measures whenever out of doors, for example: wearing sunscreen (for details about ideal features of the sunscreen itself as well as the routine of applying it, see the Figure 1 below), avoiding sun exposure (of special importance during the middle of the day, between 11 am and 4 pm, when UV radiation levels are at their highest); if you tend to do outdoor activities during these times, stay in the shade. Checking the weather forecast to find out ultraviolet index is an advisable habit to implement, covering up well (wear long-sleeved shirts and pants). Dark coloured, tightly woven materials provide the most UV protection. If possible, try to wear sun protective clothing that has an Ultraviolet Protection Factor (UPF) rating of 40-50+, wearing a hat when outdoors, wearing sunglasses (this provides the best protection to the delicate skin around the eyes-hence choose glasses that are close fitting with large lenses).
Health care personnel should educate their patients about skin cancers, the risks of sun exposure and new habits which they should incorporate into their lives. Studies have shown that patients who are well informed about their condition are paying more attention to lifestyle risk modification and are prone to sticking to the routine regarding positive sun-protective behaviour [65,66].
Measures as simple as showing patients instructive video material followed up by a questionnaire to assess their knowledge has been proven to be a successful method for the patients to retain positive sun-protective behaviour over the long haul [66]. If KTR is strictly avoiding sunlight, vitamin D deficiency may occur. Therefore, vitamin D supplementation should be considered [67].
KTRs are subjected to more frequent dermatology visits, because it is by now only effective way to detect cancer in the early stage and thereby provide successful treatment. In the first year of transplantation, KTRs should undergo a total body skin examination (TBSE) by a dermatologist and continue at least annually evaluation [68]. KTRs with a history of skin cancer should be followed closely. Skin self-examination is recommended.

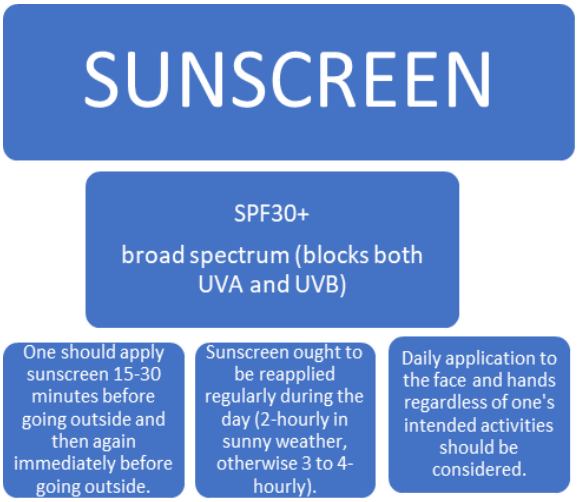

Fig. 1. What each patient should know about sunscreen

When it comes to treatments that could possibly prevent nonmelanoma skin cancer, there is not enough evidence for their efficacy and safety [69]. Some of them are retinoids (such as acitretin), imiquimod, photodynamic therapy and nicotinamide (vitamin B3) [69]. Treatment with capecitabine, orally-administred prodrug of 5-fluorouracil, significantly decreases the incidence rates of recurrent SCC, BCC and actinic keratosis in KTRs and may be considered as a secondary prevention [70]. Reduced immunosuppression dose may decrease the development of nonmelanoma skin cancer, but also increase acute graft rejection [71]. As already mentioned, use of mTOR inhibitors is suggested, especially in KTRs with a history of skin cancer [68].
There are some clinical markers that could help physicians distinguish patients who are at a higher risk of posttransplant cancer development. A UK study of renal transplant recipients developed a predictive index of skin cancer risk that includes sex, age at transplantation and eye color, and it was able to identify patients who later developed nonmelanoma skin cancer with 80% sensitivity and 75% specificity [72]. Type of immunosuppression, its dosage and duration are related with the severity of posttransplant cancer [73]. A study from Oxford Transplant Centre showed that KTRs displaying high levels of CD57 CD8+ are at increased risk of squamous cell carcinoma development [74].

Conclusion

Skin cancer is one of the major causes of morbidity and mortality in patients with kidney transplant compared

to the general population. Due to severe immunosuppression that affects DNA repairing mechnisms, reactivation of potential oncogenic viruses and other risk factors, kidney transplant recipients are at a higher risk of developing malignancies. There are still challenges associated with the adequate immunosuppression therapy usage that is efficient enough to prevent a graft rejection, but also not having a carcinogenic potential. It is important to educate patients about skin cancer, make them well aware of potential risks and provide them a regular cancer screening.

Conflict of interest statement. None declared.

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Original article

Urinary Tract Infections in Pregnant Women in Second Trimestar and the Risk of Preterm Birth

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Abstract

Introduction. Preterm birth, defined as delivery less than 37 weeks of gestation, is the most common cause of neonatal mortality and morbidity accounting for 11% of pregnancies worldwide. Pregnancy causes numerous changes in the woman's body that increase the risk of urinary tract infections (UTIs). Hormonal and mechanical changes can promote urinary stasis and vesicoureteral reflux, along with an already short urethra (3-4 cm in females) and difficulty with hygiene due to a distended pregnant belly, help make UTIs the most common bacterial infections during pregnancy predominantly with Escherichia coli, but also with Staphylococcus saprophyticus, Klebsiella, Enterobacter, Proteus, Enterococcus, etc. The aim of the study was to prove the relationship between UTIs in pregnancy and their risk to cause preterm birth (<37 g.w.).

Methods. This is a prospective case- control study, conducted at the University Clinic of Gynaecology and Obstetrics, Ss' Cyril and Methodius University, Medical Faculty, Skopje, Republic of North Macedonia at the Department of High-Risk pregnancy. The study included 103 patients with signs and symptoms of preterm labour. Patients between 28-36 g.w. were followed until the end of the pregnancy. Obstetric ultrasound on Voluson 730 pro machine was performed by two experienced ultrasound observers calculating the fetus weight, estimating the gestational week and measuring the cervical length so the variations for the measurements have been minimized.

Mid-stream urine sample was collected for cytology and culture-sensitivity.

Results. All 103 patients in the study had signs and symptoms of preterm labour. Out of the total of 103 patients 65 (63%) had a positive urine sample, and 38 (36.9%) patients had negative urinanalysis. The results showed that patients who had signs and symptoms of preterm birth were significantly different in women who had positive compared to those who had negative urine sample (p=0.0049). Microorganisms cultured in urine were predominantly gram-negative bacilli, although there were also gram-positive bacilli detected. E coli was the commonest microorganism cultured in the urine.

Conclusion. Urogenital infections contribute signifycantly to the preventable causes of preterm labor. The benefit of the study lies in detecting asymptomatic cases, so that this complication can be timely prevented. Making early diagnosis of urogenital infections and treating them adequately with the antimicrobials will help in decreasing the incidence of preterm labor, preterm births, and the associated neonatal and maternal morbidities.

Keywords: premature birth, urinary tract infections, Escherichia coli, pregnancy

Introduction

Preterm birth, defined as delivery less than 37 weeks of gestation, is the most common cause of neonatal mortality and morbidity worldwide, and a serious obstetric problem accounting for 11% of pregnancies worldwide and 5-9% in many other developed countries [1]. Infants are born preterm after: a spontaneous labor with intact membranes, preterm premature rupture of the membranes (PPROM), and labor induction or caesarean delivery for maternal or fetal indications [2]. Common reasons for indicated preterm births include preeclampsia or eclampsia, and intrauterine growth restriction. Births that follow spontaneous preterm labor and PPROM (commonly called spontaneous preterm births) are considered as a syndrome resulting from multiple causes, including infection or inflammation, vascular disease, and uterine over distension [3].

Preterm births can also be subdivided according to gestational age: about 5% of preterm births occur at less than 28 weeks' (extreme prematurity), about 15% at 28-31 weeks' (severe pre maturity), about 20% at 32-33 weeks' (moderate pre maturity), and 60-70% at 34-36 weeks' (near term) [3]. Maternal infection accounts for a main cause of preterm births, the exact mechanism has not been definitely clarified, however, it is believed

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that an inflammatory cascade is triggered resulting in an increased production of cytokines, prostaglandins and matrix-degrading enzymes that promotes uterine contractions, cervical dilatation, preterm rupture of the membranes (PROM), as well as an easier entry of pathogens into the uterine cavity [4]. Evidence suggests that infection plays a role in pathogenesis of preterm labor and delivery. An estimated 50% of spontaneous preterm births were associated with ascending genital tract infections and had positive urine and cervical cultures [5].
Pregnancy causes numerous changes in the woman's body that increase the risk of urinary tract infections (UTIs). Hormonal and mechanical changes can promote urinary stasis and vesicoureteral reflux, along with an already short urethra (3-4 cm in females) and difficulty with hygiene due to a distended pregnant belly, help make UTIs the most common bacterial infections during pregnancy [4]. Decidual invasion by the lower genital tract bacteria is associated with recruitment of leukocytes followed by cytokine production which trigger prostaglandin synthesis in the amnion, chorion, decidua, and myometrium [6]. This leads to contractions of the uterus, dilatation of cervix, membrane exposure, and entry of microorganisms into the uterine cavity. Local action of the lower genital tract bacteria produces enzymes sialidase or mucinase, which weakens the protective cervical mucosa and thus supports bacterial invasion of the upper genital tract [7]. In pregnancy, asymptomatic urinary tract infection is very common and is linked with preterm delivery. If bacteriuria without symptoms is not treated in pregnant women, then it may lead to acute cystitis and pyelonephritis [3,8]. The presence of urinary tract infection may be an indicator for abnormal vaginal flora because of the colonization of the vagina with the same pathogens as found in the urine [9].
Risk factors related to bacteriuria in pregnancy are: anatomic urinary tract abnormalities, functional urinary tract abnormalities, diabetes mellitus, sickle cell disease, low socioeconomic status, multiparity, increased frequency of sexual activity [10]. Pathogenic microorganisms associated with both symptomatic and asymptomatic bacteriuria are Escherichia coli, accounting for up to 86% of cases, Staphylococcus saprophyticus, Klebsiella spp, Enterobacter spp, Proteus spp, Enterococcus spp, group B Streptococcus, etc.
The aim of the study was to prove the relation between UTIs in pregnancy and their risk to cause preterm birth (< 37 g.w.).

Methods

This prospective case- control study was conducted at the University Clinic of Gynaecology and Obstetrics, Ss' Cyril and Methodius University, Medical Faculty, Skopje, Republic of North Macedonia at the Department of High-Risk pregnancy. The study included 103 patients, during the period from October 2018 till July 2019. Before entering in the study, all patients gave their writing consent for study participation. Patients were selected for enrolement in the study after hospitalization at High risk pregnancy department with signs and symptoms of preterm labour by the UCOG criteria as four uterine contractions in 20 min or eight in 60 min plus progressive change in the cervix; cervical dilatation greater than 1 cm; and cervical effacement 80% or greater, before 37 completed weeks. Leaking, i.e., rupture of membranes was diagnosed by speculum examination and confirmed by litmus paper (change of color from red to blue).
All patients were between 28-36 g.w. and were being followed until the end of the pregnancy. Each woman had undergone obstetric ultrasound to determine the gestational week and to confirm there are no exclusion criteria so that the patient could enter the study.
The pregnant women were followed on Voluson 730pro ultrasound machine. Ultrasound cervicometry was done and the length of cervix was measured with a vaginal transducer and results recorded on the personal document for the patient. Each patient was taken a detailed anamnesis adapted to the needs and information needed for the research.
Two experienced ultrasound observers were included in calculating the fetus weight, estimating the gestational week and measuring the cervical length so the variations for the measurements have been minimized. The **Headlock** formula was used for HC (head circumference), AC (abdomen circumference), and FL (femur length) to calculate the EFW (efficient fetal weight).
Mid-stream urine sample was sent for cytology and culture-sensitivity to the Institute of Microbiology and Parasitology-Skopje. The samples were inoculated on blood agar and MacConkey's agar using semi-quantitative method of inoculation. The culture plates were incubated at 37_C for a duration ranging from 24 to 48 h. Isolates were identified by standard methods [11].
Inclusion criteria: women with singleton pregnancy, women with signs and symptoms for preterm birth.
Exclusion criteria: Women with twin pregnancy or higher-order pregnancy, women with antepartum, hemorrhage, eclampsia, preeclampsia, women with urinary infections before the pregnancy.

Statistical analysis

A database in the statistical program SPSS for Windows 23.0 was created for the purpose of analyzing the results obtained in the research.
The numerical, i.e. the quantitative parameters are shown with an average, standard deviation, median and interquarter rank.
Qualitative i.e. attributive parameters are shown by distribution frequencies.
Mann-Whitney test was used for comparing women who gave premature birth and those who gave term birth.

Statistically significant differences were set at the level of $p<0.05$.

Results

All 103 patients in the study had signs and symptoms of preterm labour. Out of the total of 103 patients 65 (63%) had a positive urine sample, and 38(36.9%) patients had negative urinanalysis. The results showed that patients who had signs and symptoms of preterm birth were significantly different in women who had positive compared to those who had negative urine sample ($p=0.0049$). Microorganisms cultured in urine were predominantly gram-negative bacilli, although there were also gram-positive bacilli detected. E coli was the commonest microorganism cultured in the urine.
In those patients who had positive urinary tract sample, the mean gestational week at birth was 33.2 and in those who had negative urinary tract sample the mean gestational week was 35.4. The mean length of the cervix was 28 mm (26.0±3.5) in those patients with positive urinary tract sample, and 31mm (30.0±3.5) in patients with negative urinary tract sample. The BMI was 25.2 kg/m^2 in patients with UTI and 24.4 in patients with negative urinary tract sample.
From 65 patients who had positive urinary tract sample with detected UTI, pathogenic microorganisms associated with both symptomatic and asymptomatic bacteriuria were Escherichia coli, accounting for up to 86% of cases, Staphylococcus saprophyticus, Klebsiella spp, Enterobacter spp, Proteus spp, Enterococcus spp and group B Streptococcus (Figure 1).

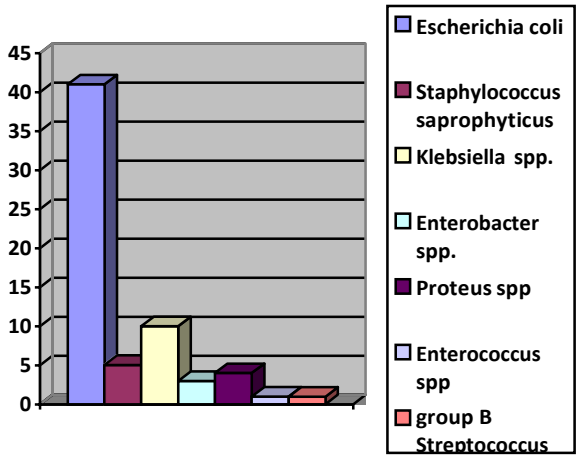

Fig. 1. Isolated microorganisms in the urinary tract samples

From the total number of 103 patients including all samples (positive and negative) in 41 urine samples we isolated Escherichia coli, in 5 samples we isolated Staphylococcus saprophyticus, in 10 Klebsiella spp was isolated, Enterobacter spp was isolated from 3 samples, Proteus spp from 4 samples, Enterococcus spp from 1 sample and group B Streptococcus was isolated from 2 samples (Table 1).

Table 1. Isolated microorganisms in urine sample by number of patients and gestational week

Isolated microorganisms	No of positive samples	Mean gestational week at birth
Escherichia coli	41	33.1
Staphylococcus saprophyticus	5	32.3
Klebsiella spp	10	32.5
Enterobacter spp	3	32
Proteus spp	4	32.5
Enterococcus spp	1	30.4
groupB Streptococcus	1	33.4

Discussion

Pregnant women are at an increased risk of acquiring urinary tract infection due to functional and anatomical changes in pregnancy. In most cases the urinary tract infection is asymptomatic. The purpose of our study was to evaluate primarily the possible association between maternal UTI during pregnancy and the increased risk of preterm birth, and in addition birth outcomes such as gestational age and isolated microorganisms. The results obtained in this study, support the expected hypothesis that the positive urine sample can increase the risk of preterm birth and affect the outcome of the pregnancy i.e. its presents leads to premature birth. The examination is more valuable since we know that 5-18% in total of the full number of births in our Clinic belongs to this group. The results have confirmed that risk factors for premature birth include vaginal and cervical infection [12], shortened cervix and presence of urinary tract infection [13].
However, the reported cultivation data and the results of our previous studies [14,15] showed a similar spectrum of microorganisms associated with significant bacteriuria. The type of microorganisms isolated in our study was quite similar to those isolated in other studies [14], but the gestational week of delivery tended to be higher in the other studies [16]. Maybe the reason for this is the fact that in those studies the parameters compared were mainly related to the risk of premature birth as cervical length, vaginal and cervical swabs and other risk factors [17-19]. In our study, E. coli was the most common organism isolated. This is in agreement with other studies carried in other countries in which E. coli was the most common organism isolated [20, 21].
E. coli is also a common microorganism in the perineum and failure to maintain personal hygiene may increase the risk of infection with E. coli [22]. In addition, gram negative bacteria have a distinct structure which enables the organism to attach, grow and invade the uroepithelium. This may result in invasive infection and pyelonephritis [21].
The benefit of our study lies in detecting the asymptomatic cases, so that this complication can be timely

prevented. Also, a prenatal check in the first trimester for detecting asymptomatic cases can increase the UTIs in pregnant women, at the same time increasing the complication of UTIs in the second trimester and adverse pregnancy outcomes such as pyelonephritis, preterm birth or low birth weight [23]. However, an emerging evidence in the past few years shows the microorganisms inhabiting many sites of the body, including the urinary tract, which has been assumed sterile in healthy individuals, might have a role in maintaining urinary health. Studies of the urinary microbiota have identified remarkable differences between healthy populations and those with urologic diseases [24]. So maybe the future studies can make difference for detecting the urinary microbiota. We live in the era of antimicrobial resistance and may live in other eras like the era of the microbiome. Thus, new insights might also provide an opportunity to prevent the overuse and misuse of antibiotics and could enable the development of innovate managing strategies [25].

Conclusion

Patients with UTI have more common complications in pregnancy especially in the second trimester. Our study has confirmed the reasons for examining UTIs as a method to discover risk in patients who have signs and symptoms of premature birth.

The patients with UTI have more common complications with an increased risk of premature termination of the pregnancy. The benefit of our study lies in detecting asymptomatic cases, so that this complication can be timely prevented. We recommend that women coming for first antenatal checkup should be investigated for the presence of asymptomatic UTI. Making early diagnosis of UTIs and their adequate antibiotic treatment helps decreasing the incidence of preterm labor, preterm births, and the associated neonatal and maternal morbidities. Moreover, an early diagnosis would lower the hospital admittance rate and associated costs, and reduce the premature births. It would be useful to create an algorithm for multidisciplinary treatment of these patients enabling the development of innovative therapeutic strategies.

Conflict of interest statement. None declared.

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Original article

Influence of Nephrolithiasis and Urinary Tract Infections on the Renal Function in Autosomal Dominant Polycystic Kidney Disease

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Abstract

Introduction. The prevalence of nephrolithiasis is considerably greater in patients with autosomal dominant polycystic kidney disease than in the general population. The anatomic factors leading to increased intrarenal obstruction, conditioning cyst growth, followed by renal tubular stasis alongside metabolic disorders, are important and may predispose stone formation. Presence of urinary tract infections in patients with adult polycystic kidney disease can influence the progression of the disease.

Methods. Forty patients with autosomal dominant polycystic kidney disease, mean age 43.2±11.8 years, 19 males and 21 females, underwent echosonography and computed tomography scan to evaluate the prevalence of nephrolithiasis in polycystic kidneys. Routine blood analysis and urine samples, including 24h urine collections were done. Patients were also evaluated from the aspect of urinary tract infection. Criteria for the presence of urinary tract infection were: more than 10 leucocytes in the urine sediment and positive urine culture.

Results. Renal stones were detected in 17 out of 40 patients (42.5%). The morphologic data presented patients with autosomal dominant polycystic kidney disease and nephrolithiasis had more renal cysts and larger predominant cyst size than patients without nephrolithiasis ($p<0.05$). Renal function expressed by creatinine clearance was also different between the 2 groups of patients (73.2±8.7 in patients with nephrolithiasis, and 96.8±7.6 in patients without nephrolithiasis). Twenty-four hours urine analysis showed that patients with nephrolithiasis had significantly lower urine volumes and levels of uric acid. Kaplan-Meier's statistical analysis demonstrated that the worsening of the renal disease depends on the presence of urinary infections in these patients. Comparing the 2 groups of patients (with and without infections), we found that patients with infections had worse renal survival ($p<0.05$). Three patients had urinary tract obstruction, ureterolithiasis with hydronephrosis, with diminished creatinine clearance, but after deopstruction and elimination of the calculi, the renal function was improved.

Conclusion. Nephrolithiasis is an important factor in further progression of renal damage in patients with autosomal dominant polycystic kidney disease accelerating its decline, especially followed by urinary tract infections.

Key words: polycystic kidney disease, nephrolithiasis, urinary tract infections

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary disease, which leads to terminal stadium of chronic renal failure and treatment with renal replacement therapy [1-3]. The disease is on the 4th place among kidney disorders, leading to chronic haemodialysis treatment. With 8-10% of these patients in haemodialysis units, adult polycystic kidney disease takes important place considering the other kidney diseases [3-5]. It is a genetic disorder characterized by formation of cysts in the kidneys. Symptoms caused by cyst formation in the kidneys include high blood pressure, pain on the sides of the body between the last rib and the hip (flank pain), hematuria and progressively loss of kidney function. In most patients, ADPKD eventually progresses to end-stage renal disease, requiring renal replacement therapy, either dialysis or renal transplantation. The disease itself is not simply a kidney disorder and other organ systems of the body can potentially be affected by the development of cysts. The specific symptoms present in each person depend on the specific organ systems involved. The liver, pancreas, the arachnoid membrane of the spinal cord and brain, the prostate, and the glands of the male reproductive tract that produce fluid that is part of semen (seminal vesicles) may become involved. Abnormalities affecting the cardiovascular system may also occur in individuals with ADPKD [2, 3].

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The prevalence of nephrolithiasis is considerably greater in patients with autosomal dominant polycystic kidney disease than in the general population [6-8]. The anatomic factors, such as cyst growth, renal tubular stasis and metabolic disorders, are important and may predispose to stone formation [7, 9-11]. Renal ultrasound may under detect nephrolithiasis, but computerized tomography, provides an excellent technique for distinguishing renal calculi from cyst calcifications in patients with ADPKD [1, 3, 12, 13].
This study aimed to assess the frequency of the presence of renal stones in patients with ADPKD, as well as to detect factors that contribute to stone formation, but also to find the influence of nephrolithiasis and urinary tract infection on the progression of the disease.

Material and methods

In order to evaluate the nephrolithiasis in polycystic kidney disease, 40 patients with autosomal dominant polycystic kidney disease, mean age 43.2±11.8 years, 19 males and 21 females, underwent echosonography and computed tomography scan. Considering the different morphology of the kidneys in adult polycystic kidney disease, we analyzed the impact of the cyst number, the cyst diameter and the renal volume, on the presence of nephrolithiasis in these patients. The clinical and laboratory evaluation included routine blood analysis, urine samples, and 24h urine collections for all the patients. Moreover, the presence of urinary tract infection was studied, which commonly is associated with nephrolithiasis. The presence of urinary tract infection was confirmed with finding of over 10 leucocytes in urine sediment, with confirmed positive urine culture. The diagnosis of ADPKD was mainly done by abdominal and renal ultrasound using HDI and 3 to 5 MHz covex-array transducer based on criteria established by Ravine [14]. The diagnostic criteria for individuals who have a 50% risk of developing ADPKD include:

- At least two unilateral cysts in one kidney or one bilateral cyst in both kidneys in individuals younger than age 30.
- At least two cysts in each kidney in individuals between 30 and 59 years.
- At least four cysts in each kidney in individuals 60 years old or older.

CT scan was performed in patients with suspected calculi, not detected clearly by ultrasonography, and where was needed to distinguish the calculi from calcification of the cysts. Urinary tract infections were detected during the period of 3 years follow up.

Statistical analysis

All the statistical analyses were performed with the SPSS software (Statistical Package for the Social Sciences, version 19.0, SPSS Inc, Chicago, IL, USA). Results were expressed as mean ± standard deviation. Categorical data were compared between groups by the chi-square test and parametric data with regular distribution by t-test. Kaplan-Meier's estimate was used to evaluate survival with "end point" on the creatinine clearance 60 ml/min equal to stage 3 chronic kidney disease.

Results

The presence of nephrolithiasis was identified in 17 out of 40 ADPKD patients (42.5%). The collected data were compared between the two groups of patients, presented on the following tables. Table 1 and 2 presents respectively laboratory findings of serum and urine of patients with and without nephrolithiasis.

Table 1. Median values of serum parameter

Parameter	Pts with NL*	Pts without NL	P Value
Creatinin clearance (ml/min)	73.2±8.7	96.8±7.6	0.67
Sodium (mmol/l)	142±3.6	138±4.2	0.83
Potassium (mmol/l)	4.3±2.4	3.8±1.4	0.52
Calcium (mmol/l)	2.4±0.9	2.1±0.5	0.33
Uric acid (μmol/l)	258±11.2	167±7.1	0.19

*NL-nephrolithiasis

Median values of the creatinine clearance were 73.2±8.7 ml/min, in patients with nephrolithiasis, and 96.8±7.6 ml/min in patients without calculi. Uric acid as important factor for composing calculi was 258±11.2 μmol/l in patients with nephrolithiasis, and 167±7.1 μmol/l in those without calculi. The electrolyte status was as follows; in the group of patients with nephrolithiasis: Na 142±3.6; K 4.3±2.4; Ca 2.4±0.9 mmol/l, and in the group of patients without nephrolithiasis: Na 138±4.2; K 3.8±1.4; Ca 2.1±0.5 mmol/l.
The values for the urinary parameters are presented in Table.2. In patients with renal calculi we found that Na was 146.7±28.1, K 42.5±13.8, Ca 3.7±1.5 mmol/l, and the uric acid 2.63±0.96 mmol/l. On the contrary in the other group of patients without nephrolithiasis Na was

Table 2. Values of urinary parameters

Parameter	Pts with NL*	Pts without NL	P Value
Na (mmol/l)	146.7±28.1	142.2±32.5	0.78
K (mmol/l)	42.5±13.8	38.8±14.1	0.26
Ca (mmol/l)	3.7±1.5	2.9±1.6	0.14
Uric acid (mmol/l)	2.63±0.96	3.99±1.05	0.46

*NL- nephrolithiasis

142.2±32.5, K 38.8±14.1, Ca 2.9±1.6 mmol/l, and also uric acid 3.99±1.05 mmol/l.
In table 3 are the morphologic data of the polycystic kidneys in patients with nephrolithiasis and without nephrolithiasis. Cysts number in patients with nephrolithiasis varies from minimum 30 to up to 40, but in

patients without nephrolithiasis is below 10 (range 5-10) present cysts. The largest renal cyst diameter varied from 4.2 cm (range 3.4-6.3) to 2.6 cm (range 1.3-3.2) respectively in patients with and without nephrolithiasis. We also noticed a difference in renal volume which varied from 801±360.8 cm^3 in patients with nephrolithiasis to 414.3±149.5 cm^3 in those without nephrolithiasis.

Table 3. Morphologic data in ADPKD and nephrolithiasis

Data	Pts with NL*	Pts without NL	P Value
Cysts number	min 30	5-10	0.048
Largest renal cyst	4.2 cm	2.6 cm	0.036
Renal volume	801.7±360.8	414.3±149.5	0.064

*NL- nephrolithiasis

The clinical parameters of the patients are presented on Table 4. We observed the presence of episodes of hematuria, flank pain, urinary tract infections in both group of patients. Hematuria was noticed in 5 patients out of 17 with nephrolithiasis and only in 1 patient out of 23 without nephrolithiasis. Flank pain had 12 patients with nephrolithiasis and only 4 without nephrolithiasis. The urinary tract infections are common in patients with polycystic kidneys, especially with nephrolithiasis. Urinary tract infection was present in 10

Table 4. Clinical parameters of the patients with nephrolithiasis

Parameter	Pts with NL*	Pts without NL
Hematuria	5 (17)	1 (23)
Flank pain	12 (17)	4 (23)
Urinary tract infections	10 (17)	3 (23)

*NL- nephrolithiasis

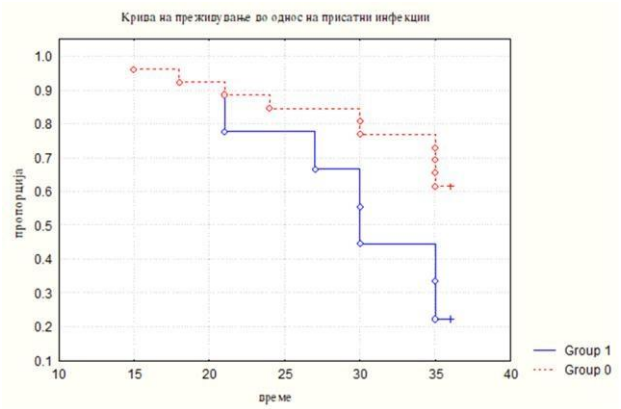

Fig. 1. Kaplan-Meier's analysis of renal survival in patients with and without urinary tract infection
Group 1: patients with urinary tract infections;
Group 0: patients without urinary tract infection

out of 17 patients with nephrolithiasis and 3 out of 23 patients without nephrolithiasis.

Figure 1 presents Kaplan-Meier's analysis of renal survival (with endpoint on the creatinine clearance 60 ml/min, equal to stage 3 of chronic kidney disease) in patients with and without urinary tract infection.

Comparing the 2 groups of patients (with and without infections), we found that patients with infections had worse renal survival than patients without urinary infections ($p<0.05$).

Discussion

Autosomal dominant polycystic kidney disease is associated with an increased incidence of nephrolithiasis [2, 3]. However, the diagnosis of nephrolithiasis in ADPKD by ultrasonography might be impaired as a result of the frequent occurrence of parenchymal or cyst wall calcifications [4]. It has been suggested that CT scan could be more efficient in detecting stones that have been missed by sonography, as well as in separating stones from renal calcifications in ADPKD [3, 12].

In our study, to diagnose renal stones, we used renal ultrasonography. CT scan was performed in patients with suspected calculi, not detected clearly by ultrasonography, and where there was a need to distinguish the calculi from calcification of the cysts. The presence of nephrolithiasis was identified in 17 out of 40 ADPKD patients (42.5%).

Flank pain or low back pain as a result of cyst enlargement, rupture or infection, as well from nephrolithiasis is commonly reported by patients with ADPKD during the course of their disease [15,17]. In this series, low back pain was much more frequent in patients with ADPKD and nephrolithiasis than in patients with ADPKD without nephrolithiasis. This was also evident in our study. Three patients had urinary tract obstruction, ureterolithiasis with hydronephrosis, with diminished creatinine clearance. However, renal function was restored after deconstruction and elimination of the calculi [15-17].

The aim of this study was to investigate whether anatomic abnormalities and/or metabolic disturbances might have contributed to the development of nephrolithiasis in this series of patients with ADPKD. With respect to anatomic abnormalities, some of the studyes disclosed a significantly higher kidney volume in patients with ADPKD and nephrolithiasis than in patients with ADPKD [7, 12]. In our study the difference of kidney volume in the group of patients with nephrolithiasis and those without nephrolithiasis is evident, but it is not statistically significant. Progressive enlargement of polycystic kidneys is inversely correlated with creatinine clearance and presumably directly related with distorted anatomy favoring stone formation, the reasons for such findings remain unclear.

The higher renal volume of patients with nephrolithiasis might have been ascribed to a higher number of cysts, because patients in this group presented more than 30 cysts in their kidneys. Grampsas *et al.* [7] also

observed a significantly higher number of cysts and larger predominant cyst size in 15 individual stone-forming polycystic kidneys compared with kidneys without stones, suggesting intrarenal anatomic obstruction as a cause for nephrolithiasis. In our study, the morphologic data presented that patients with autosomal dominant polycystic kidney disease and nephrolithiasis had more renal cysts and larger predominant cyst size than patients without nephrolithiasis ($p<0.05$). Renal function expressed by creatinine clearance, was also different between the two groups of patients (73.2± 8.7 in patients with nephrolithiasis, and 96.8±7.6 in patients without nephrolithiasis).

Twenty-four hours urine analysis showed that ADPKD patients with nephrolithiasis had lower urine volumes and levels of uric acid. Analysis of the serum and urinary parameters showed that there was not statistically significant difference between the values in both group of patients, with or without nephrolithiasis.

Torres *et al.* [18] observed that 49% of patients with ADPKD and nephrolithiasis had symptoms. Urinary tract infection was also seen only in ADPKD associated with nephrolithiasis in this sample. Considering the clinical manifestations in ADPKD patients with and without nephrolithiasis, we noticed that hematuria occurred more frequently in patients with nephrolithiasis, with urinary tract infection, or cyst rupture. Sometimes hematuria and urinary tract infections were recurrent, but the correct treatment decreased their frequency. In our study comparising the 2 groups of patients (with and without infections), we found that patients with infections had worse renal survival than patients without urinary infections ($p<0.05$). Kaplan-Meier's statistical analysis demonstrated that the worsening of the renal disease, depends on the presence of infections in these patients.

Conclusion

Authors consider that nephrolithiasis is important factor for the progression of renal damage in patients with autosomal dominant polycystic kidney disease. This may be due in part from the complications that may accelerate worsening of the renal disease, especially joined by urinary tract infections.

Conflict of interest statement: None declared.

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Original article

Drug Dosage Adjustment in Hospitalized Patients with Renal Failure

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Abstract

Introduction. Dose adjustment for certain drugs is required in patients with reduced renal function to avoid toxicity as many drugs are eliminated by the kidneys. The aim of this study was to assess whether appropriate dosage adjustments were made in hospitalized patients with renal failure.
Methods. A prospective study was carried out in the ward Service of Nephrology, University Hospital Center "Mother Teresa", Tirana. The patients admitted to hospital between October and December 2019 were included in the analysis. Data regarding serum creatinine level, age, gender, prescribed drugs and their dosage was collected from the patients' medical records. The estimated creatinine clearance was calculated using the Cockcroft-Gault equation. Guideline for Drug prescribing in renal failure provided by the British National Formulary was used as the standard for dose adjustment.
Results. There were 589 prescription entries for 74 patients with renal impairment. Dose adjustment was required in 56% (331/589) of prescription entries and 49.8% (165/331) prescription entries requiring dose adjustment were found to be inappropriate. Eleven (14.9%) patients had all of their drugs appropriately adjusted while 53 (71.6%) patients had some drugs appropriately adjusted, and 10 (13.5%) of patients had no drugs appropriately adjusted.
Conclusion. The findings indicate that dosing errors were common among hospitalized patients with renal failure. Improving the quality of drug prescription in patients with renal impairment could be of importance for improving the quality of care.

Keywords: dose adjustment, renal failure, prospective, creatinine clearance

Introduction

The progress in the field of medicine, both in terms of diagnostic and therapeutic goals, has led to a significant increase in the average age of the population, an increase in the number of patients affected by diabetes, obesity, cardiovascular diseases, which are subject to an increased incidence and prevalence of renal failure [1]. Drug dosing errors are today one of the major problems faced by patients with renal insufficiency. Most of the drugs and metabolites are excreted through the kidney. Therefore, a normal renal function is important to avoid potential toxicity in these patients. The impairment of kidney function has a significant influence on pharmacokinetic and pharmacodynamic parameters. Administration of specific doses of the drugs in patients with kidney deficiency is one of the most important steps in avoiding side effects of drugs as well as providing optimal efficacy [2,3]. Despite this important fact about regulating doses in patients with renal failure (RF), these adjustments in most cases are not taken into consideration. Consequently, if adequate dosing regimens are not used in patients with kidney failure, accumulation and immediate toxicity may occur [4].
The purpose of this study is to evaluate if appropriate dosage regimens are applied for the drugs administered to hospitalized patients with renal impairment at the Service of Nephrology and to identify and analyze factors that may influence the correct or not medication prescription in these patients.

Materials and methods

The present study for the assessment of the implementation of correct dosing regimens in patients with RF, included patients hospitalized during the period October-December 2019 at the Nephrology Unit, University Hospital Center "Mother Teresa", Tirana.
Clinical and demographic data were exported from the medical records of hospitalized patients during this period. The study protocol was approved by the head of the nephrology unit and by the Ethics Committee of Clinical Studies.
In total, 74 patients were hospitalized and diagnosed with renal failure (RF), out of which 11 were with acute renal failure (ARF) and 63 with chronic renal failure (CRF).

Data collection
To complete this study, a two-part questionnaire was drafted, the first part consists of patient's general data,

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and the second part includes clinical data obtained from the medical records. From all drugs administered to these patients, the second part of the questionnaire considered mainly those metabolized and excreted in the renal route, excluding drugs that are excreted and metabolized in other extrarenal pathways.

Statistical analyses

The data collected were analyzed using the Statistical Product Service Solutions (SPSS) statistical program and part of the data was analyzed with Excel. Results are reported as mean±SD.
The descriptive analysis focuses on the analysis of the distribution of the variables in the study population, including demographic factors, comorbidities and the reason for drug administration.
The search for significant statistical links was made through several tests where for values of $p<0.05$, correlation between the variables is statistically significant.

Results

From October 2019 to December 2019, 74 patients undergoing acute renal failure (ARF) and chronic renal failure (CRF), from stage I to pre-dialysis stage V, were included in the study. Patients in chronic dialysis and those hospitalized for other diagnoses were excluded from the study.
Data were received from the medical records of 74 patients. From these patients included in the study, 63 (86.5%) were diagnosed with CRF, while 11(13.5%) were diagnosed with ARF.
From the 74 patients diagnosed with renal failure (RF), 30 (40.6%) are female and 44 (59.4%) are male. The mean age of patients diagnosed with RF was 52.6 years, from the target age group 18-83 years.
Evidently, the CRF pre-dialytic stage V was predominant, with 48% of the cases, followed by CRF stage III and IV with 20% of cases each. While the cases in CRF stage I and II were present in 12 % of cases. These results are shown in Figure 1.

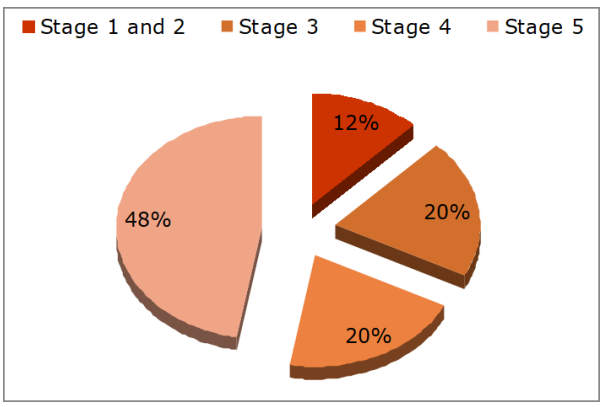

Fig 1. Distribution according to the stages of chronic renal failure

In the unit where the study was conducted, there were patients from all over Albania, although the presence of patients from the other municipalities was predominant compared to patients from Tirana. Other diseases such as arterial hypertension, diabetes mellitus type 2, secondary anemia, dyslipidemia, thrombosis, were present in 62/74 (83%) of patients with RF. 58/74 (78.3%) suffered HTA and dyslipidemia, 34/74 (45.9%) secondary anemia and 15/74 (20%) diabetes mellitus. Information about the stage of the disease was obtained from the patient medical records.
The total number of drugs administered in the patients included in the study was 589. The number of drugs used by the patients ranged from 3 to 18 drugs, showing a high presence of polypharmacy in this category of patients.
The average of the drugs used was 7 drugs per patient per day, with a distribution of the number of drugs per day as shown in Figure 2.

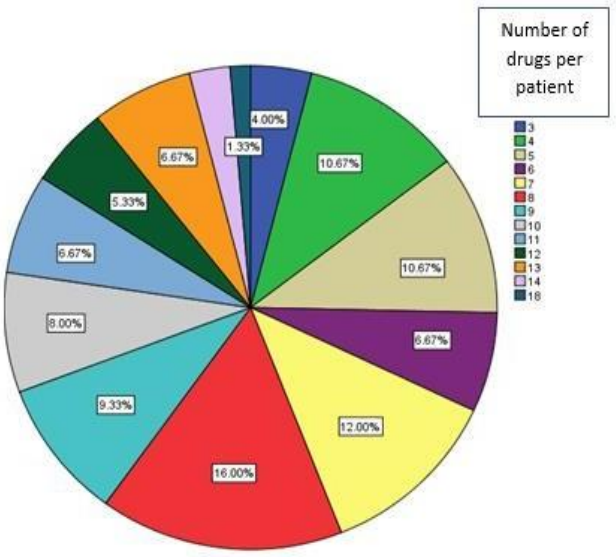

Fig. 2. Distribution of number of drugs /day in patients with RF

The prescribed therapy consisted in drugs for the treatment of renal symptoms or in drugs for the treatment of both renal and non-renal symptoms. The renal therapy consisted in drugs to correct conditions such as hy-

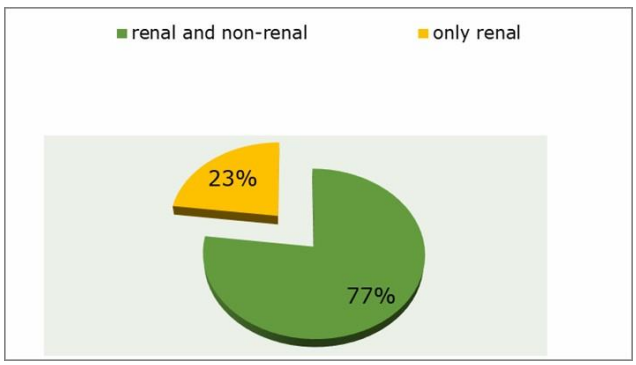

Fig. 3. Distribution of prescription according to the pathology

perphosphatemia, acidosis, hyperkalemia, RF caused by uremia. Nevertheless, renal and non-renal therapy consisted in the treatment of above mentioned health conditions as well as other accompanying diseases. Prevalence of renal and non-renal therapy was 77%, followed by renal therapy only with 23% (Figure 3).

Using the guidelines and the British National Formulary (BNF) [5], each of these drugs was examined and carefully dosed according to renal failure stage. The study excluded drugs that were metabolized and excreted in a large scale by hepatic pathways. These drugs included folic acid 5 mg, iron fumarate 350 mg and erythropoietin administered to control anemia, sodium bicarbonate to correct acidosis, sevelamer carbonate to correct the increase in phosphate levels, keto-acids to correct the deficiency of amino acids and high levels of creatinine in the blood.

On the other hand, more attention was given to large-scale kidney-excreted drugs. For each drug used, its dose was accordingly adapted for each patient.

Out of the 589 medications taken into consideration, 258 drugs (43.8%) did not need dose adjustment, while in 331 (56.2%) it was necessary to adjust the dose before administration to these patients.

Out of these, about 331 drugs that needed a regulated dosage system, only for 166 (50.15%) drugs the doses were appropriately adjusted based on the creatinine clearance values of the patient and for 165 (49.85%) drugs the doses were not appropriately adjusted. The scheme in Figure 4 describes this fact.

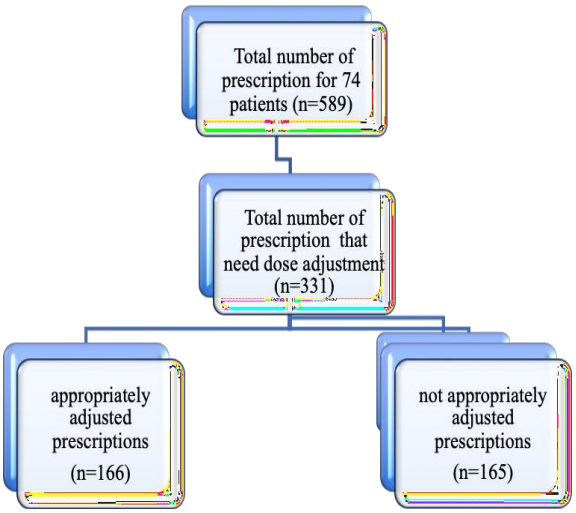

Fig. 4. Appropriateness of prescription in patients (n = 74)

In only 11 out of 74 patients (14.9%) all drugs doses were appropriately adjusted. While at 10 (13.5%) none of the medicines resulted to be adjusted. In most patients, 53/74 (71.6%), only a part of the prescribed drugs had an appropriate dosing regimen.

Based on a detailed assessment of the drugs used in the patients under study, the most prescribed drug was ranitidine. It is mainly excreted in the renal pathway and dose adjustment in patients with damaged renal function is very important. But only in 11/53 (21%) cases an appropriate dosing system was performed while in 42/53 (79%) dosing resulted improper. Nebivolol was administered to 33/74 patients and the dose was appropriately adjusted in only 8/33 (24%) of patients, moxonidine was administered to 17/74 patients where only in 7/17 (41%) cases a suitable dosing system is implemented. Ciprofloxacin administered to 20/74 (27%) patients is the only drug whose dose was appropriately adjusted in all cases (Table 1). We can also say that in patients with RF, hospitalized in the Nephrology Service, there is a tendency to use drugs, whenever possible, that do not need dose adjustment (extrarenal excretion) such as ceftriaxone, cefuroxime etc.

Table 1. The most prescribed drugs

Drugs administered	Number of patients treated with drug	Number of patients treated with the adjusted drug dose
Ranitidine	53	11
Nebivolol	33	8
Moxonidine	17	7
Ciprofloxacin	20	20
Nitrofurantoin	25	17

According to the RF stage, we noticed that in CRF stage III, out of 73/331 administered drugs, about 5/73 (61.6%) were dosed appropriately and in 28/73 (38.4%) of drugs, the dosages are not adjusted properly. In the CRF stage IV, out of 65/331 administered drugs, about 37/65 (56.9%) were dosed appropriately and in 28/65 (43.1%) of drugs, medication doses have not been appropriately adjusted. While in patients in CRF pre-dialysis stage V, there were administered about 162/331 of medicines, where 59/162 (36.4%) of the medication was subject to anappropriate dose adjustment and in 103/162 (63.6%) of the administered medicines, the medication doses were not appropriately adjusted.

Creatinine data was obtained from patient medical records and was calculated using the ClCr values using

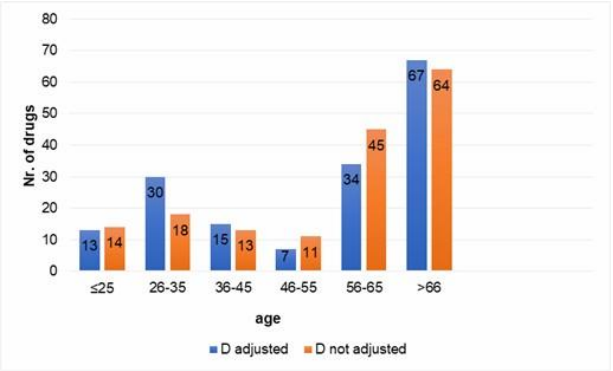

Fig. 5. Distributions of drugs according to age

Cockcroft/Gault formula for each patient. The mean creatinine clearance value (ClCr) was 27.9 ml/min. This confirms once again the severity of the condition in hospitalized patients in this Service and the predominance of the patients in pre-dialysis stage V. It was noticed that the lowest value of ClCr (under 30 ml/min) is present among 50 to 70 years old patients. The largest number of drugs was prescribed in age groups >66 years, and in most cases (67 drugs) the dose was adjusted appropriately, while in 64 drugs the dose did not appear to be adjusted, as shown in Figure 5. According to the stage of the disease, we evidenced the highest number of drugs used in the CRF pre-dialysis stage V, but also the highest number of unproper dose recommendation (59 out of 162 drugs unproperly dosed). On the other hand, in CRF stage III and IV predominate drugs whose dose was adjusted in comparison to those that were not adjusted (Figure 6).

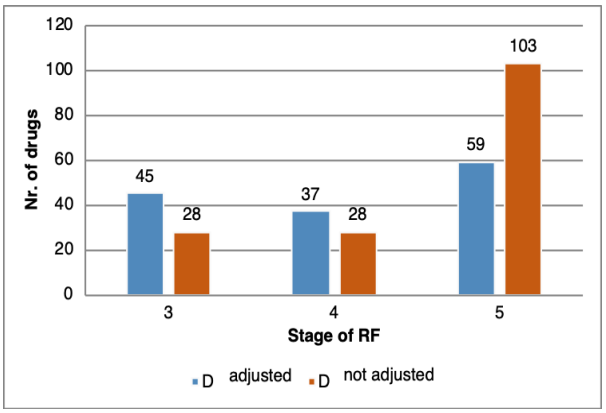

Fig. 6. Distributions of drugs according to renal failure stage

Overall, 18.1% of patients have a prescription for any relatively contraindicated drug like acarbose, chlorpropamide, nitrofurantoin or any non-steroid anti-inflammatory drug (NSAID), aminoglycosides, prescribed from family doctor. Ibuprofen and naproxen are the most commonly used NSAID, while from aminoglycosides gentamycin is the most frequent used drug. The last drug classes are the causes of ARF. From patients complicated with ARF only two of them required dialysis.
This fact need to be evaluated and to reinforce further continuous professional education for family doctor.

Discussion

This study can be considered representative of the Albanian population as it includes patients from all over Albania in the University Hospital in Tirana. The prevalence of RF was higher in males compared to females, namely 40.6% of patients were females and 59.4% males. This datadoes not accord with other similar studies indicating that women's prevalence is higher [5-13]. Meanwhile, 6.1% of British population, from the age 16 suffer from RF, from this percentage, namely 1 in 5 males and 1 in 4 females [8]. Also, the largest percentage is represented by the elderly population, over 65 years of age, with predominance of CRF stage III-IV [8,9]. Renal insufficiency is becoming a global problem, and this is due to the growth of diseases such as hypertension, type 2 diabetes mellitus and obesity considered to be its main causes [14,15].
Several studies focused on renal disease conclude that in the last 20 years, the number of people with RF is growing dramatically. Our study has performed an assessment of dosing regimens in patients diagnosed with RF. In this way, it identified the percentage of administered medications at the right dose in RF patients. Our study points to the fact that dose adjustments were necessary in 56.2% of the prescribed drugs, based on the value of ClCr. While 43.8% of the administered drugs in these patients were not considered as dose-necessary adjustment. This may be the influence of prescribed drugs that do not need dose adjustment (excretion in the extrarenal pathway), nephrotoxic drugs are avoided, and/or prescription of drugs with a wide therapeutic index (safe drugs) [16]. In our study, only 11 out of 74 patients (14.9%), were correctly adjusted for drug doses. While in 10/74 (13.5%) patients none of the medicines was adequately regulated. In most patients, 53/74 (71.6%), only a part of the prescribed drugs had an appropriate dosing regimen.
In a study conducted in Spain by Paula Arrabal-Duran *et al.* 13 in 2014, it turned out that it was necessary to apply a regulated dosage system to the 221 prescribed drugs. In 65.6% of cases it was recommended a dose reduction, while in the 26.7% the dosing interval was prolonged and at 7.2% the use of the drug was avoided. Antimicrobials comprised the category of drugs that had the highest dose modification [17] and the dose of the drug that was mostly modified was levofloxacin. On the other hand, even in the study conducted in our country, it turned out that the levofloxacin analogue drug, ciprofloxacin was the drug more precisely dosed in all administered cases. In a study conducted by Baum S *et al.* [17], Institute of Clinical Pharmacology, Germany, in 2010 showed that in 55/85 patients, on average one of the prescribed drugs was not regulated by protocol. Out of the 220, which was the total number of drugs prescribed, 46.0% of the medication was needed to adjust the dose. Henok Getachew *et al.* [18] showed in their study that only a limited number of patients had a correct dosing system. Thus in 15/54 (28%) patients the dose of drugs was adequately adjusted, in 22/54 (41%) patients only in some drugs the dose was adjusted, while in 17/54 (31%) did not result in the application of an appropriate dosing regimen [18]. Markota NP *et al.* [19] in their study showed that 161 out of 712 patients altogether had 874 drugs out of which 171 (19.6%) of the prescriptions were found to need dose adjustment. From where in 81/171 (47.4) cases dose adjustment was done in accordance with protocols and in 90/171 (52.6%) this was not realized [19]. In another study in the Netherlands by Van Dijk EA *et al.*

[20] involving 647 patients under study, 237 (36.6%) of them needed dose adjustment. The total number of prescribed drugs was 1718 out of which 411 of them (23.9%) was necessary to adjust the dose. The drugs were correctly dosed at 242/411 (58.9%) of the prescriptions and in 169/411 (41.1%) doses were not adjusted.

Above mentioned studies and other similar ones in the literature indicate that much work is still needed to achieve appropriate dose regimens in most RF patients.

Calculation of the ClCr not only enables an assessment of the patient's condition but creates a positive impact, as it allows medical personnel to prescribe more safe medicines for patients. The median of the medicines prescribed for the patient in our study resulted in 7 medications per patient and in 83.7% of patients it resulted in 5 drugs. The reason for the administration of the drugs was renal and non-renal or only renal, with 77% of the renal and non-renal followed by 23% of renal failure.

This situation is related to the fact that patients with RF have other accompanying diseases such as HTA, secondary anemia, dyslipidemia, diabetes, which aggravate the patient's condition as a whole.

As a consequence, the number of drugs used by the patient increases and the reason for their use is not only renal [6] In many cases, drugs prescribed in the nephrology unit do not need dosing regimen modifications, in particular antibiotics such as ceftriaxone, cefuroxime. Also, there were prescribed drugs metabolized and excreted in a large percentage by hepatic pathways and less by renal pathways. The drug administered at a high frequency was ranitidine, and mainly the dose was kept unchanged despite creatinine clearance and RF Stage values. In an analogue study conducted by Henok Getache *et al*. regarding dosage regimens it was found that cimetidine, the ranitidine analogue, was administered at a high frequency and the dose of which was also not adjusted according to ClCr. It was also noted that V-RF stage predominated, with 47% involving mainly dialysis patients, kidney transplant patients, and patients with severe V-stage [18]. This may be for a variety of factors such as late diagnosis of the disease, use of nephrotoxic medications for long periods of time, late referral to nephrologists, use of medicines for the treatment of accompanying diseases, and the genetic factor plays a role as well. Over the years these diseases reduce renal function by precluding renal failure. Also, in our study it was found that 78.6% of patients hospitalized in the nephrology unit at the stated period had HTA accompanying disease and 20% diabetes. Promoting health and early screening of these diseases can reduce the incidence of RF [14].

The latest data conclude the importance of cooperation between healthcare providers for a successful metabolic therapy in patients with renal failure. The steady increase in the list of drugs makes it difficult for the medical staff to stay informed. In this way, clinical pharmacists who have the proper knowledge about pharmacokinetics, pharmacodynamics and drug action mechanisms can be helpful [21-23].

Our study presents some limitations. The number of patients analyzed and the period of study was limited. In this study is used the Cockcroft / Gault formula for the Calculation of ClCr. It should be noted that the Calculation of ClCr value, which is used as an indicator of how the dosage regimens need to be adjusted, is achieved using the average weight in both females (60 kg) and males (70 kg). Consequently, the values of ClCr may have deficiencies, which are then reflected in the dosage regimens and automatically in the number of medications adjusted under this regime. Also, doctors who prescribe drugs may have used different guidelines from ours. When the clearance values are below 10mL, the doses of the drugs are difficult to adapt, since there is a risk of undergoing ineffective therapeutic concentration. But on the other hand, there is a risk of not reducing the dose depending on the creatinine clearance values, the drug accumulates in the body and causes toxic effects.

This study did not take into account patient compliance that could increase drug complications. Previous studies have evidenced a considerable level of inappropriate drug use by Albanian patients [24,25], which should be further explored and taken into consideration while interpreting the data.

The distribution drug system in the hospitals is often the reason for the prescription of certain drugs. The choice is therefore made upon the availability of the drugs in the hospital. This may be also the cause for the prescription of ranitidine and not of other similar drugs.

Conclusion

The results of this study show that drug therapy in renal failure in Albania is analogous to other countries. Prescription of drugs in this category of patients was only partially performed according to the rules and protocols of the field. Among the major causes that complicate the regulation of dosage regimes is the presence of a large number of medications, which is due to the age of patients developing the disease and the presence of other diseases. One way to improve dosage regimens would be to reduce the number of medications to the possible minimum and to prescribe extrarenal excretion drugs. Collaboration between the medical doctor and the pharmacist is essential to achieve safe, effective medication therapy with as few side effects as possible for the patient.

Conflict of interest statement. None declared.

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Original article

Assessment of Luminex Mean Fluorescence Intensity Values with Complement-Dependent Cytotoxicity Results in Detection of Antibodies Against Human Leucocyte Antigen

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Abstract

Introduction. Antibodies to human leukocyte antigens (HLA) are one of the major immune barriers to successful organ transplantation. In addition to complement-dependent cytotoxicity (CDC) assay as a standard method for the HLA antibodies detection, more sensitive solid-phase assays like Luminex were introduced. The aim of this study was to define mean fluorescence intensity (MFI) cutoff values of HLA Class I antibodies detected by Luminex from those detected by CDC given the possibility to use only Luminex assay in HLA antibody screening.
Methods. This is a retrospective analysis of the HLA antibody screening results of patients on a kidney transplant waiting list, performed at the Tissue Typing Laboratory, Clinical Hospital Center Rijeka, Croatia, from January 2012 to July 2019. The study included 1,665 sera tested in parallel by CDC and Luminex techniques.
Results. Almost half sera contained HLA antibodies (47.9%), significantly more detected by the Luminex than a CDC method. Antibodies against HLA-A and HLA-B molecules had higher MFI values, relative to the HLA-C antibodies, as well as antibodies detected by Luminex and CDC than those detected by Luminex alone. A cutoff MFI≥9,204 for Luminex detected HLA Class I antibodies correlated with positivity in the CDC assay. Besides MFI, several factors need to be taken into consideration in interpreting test results to identify unacceptable antigen mismatch.
Conclusion. The results of this analysis suggest that the current features of the Luminex technique provide the most benefit in the HLA antibody screening when combined with other methods, like CDC.

Keywords: Antibodies, CDC Test, HLA antigens, Kidney Transplantations, Luminex

Introduction

Kidney transplantation is the best therapy for patients suffering from end-stage renal disease [1]. The most important immune factors that affect graft survival are the ABO blood group system and the human leukocyte antigen (HLA) system [2,3].
The polymorphism of HLA system always causes some degree of mismatch (MM) between the donor and the transplant recipient, except in identical twins [4]. Donor HLA antigens that the recipient does not have induce cellular or humoral immune response. Today in solid organ transplantations, the cellular response can be controlled by immuno-suppressive therapy, while the humoral response still constitutes a major cause of immune-mediated graft rejection [5]. Therefore, HLA antibody monitoring in the recipient and determining unacceptable antigens is an important prerequisite for a successful transplantation [6].
HLA alloimmunization can result from prior contact with foreign HLA antigens via blood, after tissue or organ transplantation, transfusion of blood products and/or pregnancy [7].
Basic methods for detecting the presence of HLA antibodies are screening of patients' serum and crossmatch between recipients' serum and donor lymphocytes. The "gold standard" in an HLA antibody detection for many decades was a complement dependent cytotoxicity test (CDC) assay [8]. The knowledge of clinical relevance of HLA antibodies in organ transplantation has prompted the development of new techniques, since one of the major drawbacks of CDC method was its low sensitivity. By modifying cell-based assays, the highest sensitivity was achieved by flow cytometry, while in the 90s, solid phase assays (SPA) like Enzyme-Linked Immunosorbent Assay and, currently the most sensitive, Luminex technique have been introduced [9].

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Luminex technology became an important technique in HLA antibody screening, significantly reducing the use of CDC method. Some laboratories decided to exclude CDC method out of the HLA antibody screening and to rely solely on single antigen bead (SAB) assays [10].
The aim of this study was to assess median fluorescence intensity (MFI) cutoff values of HLA antibodies detected by Luminex only from those detected by both, Luminex and CDC assays in the light of possible replacement of CDC method by Luminex technique in the HLA antibody screening. In a clinical setting, antibodies positive by both methods (MFI values above cutoff) can predict positive CDC crossmatch result with, consequently, refusal of the kidney offer and prevention of the futile organ shipment in the recipient centre. In the case of antibodies that have MFI below the established cutoff and that are likely to be detected by Luminex only, we reassess criteria and parameters beside MFI that should be considered in the accurate and reliable identification of clinically relevant unacceptable HLA antigen mismatches (UAM).

Material and methods

This study is a retrospective analysis of HLA antibody screening results performed at the Tissue Typing Laboratory (TTL), Clinical Institute for Transfusion Medicine, Clinical Hospital Center (CHC) Rijeka, Croatia, from January 2012 to July 2019. Out of 1,729 pre-transplant sera, 1,665 native sera tested in parallel by two methods (CDC and Luminex) were analysed. In the study were not included 64 sera, as 54 were tested partially, by CDC or Luminex method only, and 10 were positive in patients not exposed to any immunizing event. Positivity was detected only by Luminex as an isolated finding and interpreted as false positive. Data were obtained from TTL and CHC documentation related to HLA antibody screening including worksheets, final interpretations of test results and informations about patients' clinical status and exposure to immunizing events.
In the TTL Rijeka, HLA antibody screening is performed quarterly at regular intervals, by the two methods in parallel-CDC and Luminex. Serum collected two to four weeks after the immunization event are also included in the regular screening scheme. CDC method is a three-step assay in which donor lymphocytes, with specific HLA antigens expressed on their surface, are incubated with patient serum. If recipient has donor-specific antibody, it will bind to the complementary antigen, forming an immunocomplex. By adding rabbit serum as a source of complement, the activation of complement components leads to formation of a membrane attack complex (MAC) that damages cell membrane. Vital dyes are used for visualization of dead lymphocyte cells making it visible under the microscope. The degree of lysed cells is expressed as the percentage of panel reactive antibody (%PRA) [11,12]. In our laboratory HLA antibody screening by CDC method is performed using unseparated T+B lymphocytes consisting of a panel of 50 cells, with and without dithiothreitol (DTT) addition in order to distinguish IgG from IgM antibodies. A serum with PRA>5% is considered positive.
Along to CDC method, HLA antibody detection is performed with Luminex technology that is based on the principles of flow cytometry using polystyrene microspheres (instead of cells) to which purified glycoproteins (antigens) HLA Class I and/or II are conjugated [15]. The basic principle of this method is that after incubation of beads with the patient's serum, the eventually present IgG antibodies bind to complementary bead-conjugated HLA antigens while the unbound antibodies are washed. Bound HLA antibodies are detected by use of a phycoerythrin (PE) labelled anti-human IgG antibody. Polystyrene microspheres contain two fluorochromes in different ratios making each set of beads unique in their spectral signature, allowing simultaneous detection of up to 100 different sets of beads. The Luminex fluorocytometer uses a system of two lasers-green (wavelength 532 nm) and red (wavelength 650 nm). After measuring signals by detectors, results are processed in a computer program [13]. The degree of fluorescence is expressed as mean fluorescence intensity. MFI value greater than 1,000 is considered as a positivity cut-off.
HLA antibody screening by Luminex technology in the TTL Rijeka is performed at two levels according to the guidelines of test manufacturer-Immucor GTI Diagnostics, Inc. (Waukesha, WI, USA; formerly: GenProbe). The first phase is a Lifecodes LifeScreen Deluxe (LMX) assay. It is a qualitative assay and the results are expressed as positive or negative depending on the presence or absence of HLA antibodies. In the case of a positive result, testing is continued with Lifecodes Single Antigen (LSA) I and/or II assays [1,14]. Once specificities have been established, SAB tests are performed once a year or after immunization event. Testing is carried out on a LABScan 200 Flow Analyzer (Luminex, Austin TX, USA). The results are analysed using the computer program Lifecodes® MatchIt software manufactured by Immucor GTI Diagnostics, Inc. (Waukesha, WI, USA).
All analysed sera were divided into four groups according to test results: CDC+LUM+ (positive test result by CDC and Luminex); CDC-LUM+ (negative test result by CDC and positive result by Luminex technique); CDC+LUM- (positive result only by CDC method and negative test result by Luminex) and CDC-LUM- (negative test result by both methods).
Furthermore, the results of 174 sera tested by CDC method and Lifecodes Single Antigen I (LSA I) kit were analysed and then compared between CDC+LUM+ and CDC-LUM+ groups. This comparison was limited by several factors. Firstly, CDC screening was performed with unseparated T+B lymphocytes so HLA Class II antibodies could not be detected by this method. In CDC positive sera, HLA-A and HLA-B antibody spe-

cificities were identified, however, specificities of antibodies against HLA-C molecules were difficult to interpret. Thus, comparing the results obtained by CDC and Luminex method for Class II and HLA-C antibodies would not be plausible given the aim of this study. Secondly, in the CDC+LUM- group HLA IgG antibodies were not confirmed by Luminex, so sera from groups CDC+LUM- and CDC-LUM- were not further analysed.

Statistical analysis

Frequency differences with respect to the method and HLA antibody screening results were calculated by the Hi-square test ($\chi 2$). If $\chi 2$ was significant, differences between the groups were checked by T-test for proportions. Numerical values are presented with median, 5th and 95th percentiles and range, as data were not distributed normally. The medians of maximum MFI values between the CDC+LUM+ and CDC-LUM+ groups were compared using the Mann-Whitney U test. The results are presented graphically by box and whisker diagrams.

Cutoff MFI values for the HLA Class I antibodies detected by CDC method and Luminex technique were determined by using receiver-operating characteristics (ROC) curve and cutoff analysis. In the range of limit value, antibodies having MFIs above cutoff will be detected by CDC method and Luminex, while antibodies with MFI values below cutoff will be detected by Luminex technique only. Statistical analyses were performed using MedCalc® v18.2.1 (©1993-2017, MedCalc Software bvva, Ostend, Belgium). All results with the level of $P \leq 0.05$ were considered statistically relevant.

Results

During the study period, 1,665 sera of 296 patients awaiting kidney transplantation were screened for HLA antibody presence by CDC method and Luminex technique in parallel. Almost half sera were antibody negative (n=867; 52.1%). Among positive sera, significantly more antibodies were detected by Luminex technique than by CDC method ($\chi 2$=58.95; P<0.001) (Table 1).

Table 1. Results of HLA antibody screening performed by the CDC method and Luminex technique

Methods of HLA antibody screening	LUM – N (%)	LUM + N (%)	Total N (%)
CDC -	867 (52.1)	467 (28.0)	1.334 (80.1)
CDC +	58 (3.5)	273 (16.4)	331 (19.9)
Total	925 (55.6)	740 (44.4)	1.665 (100)

Abbreviations: CDC, complement-dependent cytotoxicity; LUM, Luminex technique

The presence of antibodies against HLA Class I and/or II antibodies were analysed and compared between CDC+LUM+ and CDC-LUM+ groups. HLA Class I antibodies were only detected in twice as less sera as Class II antibodies (16.1% and 34.4%, respectively), while Class I/II antibodies were detected in almost half sera (49.5%). In total, a slightly more HLA Class II than Class I antibodies (56.1% vs. 43.9%) were detected in the pre-transplant patients' sera.

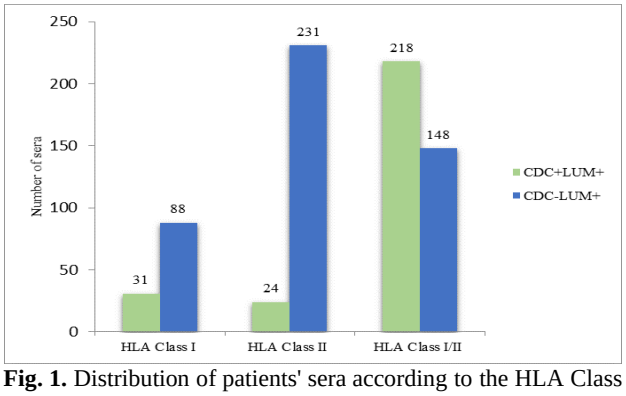

Fig. 1. Distribution of patients' sera according to the HLA Class I and/or II antibody specificity. Abbreviations: CDC, complement-dependent cytotoxicity; LUM, Luminex technique

In the CDC+LUM+ group, most sera (N=218; 79.9%) contained HLA Class I and II antibodies while in the CDC-LUM+ group, most sera (N=231; 49.5%) gave a positive result for HLA Class II antibodies only (Figure 1).

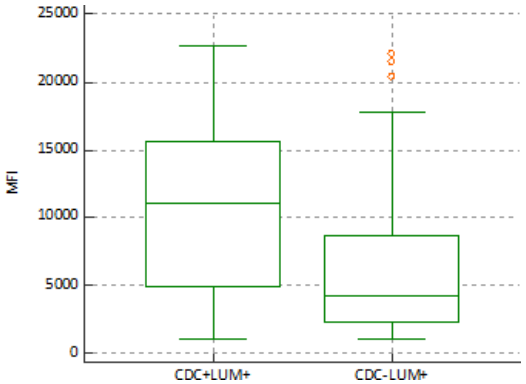

Fig.2. Comparison of median and interquartile range for the highest MFI values from SAB assays for HLA Class I antibodies between CDC+LUM+ and CDC-LUM+ groups (the plot represent from bottom: minimum, first quartile, median value, third quartile, maximum value and outliers). Abbreviations: CDC, complement-dependent cytotoxicity; LUM, Luminex technique; MFI, mean fluorescence intensity; SAB, single antigen bead

For Class I antibodies, median of the highest MFI values between groups CDC+LUM+ and CDC-LUM+ showed statistically higher MFI value in CDC+LUM+ group (median=11,022) than in CDC-LUM+ group (median= 4,293), (P<0.001) (Figure 2).

Medians of the highest MFI values from antibodies against HLA-A, HLA-B and HLA-C molecules were compared. While the results for HLA-A and HLA-B antibodies were similar, almost twice as low was median of MFI for HLA-C antibody specificities (Table 2).

Table 3. shows the evaluation of pre-transplant HLA antibody MFI values separately by HLA-A and HLA-B loci in CDC+LUM+ and CDC-LUM+ groups. MFI values of both, HLA-A and HLA-B antibodies were statistically higher in group of sera with antibodies detected by CDC and Luminex methods in comparison to the sera in which antibodies were detected by Luminex only. In the CDC-LUM+ group, MFI values of antibodies against HLA-A*24:02, A*03:01, A*36:01, A*32:01, A*02:03 and HLA-B*07:02, B*27:05, B*27:08, B*07:02, B*08:01, B*81:01, B*56:01, B*44:03, B*37:01 were higher than the median MFI value of antibodies of respective locus in the CDC+LUM+ group (listed in Table 3.).

Table 2. Medians of the highest MFI values for antibodies against HLA-A, HLA-B, and HLA-C molecules

HLA locus	Median	Range (min-max)	(5^{th} – 95^{th} percentile)
A	8.559	1.008-22.215	1.493-20.488
B	9.402	1.033-22.493	1.260-20.621
C	4.018	1.138-22.611	1.317-18.597

Abbreviation: MFI, mean fluorescence intensity

Table 3. Comparison of the median MFI values for HLA-A, HLA-B and HLA-C antibody specificities identified in pre-transplant sera between CDC+LUM+ and CDC-LUM+ groups

	CDC+LUM+ MFI				CDC-LUM+ MFI				Mann-Whitney U test	
Locus	**N**	**Median**	**Range (min-max)**	**(5^{th}-95^{th} percentile)**	**N**	**Median**	**Range (min-max)**	**(5^{th} – 95^{th} percentile)**	**U**	***P***
A	79	11.412	1.704-22.215	1.954-21.046	58	4.122	1.008-21.477	1.325-3.975	906	<0.001
B	79	12.802	1.078-22.493	1.453-21.429	62	5.927	1.033-22.057	1.167-7.678	1.433	<0.001

Abbreviations: CDC, complement-dependent cytotoxicity; LUM, Luminex technique; MFI, mean fluorescence intensity

Cutoff MFI value for HLA antibodies Class I was determined by the Receiver operating characteristic (ROC) curves that represent the graphical relationship between sensitivity and specificity of Luminex technique versus CDC method. Antibodies having MFIs above cutoff will be detected by CDC method and Luminex, while antibodies with MFI values below cutoff will be detected by Luminex technique only. For HLA Class I antibodies cutoff MFI value is 9,204. Area under the ROC curve is 0.720 (P < 0.001).

Discussion

HLA antibody screening and identification is an important part of an immunogenetic assessment of potential organ transplant recipients and also one of the most challenging procedures in the TTL. Low sensitivity of the CDC method in HLA antibody detection encouraged development of new techniques and methods among which microbead based platforms such as Luminex, emerged as the most sensitive ones [15]. Higher sensitivity of Luminex technology in HLA antibody detection reported by many researches was also confirmed by this study [10,16,17]. Presence of antibodies in significantly higher number of sera was demonstrated by Luminex technique than by CDC method (44.4% vs. 19.9%; P < 0.001).

Based on the method used, the prevalence of HLA Class I/II antibodies between two groups of sera (CDC+LUM+ and CDC-LUM+) was compared. In the CDC+LUM+ group, antibodies were mostly HLA Class I and II specificities, while in the CDC-LUM+ group most sera contained HLA Class II antibodies. HLA Class I antibodies react with B and T lymphocytes while HLA Class II antibodies react with B cells expressing Class II molecules. Without T and B lymphocyte separation, HLA Class II antibodies cannot be entirely detected by the CDC, so the result of their highest proportion in sera with antibodies detected by Luminex only (CDC-LUM+) was as expected (90.6%). Elegant detection of HLA Class II antibodies which in CDC assay require lymphocyte separation, is one of the great advantages of Luminex technique. Clinically, the highest risk of organ rejection and graft loss have patients with both HLA Class I and II antibodies, which are in this study mostly present in the CDC+LUM+ group [18,19].

The output of Luminex assay is a semiquantitative measure referred as the mean fluorescence intensity (MFI) [12]. According to the MFI values, Class I antibodies detected by CDC and Luminex methods have a significantly higher MFI value than HLA antibodies proven by the Luminex technique alone (P<0.001) which has also confirmed higher sensitivity of Luminex technique.

Analysing each HLA Class I locus separately, median MFI values of HLA-A and HLA-B antibodies are twice as high as MFI for HLA-C locus. Peripheral blood lym-

phocytes express HLA-A and HLA-B proteins at similar levels, which are several times higher than HLA-C molecule expression [20]. HLA-C locus has relatively reduced diversity with regard to HLA-A and HLA-B loci [21]. Therefore, the mentioned differences in MFI values, so as difficult interpretation in CDC assay, may be explained by the influence of cell surface expression and polymorphism level of HLA molecules on the occurrence and strength of the antibody.

According to literature, antibodies with MFI value above 7.000-8.000 will give a positive reaction in CDC assay [22,23]. In this study, MFI cutoff value for HLA Class I antibodies was above 9.204, which is consistent with literature data. The median MFI value for HLA-A was 8.559, for HLA-B 9.402. The highest MFI median value was determined for HLA-B antibody specificities as the HLA-B locus is the most polymorphic, it has over 3.000 allelic variants, and the highest number of epitopes are shared by HLA-B locus antigens in comparison to the HLA-A (or HLA-C antigens) [24-26].

The antibody-reactivity pattern against epitope is the basic setting of determining HLA matching at the epitope-level which became a recent topic in organ transplantation. If the recipient and donor share the same epitopes, no HLA antibody will be generated. Although each HLA molecule has a unique set of epitopes, some of them may be present on multiple antigens of different specificities [27]. Today, there are computer programs that determine epitope-level matching identifying mismatched HLA molecules that will not lead to the generation of antibodies in the organ recipient (HLA Matchmaker, Predicted Indirectly ReCognizable HLA Epitopes; PIRCHE, etc.), thereby achieving better match between recipient and organ donor [28]. Although the concept is promising, there are some issues to be addressed before its integration into organ allocation programs. The most important is a system for defining a complete epitope spectrum of all HLA antigens and determination of the biological significance as well as immunogenicity of all eplets [29].

One of the advantages of implementation of Luminex technique into patients' screening at the TTL Rijeka is the increase in organ allocation of highly immunized patients (PRA>85%), while UAMs in such patients are difficult and sometimes impossible to characterize by CDC technique. Also, given the limitation of CDC method in detection of HLA Class II antibodies, the use of Luminex technique has made a significant shift in the identification of lymphocytotoxic HLA antibodies, which ultimately resulted in better immune matching when selecting organs for transplantation. Development of Luminex technology and SAB assays enabled implementation of the "virtual crossmatch" which allows exclusion of donors with UAM based on HLA antibody specificities defined by solid phase assay [30]. This reduces the time of cold ischemia, which in cadaveric transplantation is one of the most important factors affecting graft survival [31].

The higher sensitivity of Luminex technique in detection of HLA antibodies is of great advantage on the one hand, but it raises many questions and concerns on the other. The strong limitation of Luminex technique is certainly the impossibility to standardize the universal cutoff MFI value that would ensure the detection of only clinically relevant HLA antibodies in the patient's serum. The most common MFI value taken as a positivity cutoff in the literature is MFI>1.000 (the value also used in this study), although each laboratory sets its own limit values based on their laboratory and clinical results [12,32]. MFI values depend on the amount (titre) of antibody, affinity for the antigen, antigen density on the beads that varies within same assay and between manufacturers, non-specific binding of serum components to microspheres and on technical performance of the test, which varies between laboratories and operators [17,21]. Artificially antigen binding to a bead may cause conformational change of protein leading to formation of denatured HLA molecules. The result may be exposure of cryptic epitopes which are normally inaccessible to an antibody, formation of neoepitopes or concealment of immunologically relevant epitopes [17]. In this study, in sera without panel reactive antibodies, some antibody specificities expressed high MFI. This finding raised the question of whether these antibodies were complementing binding? If not, what role do they have in transplant outcome given the high MFI value? Recent modifications of SAB assays allow detection of HLA antibodies that have the capacity to bind C1q or C3d as indicators of complement activation. Published studies are inconsistent regarding clinical relevance of complement fixing characteristics of IgG DSA detected by SAB [4,19,33,34].

The role of HLA antibodies detected by Luminex technique only in the graft rejection has not yet been fully elucidated. Although some studies have shown their harmfulness in terms of increased risk of rejection or adverse effect on graft survival, other studies have failed to confirm this [35-37]. Thus, HLA antibodies detected exclusively by Luminex technique (without confirmation by CDC method) are more considered to be a relative rather than an absolute contraindication for transplantation [4,24,38]. The experience of our laboratory in patients' serum screening with Luminex technique is relatively short. In a study that monitored the outcome of 109 kidney transplants performed at the CHC Rijeka from 2012 to the end of 2015, no significant effect of HLA antibodies detected by Luminex method on one-year graft survival was demonstrated (unpublished observation). Also, of all transplants performed at the CHC Rijeka in the period followed by this study, only three recipients were transplanted across donor specific antibodies. Therefore, views on the

clinical relevance of antibodies detected by Luminex technique in our center are largely based on literature data. Although the group of CDC+LUM- sera were not analysed, these results (found in 3.5% of sera) may have an important impact on final transplant decision giving the possibility of positive crossmatch with a potential donor. Possible reasons may be false results (prozone effect, technical reasons), IgM antibodies, non-HLA antibodies, previous treatment by rituximab or antithymocyte globulin, etc. Some of those doubts can be resolved only by comparing the results of Luminex and CDC assays.

There are some limitations of this study. Most references cited for possible MFI cutoffs present results produced by kits from another manufacturer. Comparative analysis showed they have a similar, but nonidentical, ability to detect HLA antibodies [39]. Over the tested period a variety of lots have been used for Luminex testing so the possible variations in MFI values due to new lots were not considered. False negative Luminex results due to potential interaction of complement with IgG HLA antibodies as the prozone effect could not therefore be avoided in some cases as serum pretreatment or dilution were not performed.

Conclusions

Implementation of advanced technologies brought new insights in the patients' immunization status and the influence of HLA antibodies on organ transplantation outcome. Today, Luminex technology is the most sensitive SPA in HLA antibody detection, accompanied by numerous advantages, but not without challenges that need to be overcome, most notably regarding test performance and data interpretation. MFI values are often used as quantitative assessment of antibody strength and used to monitor patients' clinical status. Though it is a useful tool, there are many more factors to consider in test interpretation. Consequently, MFI values of HLA antibodies represent just a tip of an iceberg and we can partially rely on it. Taking the benefits and limitations of Luminex technique into consideration, according to our results, determination of clinically relevant antibodies pre-, as well as post-transplantation cannot be based on SPA only. Sensitive Luminex technique must be combined with CDC method and the final interpretation of results needs to be based on patient's immunological history and clinical status requiring close collaboration between clinicians and tissue typers.

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Case report

Takotsubo Cardiomyopathy-Unusual Cause of Chest Pain in Patients on Hemodialysis

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Abstract

Takotsubo cardiomyopathy is rather rare entity among patients on hemodialysis with very few cases reported in the literature to date. This syndrome is characterized with transient left ventricular dysfunction and apical motion abnormality with the absence of coronary artery disease and by clinical features it is mimicking acute coronary syndrome. It almost always follows high level of emotional or physical stress. Although rarely described, it should be considered as a differential diagnosis in patients on hemodialysis presenting with the symptoms of acute coronary syndrome, especially in the setting of acute stress. It is possible the condition is underdiagnosed giving the wide range of its clinical presentations.
To the best of our knowledge there are 11 cases and here we present 12th case and the review of the literature.

Keywords: Takotsubo cardiomyopathy, hemodialysis, physical stress

Introduction

Takotsubo cardiomyopathy is characterized by transient left ventricular dysfunction and apical motion abnormality with the absence of coronary artery disease. Its clinical presentation resembles acute coronary syndrome [1]. It is rather rare entity among patients on hemodialysis with very few cases reported in the literature to date [2]. To the best of our knowledge there are 11 cases and here we present 12th case and the review of the literature.

Case report

At the age of 61 patient with end stage renal disease due to polycystic kidney disease started peritoneal dialysis treatment. After 11 months of treatment, she developed umbilical hernia and was switched to hemodialysis. In the mean time she started workup to be listed for kidney transplantation. Due to repeated urinary tract infections followed by hemathuria as well as the size of her polycystic kidneys right sided nephrectomy was performed followed by left sided nephrectomy after 9 months. After 14 months on hemodialysis she presented in emergency room with left sided chest pain and general weakness one day after her regular dialysis treatment. Initial ECG showed 2 mm ST elevation in V2-V6 leads (Figure 1) with elevated cardioselective enzymes. Acute coronary syndrome was suspected and coronary angiography followed revealing no significant obstruction of coronary arteries. Echocardiography revealed akinesia of all apical myocardial segments and significant systolic dysfunction (LV EF=30 %) (Figure 2). She was diagnosed with Takotsubo cardiomyopathy and treated with β-blocker, ACEi and ASK. She remained stable, with no chest pain, no significant arrythmias recorded and follow up echocardiography performed on day 4 after admission showed almost complete systolic function recovery (LV EF=55%) with minimal apical akinesia, and ECG finding showed no abnormalities. Further follow up echocardiogram after 1 month showed normal systolic function with no apical akinesia whatsoever. Seventeen months later she was successfully transplanted with excellent outcome. This is the 12th case of Takotsubo cardiomyopathy in patients on hemodialysis followed by complete recovery and successful kidney transplantation.

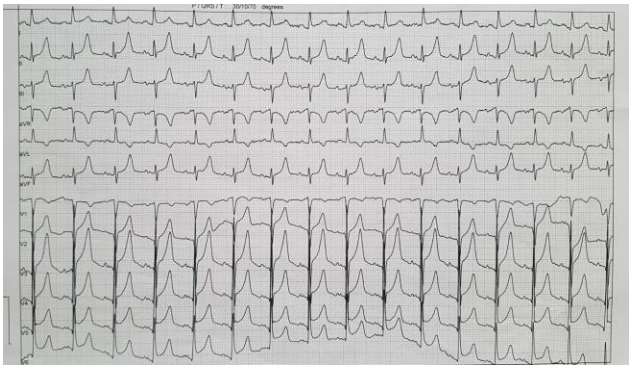

Fig. 1. ECG finding finding at the time of diagnosis

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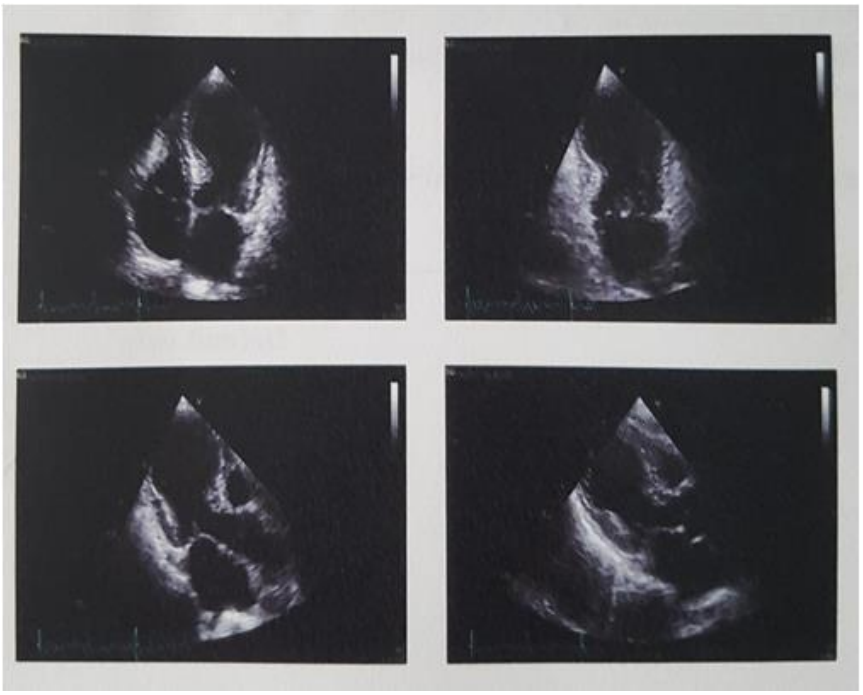

Fig. 2. ECHO finding at the time of diagnosis

Discussion

Takotsubo cardiomyopathy is syndrome characterized with clinical signs mimicking acute coronary syndrom, typical transient left ventricular apical ballooning with left ventricular dysfunction, ECG changes by means of ST segment elevation or T-vave inversion and elevated levels of cardioselectove enzymes without any angiographic evidence of obstructive coronary artery disease (3). It almost always follows high level of emotional or physical stress and predominantly affects postmenopausal women [4,5]. The term takotsubo has been introduced in 1990. It is the Japanese term for ancient traps for octopus (tako=octopus, tsubo=a pot) introduced to describe typical left ventricular silhouette in patients presenting with the syndrome [6,7]. Giving all this features the syndrome is also known as broken heart syndrome, stress induced cardiomyopathy and transient left apical ballooning syndrome [8-10]. The prevalence has been reported to be 1-2% of all patients presenting with ACS and it has been shown to increase with the increased awareness and access to early invasive coronary angiography [11].

The exact pathophysiological mechanism remains undetermined but it has been postulated that sympathetic hyperactivity with elevated catecholamine levels play key role in pathogenesis of Takotsubo car-

Table 1. Data from patients reported into the literature

Author	Sex	Age	Symptom	Inducing factor	HD (years)	Outcome
Tsigaridas	f	55	Chest pain and substernal heaviness during dialysis	N/A	2	recovered
Bhogal	f	75	Chest pressure and heaviness during HD session	Family illness	5	recovered
Garea Garcia-Malvar	f	55	Chest pain-middle chest after HD session	Seizure	N/A	recovered
Shin 1	f	54	Dyspnoea	Pneumonia	N/A	recovered
Shin 2	f	68	Dyspnoea	Infectious colitis	N/A	recovered
Shin 3	m	N/A	Dyspnoea	Pneumonia	N/A	recovered
Muratsu 1	f	63	No symptoms	Seizure	32	recovered
Muratsu 2	f	59	Fatigue	Family illness	12	recovered
Takemoto	f	61	Chest pain and dyspnoea during HD session	Cervical spondylosis surgery	20	recovered
Fukui	f	84	Chest discomfort	Abrupt smoking cessation after 60 y	2	recovered
Kusaba	m	65	left shoulder pain	Headache and fever-MRSA meningitis	9	recovered
Our patient	f	63	Chest pain	Family situation	1	recovered

diomyopathy [12,13]. Patients with chronic kidney disease and on hemodialysis are at increased risk for cardiovascular morbidity and mortality, predominantly due to coronary artery disease [14]. In addition, sympathetic activity and catecholamine levels are increased in these patients [15,16]. Furthermore, patients on hemodialysis exhibit significant level of psychological stress as a consequence of physiological changes due to their illness that significantly affects their lifestyle and quality of life, and many of them also exhibit anxious or depressive symptoms [17,18]. Thus, patients on hemodialysis could be a group of patients with increased risk for Takotsubo cardiomyopathy in a setting of additional acute physical and/or emotional stress [2,19].

To the best of our knowledge there are 11 cases of Takotsubo cardiomyopathy in patients on maintenance hemodialysis in the literature to date. Vast majority of patients, including our case were female [10-12]. The age ranged from 54 to 84 years. In most of the patients there was physical or emotional preceding stressful event. Most of the patients presented with symptoms: half of them presented with chest pain, 3 patients had dyspnoea, 1 patient presented with severe left shoulder pain while only 2 patients were asymptomatic. All patients recovered completely [20-27] (Table 1).

Conclusion

Takotsubo cardiomyopathy should be considered as a differential diagnosis in patients on hemodialysis presenting with the symptoms of acute coronary syndrome, especially in the setting of acute stress. It is possible the condition is underdiagnosed giving the wide range of its clinical presentations. In this group of patients with otherwise high cardiovascular mortality it has favourable prognosis.

Conflict of interest statement. None declared.

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Case report

Radial Artery Aneurysm Following Coronarography in Hemodialysis Patient: A Challenging Complication of Catheterization

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Abstract

Percutaneous radial arterial cannulation is routinely used in cardiac surgery and in hemodynamic monitoring in intensive care units. As for coronarography, it is now considered superior to femoral artery access as it has lesser vascular complications. Nevertheless, this procedure can have many consequences, though aneurysm is an exceptional one. Its occurrence is highly correlated with multiple risk factors such as advanced age, longer duration of catheterization, hospitalization duration, and infection with Staphylococcus aureus. We report an uncommon case of radial artery aneurysm following coronarography in hemodialysis patient for which he underwent successful surgery.

Keywords: radial artery, aneurysm, coronarography, hemodialysis, catheterization

Introduction

The transradial approach to vascular access in interventional cardiology is now commonly used in different parts of the world [1]. It reduces vascular complications and improves patient comfort. However, it is not without side effects. Its main complications may include hematoma at the puncture site (10%), arterial thrombosis (20 to 38%) and infection at the puncture site (0.4-4%) [2] Arteriovenous fistula, dissection or aneurysm are exceptional complications, but may lead to dramatic consequences. [3,5] We report a case of a right radial artery aneurysm following coronary angiography in a patient on chronic hemodialysis.

Case report

An 80-year-old patient with type 2 diabetes with end-stage chronic renal failure receiving hemodialysis treatment for the duration of one year. She had a left radial fistula. The patient presented at the emergency department with acute coronary syndrome. Coronary angiography was performed using the right radial approach. A compression bandage was systematically put in place for 12 hours. On the tenth day, we observed a minimal pulsatile mass and an ulcerative lesion at the

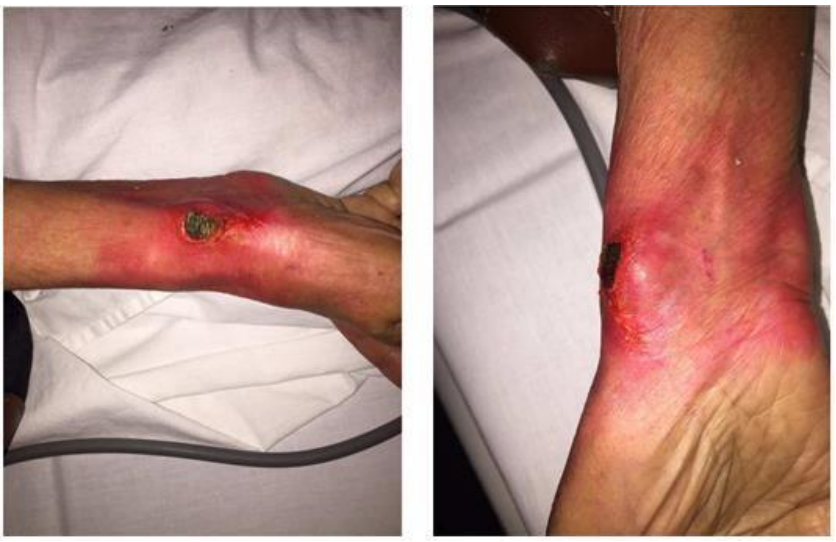

Fig. 1. Pre-operative view of the radial aneurysm, pulsatile mass of 2,5 cm.

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puncture site of the radial artery. Local and systemic antistaphylococcal antibiotics were administered to the patient. Ultrasonography was performed and showed an aneurysm 8 mm in diameter (radial artery was 3 mm in diameter). No intra-aneurysmal thrombus was observed. The distal artery was patent with good Doppler flow quality. As no more complications were present, therapeutic abstinence with a close clinical follow-up was recommended. The mass grew slowly over time, and two months following coronarography, physical examination revealed a soft pulsating mass of 6 cm in diameter with no sign of ischemia (Figure 1). Doppler ultrasound showed no interruption in the radial artery flow. The aneurysm was partially thrombosed; however, the deep and superficial palmar arches were patent. Based on aneurysm size and the high risk of embolization, the medical team and the patient elected to undergo surgical treatment. The aneurysm was exposed and then resected. Intraoperatively, an intra-aneurysm thrombus was observed. On direct examination and in culture, no germs were detected on the aneurysmal shell, the thrombus nor the radial artery. One month after the intervention, the patient presented no complications.

Discussion

The radial artery approach to vascular access is increasingly preferred to the femoral one in coronary angiography [6]. The complications of this catheterization are not uncommon, though they are often benign. Ischemic are rare [6,7]. Arteriovenous fistulas and arterial dissections are exceptional accidents. The post-catheterization radial aneurysm is very rare as well (0.05% of radial catheterizations) and is frequently associated with vascular site infection with staphylococcus aureus, whether it is local or general [4]. Other predisposing factors include age and multiple puncture attempts [4]. In our case report, two factors were noted: a general vascular pathology [8], and a local staphylococcus aureus infection [4]. The latter is a highlighted factor in a significant study: nine of the ten aneurysms reported in the series presented with staphylococcal sepsis. [4] If the infection is evident on direct examination or culture in the majority of cases, they may be no laboratory evidence in many others: samples, either endoluminal material or aneurysmal shell were negative in four out of nine general infections for the series of Falk and al [4]., as well as in our case. Consequently, the aseptic nature of the thrombus or blister is likely not to rule out an infectious etiology, most regularly caused by Staphylococcus aureus. Despite their rarity, post-catheterization aneurysms may have dramatic complications, which may comprise ischemic embolus, rupture, and even gangrene. On account of these life-threatening consequences, these aneurysms must be acknowledged and treated, ideally by resection, or at least by exclusion. In the case of our patient, the aneurysm was resected with no postoperative complications. This case report rises an important issue regarding radial catheterization in hemodialysis patients, whose vascular capital is utterly valuable. Albeit its superiority to the femoral access in coronarography, the radial artery approach in hemodialysis patient must be assessed thoroughly, as it may, if complicated, compromise potential confection of arteriovenous fistulas for vascular access. For instance, if our patient, who already suffers from severe diabetic angiopathy, presented with malfunction of her left radial fistula, with insufficiency of the vascular capital in the left upper limb, creating an arteriovenous fistula using the right radial artery would be very challenging following the multiple interventions it underwent.

Conclusion

Radial artery aneurysm is a rare post-catheterization complication [8]. It is often linked to Staphylococcus infection and advanced age. However, despite its superiority to the femoral artery in terms of general vascular complications, its use in hemodialysis patients should be questioned as it may compromise the creation of potential arteriovenous fistulas whenever necessary.

Conflict of interest statement. None declared.

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Case report

Urinalysis May Solve the Diagnostic Dilemma in Vasculitis with Pulmonary Involvement Mimicking Tuberculosis

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Abstract

Introduction. Tuberculosis is an endemic disease in developing countries and more prevalent than vasculitis. Patients with vasculitis may be intermingled due to similar findings at the time of presentation. Herein, we presented three cases of vasculitis emerging with rapidly progressive glomerulonephritis referred from the department of pulmonary diseases of another hospital within the same month.

Cases. All of the patients presented had constitutional symptoms. Two of them were in the second month of antituberculosis treatment while the other had only nonspecific antibiotherapy. Two patients had no urine analysis during the period spent in the department of pulmonary diseases. All were referred to our hospital due to the need for hemodialysis at that time proteinuria and hematuria were detected in urine analysis of the three patients. With the proper diagnostic and therapeutic approach, two of the patients became free of dialysis, and pulmonary symptoms and signs recovered in all three.

Conclusion. Vasculitis with lung involvement should be in the list of differential diagnoses of the cases with clinical and radiological findings of tuberculosis without microbiological evidence. Therefore, the addition of urinalysis to the tuberculosis guidelines may be suggested solving this diagnostic problem.

Keywords: tuberculosis, vasculitis, lung, urine examination

Introduction

Pulmonary tuberculosis and vasculitis with pulmonary involvement may be intermingled due to similar findings at the time of presentation. As tuberculosis is more prevalent than vasculitis in some countries, patients with vasculitis may be missed. Herein, we presented three cases of vasculitis emerging with rapidly progressive glomerulonephritis referred from the department of pulmonary diseases of another hospital within the same month.

Case-1. 56 years old male admitted to the department of pulmonary diseases due to fatigue, lack of appetite, and weight loss two months ago. He had bronchoscopic examination after finding bilateral diffuse micronodular opacities resembling miliary tuberculosis on chest X-ray and computed tomography (CT). Acid-fast bacilli were not detected in bronchoalveolar lavage fluid. As the pathological examination of the transbronchial biopsy revealed granulomatous lesions, he was started antituberculosis treatment. He gained weight with improved appetite and fatigue within the first month of treatment. But the presenting symptoms reappeared in the following weeks. Meanwhile, the culture of the bronchoalveolar lavage and sputum ended with no sign of tuberculosis. There was no elevation in serum transaminase levels during follow up. He was referred to our clinic due to elevated serum urea (260 mg/dl) and creatinine (16.5 mg/dl) levels detected during evaluation for severe fatigue, nausea, and vomiting. Hemodialysis treatment through a jugular catheter was started together with etiological evaluation. The chest X-ray was normal. The transbronchial biopsy specimen revision revealed neither granuloma nor findings consistent with vasculitis. Urine analysis showed proteinuria and the urine sediment revealed many erythrocytes per high power field. Cytoplasmic anti-neutrophil cytoplasmic antibody (c-ANCA) was negative while perinuclear anti-neutrophil antibody (p-ANCA) and anti-glomerular basement membrane antibody (Anti-GBM) were positive. Renal biopsy revealed fibrocellular crescents in six and cellular crescents in six of the 15 glomeruli detected in the biopsy sample. Immune fluorescence examination showed diffuse IgG staining on the glomerular basement membrane which was a diagnostic finding for anti-glomerular basement membrane disease. He was treated with pulse methylprednisolone intravenously followed by oral treatment, pulse intravenous cyclophos-

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phamide, and 15 sessions of plasmapheresis. On follow-up examinations, p-ANCA and anti-GBM turned negative but renal functions did not improve and the patient remained hemodialysis dependent.

Case-2. 55-year-old male was referred to the department of pulmonary diseases due to diffuse interstitial pattern on chest CT performed due to symptoms of fatigue, weakness, and weight loss. In the patient's history, he has been on antituberculosis treatment for six months in 2005. After the direct examination of sputum that resulted negative for acid-fast bacilli, he had a bronchoscopic examination which did not reveal any sign of consistent with malignancy or tuberculosis. The patient was referred to our clinic after the realization of increasing serum creatinine levels from 1.7 mg/dl to 4.7 mg/dl during hospitalization. He had four sessions of hemodialysis due to uremic symptoms and clinically overt hypervolemia. Urine analysis revealed proteinuria and microscopic hematuria. p-ANCA was found positive while c-ANCA was negative. A kidney biopsy could not be performed due to the lack of compliance of the patient. He was thought to have microscopic polyangiitis (MPA) with the clinical findings. Intravenous pulse corticosteroid and cyclophosphamide treatments were started concomitant with 10 sessions of plasmapheresis. After the onset of immunosuppressive treatment, the need for hemodialysis disappeared with the serum creatinine level decreasing to about 1.5 mg/dl.

Case-3. A 41 years-old woman was hospitalized in the department of pulmonary diseases two months ago due to the symptoms of coughing, bloody sputum, and fatigue. A cavitary lesion of 1.5 cm on the lower lobe of the left lung, and an area of infiltration involving cavitary lesion on the upper lobe of the left lung were detected on chest CT. Acid-fast bacilli were not detected on the direct examination of the sputum and bronchoalveolar lavage. Antituberculosis treatment was initiated due to granulomatous lesions found in the examination of the transbronchial biopsy specimen. She was referred to the department of nephrology due to increased intensity of hemoptysis which turned massive and elevated serum levels of urea (148mg/dl) and creatinine (5.9 mg/dl). Urine analysis revealed mild proteinuria and microscopic hematuria. c-ANCA was found positive. Erythrocyte transfusion was needed due to deep anemia resulting from massive hemoptysis. With the clinical diagnosis of granulomatosis with polyangiitis (GPA), she was given pulsed methylprednisolone and cyclophosphamide together with 12 sessions of plasma exchange using fresh frozen plasma. Hemoptysis disappeared following the institution of plasmapheresis. A renal biopsy was performed after the patient has been stabilized clinically. There were 13 fibrous-fibrocellular crescents among the 21 glomeruli present in the biopsy sample. Global collapse and sclerosis were observed in nine of the glomeruli besides focal tubular atrophy and hyalinization and hyperplasia of the arterioles. Renal functions recovered following treatment with createnine level decreasing to 1.4 mg/dl.

Discussion

Pauci-immune glomerulonephritides, namely GPA, MPA, and Churg-Strauss syndrome (CSS), are most frequent in the fifth to seventh decade of life although they can be seen at any age. They are more frequent in males. Constitutional symptoms like fatigue, anorexia, sweating, and weight loss are common to almost all patients [1]. Goodpasture's disease is characterized by an acute presentation with hemoptysis in the absence of systemic findings except for anemia. Constitutional symptoms are more common in the presence of ANCA positivity together with Goodpasture disease as in the first case presented [2]. Hemoptysis is an ordinary finding besides these symptoms. Patchy infiltrations, nodular and cavitary lesions may be detected radiologically in GPA and CSS due to necrotizing granulomatous inflammation. Diffuse alveolar pattern, consolidation, ground-glass appearance, and increased bronchovascular images are more common radiological findings in MPA and Goodpasture's disease characterized by pulmonary capillaritis [1,3,4]. Diagnosis of patients with ANCA-associated glomerulonephritides may be confused with tuberculosis due to these common constitutional symptoms, hemoptysis and similar radiological findings. In countries with high prevalence of tuberculosis, these findings may be interpreted as tuberculosis usually. The lower identification rate of acid-fast bacilli on direct examination and culture methods, as 60% and 33.5%, respectively in cases of pulmonary tuberculosis may be another contributing factor for this approach [5]. All our patients presented had constitutional symptoms (Table 1). Two of them were in the second month of antituberculosis treatment while the other had only nonspecific antibiotherapy. They had no urine analysis before starting antituberculosis treatment. All were referred to our hospital due to the need for hemodialysis and at that time proteinuria and hematuria were detected in urine analysis of the three patients. With the proper diagnostic and therapeutic approach, two of the patients became free of dialysis, and pulmonary symptoms and signs recovered in all three. World Health Organization reported in the Global Tuberculosis Report 2017 that tuberculosis incidence in Turkey in 2016 was 18 per 100.000 population [6]. The prevalence of tuberculosis in Turkey seems to decrease. According to the report of the Turkish Ministry of Health in the 2018, the incidence of tuberculosis decreased from 29,4 per 100.000 population in 2005 to 15,3 per 100.000 in 2016 [6]. Although the prevalence of vasculitides in Turkey is not known, the prevalence rates for MPA, GPA, and CSS in Europe have been

Table. Summary of the clinical and laboratory characteristic findings of the patients

	CASE-1	**CASE-2**	**CASE-3**
Age/Gender	56/male	55/male	41/female
Symptoms	Fatigue, lack of appetite, weight loss	Fatigue, weight loss	Lack of appetite, massive hemoptysis
Laboratory findings	Urea: 260 mg/dl, Creatinine: 16 mg/dl Urine analysis: Protein (++), erythrocyte (+++)	Urea: 90 mg/dl, Creatinine: 4,6 mg/dl Urine analysis: Protein (+), erythrocyte (++)	Urea:148 mg/dl, Creatinine: 5,9 mg/dl Urine analysis: Protein (+), erythrocyte (+++)
Chest CT	Bilateral diffuse micronodular images resembling miliary involvement	Bilateral interstitial infiltrates on lung parenchyma	Cavitary lesion on the lower lobe, infiltration involving cavitary area on the upper lobe of the left lung
Serology	pANCA: (+) cANCA: (-) Anti-GBM: (+)	pANCA: (+) cANCA: (-) Anti-GBM: (-)	pANCA: (-) cANCA: (+) Anti-GBM: (-)
Treatment	Pulse methylprednisolone and cyclophosphamide, 15 sessions of therapeutic plasma exchange	Pulse methylprednisolone and cyclophosphamide, 10 sessions of therapeutic plasma exchange	Pulse methylprednisolone and cyclophosphamide, 12 sessions of therapeutic plasma exchange
Dialysis	Remained hemodialysis dependent	Four sessions	Three sessions
Last laboratory findings	On hemodialysis program	Urea: 66mg/dl, Creatinine:1,4mg/dl Urine analysis: Protein (-), erythrocyte (-)	Urea:72mg/dl, Creatinine:1,4mg/dl Urine analysis: Protein (-), erythrocyte (-)

reported to be 2.5/100.000, 2.5/100.000 and 1/100.000, respectively [1]. The sum of these values makes 6/100.000 and this is not so far from the incidence rate of tuberculosis (15/100.000). Besides, Goodpasture disease with an incidence of 1/1.000.000 should not be forgotten [2]. So, vasculitic syndromes should always be in the list of differential diagnoses considering above mentioned clinical and radiological findings.

In cases of clinical and radiological findings without microbiological evidence of tuberculosis, the finding of active urine sediment and decreased renal functions should be alarming to the physician. Every patient without a definite diagnosis of tuberculosis may have renal function tests at least including urine analysis before anti-tuberculosis treatment in countries those endemic for tuberculosis. Urine analysis is a cheap and common diagnostic method all over the world. Guideline of the diagnosis and treatment of tuberculosis reported by the Turkish Ministry of Health in 2019 and treatment of tuberculosis guideline of The American Thoracic Society, Centers for Disease Control and Prevention, and Infectious Diseases Society of America jointly in 2016 recommended routine control of transaminase and creatinine levels, but not urine analysis [7,8]. Similarly, WHO and European Tuberculosis Guideline also did not mention any suggestions on the topic [9-11]. Besides the overlap between clinical and radiological findings at the time of presentation, it should be kept in mind that rifampicin and isoniazid may trigger vasculitis. Hence renal function tests and urine analysis should be performed at both onsets of therapy and also in case of any deterioration in the clinical status of the patient.

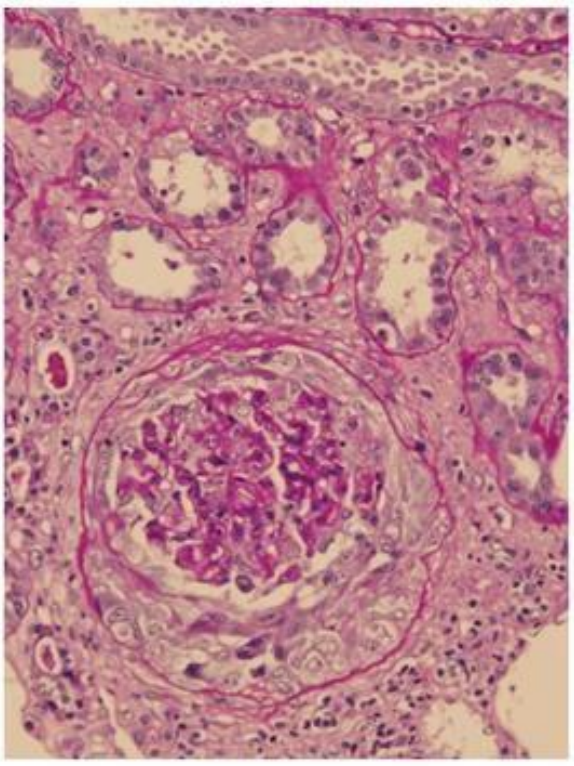

Fig. 1a. Cellular crescent formation in Bowman space, collapse of the glomerular tuft and karyorrhexis (HE 100x)

Even in case of need for hemodialysis at the time of diagnosis, with combined treatment (corticosteroids, cyclophosphamide and therapeutic plasma exchange) renal functions may recover as in two of the cases presented. But the improvement in renal functions is not

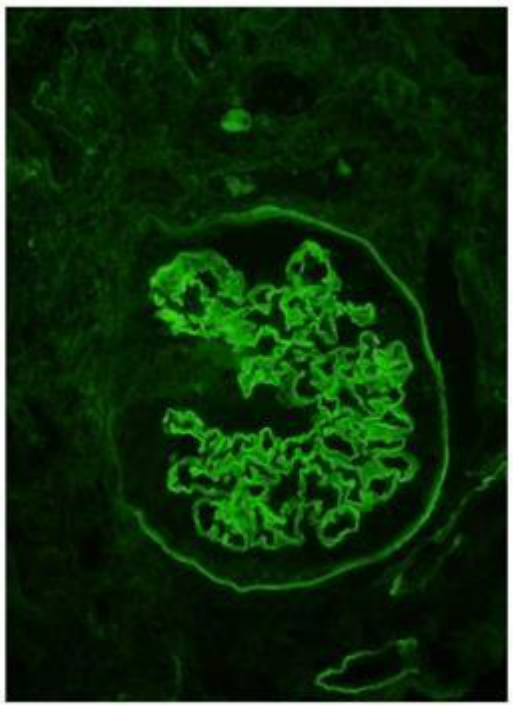

Fig. 1b. Diffuse linear staining of the glomerular capillary basal membrane and Bowman capsule with immune fluorescence staining (Anti-IgG FITC 100x)

expected in patients with Goodpasture disease who need hemodialysis at presentation. Although there are some studies reporting better prognosis for Goodpasture syndrome together with ANCA positivity compared to patients with only anti-GBM positivity, there are contrasting studies also [12]. The presented case with both pANCA and anti-GBM positivity needed hemodialysis at the time of admission to the nephrology clinic. Although pathological findings were better compared to the third case, renal functions did not recover with treatment protocol involving corticosteroids, cyclophosphamide, and therapeutic plasma exchange.

Conclusion

In conclusion, pauci-immune glomerulonephritides and Goodpasture disease should be in the list of possible diagnoses in cases with clinical and radiological findings of tuberculosis without microbiological evidence. Hence, we suggest the addition of urine analysis to the tuberculosis guidelines may help to solve this diagnostic dilemma.

Conflict of interest statement. None declared.

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Case Report

Citrobacter Freundii Peritonitis in a Patient on Peritoneal Dialysis: A Case Report

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Abstract

Peritonitis is the most common complication of peritoneal dialysis (PD). This condition is associated with morbidity, catheter loss, transition to hemodialysis and mortality. Gram negative agents are less observed than gram positive factors as a factor [1]. In this article, we presented a case of peritonitis, which is a rare cause of peritonitis caused by Citrobacter freundeii from the enterobacteriaceae family, resulting in peritoneal catheter loss despite sensitive antibiotic treatment.

Keywords: Citrobacter freundii, gram-negative organism, PD-related peritonitis

Introductıon

Despite all the developments, peritonitis is the most common complication of peritoneal dialysis (PD) [1]. The most common etiological factors are gram-positive bacteria. Gram negative bacteria are less common. Citrobacter freundii is a rare factor of peritonitis and its results are poor. It is a gram negative, motile, facultative anaerobe, sports-free, enterobacteriaceae bacillus found in the gastrointestinal tract of humans and other animals [2]. Biofilm formation structured by gram negative agents during peritonitis makes antibiotics less sensitive to factors [3].

Case report

A 66-year-old female patient who had continuous outpatient peritoneal dialysis (CAPD) for five years due to end-stage renal failure secondary to diabetes was admitted to the clinic with complaints of abdominal pain, nausea, vomiting and blurred peritoneal fluid that began 10 hours ago.

In the physical examination of the patient who had no peritonitis attack in the last two years, temperature was 38.2^0C, blood pressure was 100/60 mmHg. The heart rate was 94/min and the abdomen was diffusely sensitive. There was no evidence of infection in the catheter exit area. In the laboratory results, hemoglobin 7 g/dL, white blood cell count 6620/mm^3, sedimentation 89 mm/hour, CRP 187.8mg/dL, peritoneal cell count 1600/mm^3, neutrophil dominance was 100%. No bacteria was seen in gram staining. After peritenael culture, intraperitoneal 1g loading and 4x250 mg maintenance of cefazolin, 16 mg loading and 2x8 mg maintenance of gentamicin treatment was started. At the 48th hour of treatment, 3x2.25 g of piperacillin/tazobactam IV was started due to the fever again. Citrobacter freundii was grown in culture. Treatment was continued because of the sensitivity on the antibiogram. The cell count was 240 after 48 hours and was 20 on the fifth day. The patient was followed up with fluctuations in cell count, and the treatment was completed in 21 days, and the peritoneal catheter was removed. Fistula, graft and catheter that have been tried in the past for hemodialysis for the patient, and due to the absence of vascular access, peritoneal catheter was inserted to the patient again from the right abdomen area after 36 hours. Two years later with this new catheter, it continues to peritoneal dialysis after the treatment of staphylococcus epidermidis and vancomycin.

Discussıon

Peritonitis is still the most important complication of peritoneal dialysis [1]. Gram-positive bacteria are the most common factor, but the results of gram-negative factors may be worse [4]. The International Society of Peritoneal Dialysis (ISPD) SPICE (Serratia, pseudomonas/Pprovidencia, indole positive proteus/acinetobacter/morganella, citrobacter, enterobacter and hafnia) organisms recommend sensitive bilateral antibiotics for three weeks due to high mortality and morbidity [1,2]. Cephazoline and gentamicin were started as dual antibiotics in the case, piperacilline/tazobactam was switched due to deterioration in clinical findings until culture result was found and it was found sensitive in the antibiogram.

Peritonitis with members of the Enterobacteriaceae family is the most important cause of morbidity and mortality in PD patients. Citrobacter, one of the members of this family, has not been associated with pe-

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ritonitis until recently. Citrobacter freundii has been associated with urinary or superficial infection and bacteremia, especially in elderly, immunocompromised and hospitalized patients [4,5]. It can be translocated to the circulation in severe peritonitis with changes in bowel habit. Comorbid conditions such as the age of the patient, pressure of the immune system due to chronic renal failure, diabetes mellitus, and coronary artery disease posed a risk for citrobacter.

There was a short-term history of diarrhea and frequent constipation that she did not define precisely. Of 5-10% transmural migration seen in other microorganisms is 45% with citrobacter. Of 46% case series are with diarrhea and/or constipation. Citrobacter peritonitis mortality rate is 18% and dialysis modality has changed by 89% in 12-month follow-up in living patients [4-6]. In the presented patient, peritoneal catheter had to be withdrawn with sensitive antibiotic treatment. A new peritoneal catheter was applied from a different area after 36 hours because no other route was found. During her 2-year follow-up, she continues to peritoneal dialysis.

We presented a case of peritonitis, which is a rare peritonitis agent that develops with Citrobacter freundii and causes peritoneal catheter to be removed. The clinical significance of pathogens such as citrobacter freundii appears to increase in the future in patients with compromised immune systems such as kidney failure.

Conflict of interest statement. None declared.

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Case Report

COVID INFECTION AS DEVASTATING POST-TRANSPLANT COMPLICATION

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Abstract

In march 2020 Covid 19 was declared as a pandemic by World Health Organisation. The marked risk group were older patients and patients with comorbidity such as hypertension, DM, obstructive pulmonary disease and chronic kidney disease. Patients on dialysis and kidney transplant recipients are among highest risk groups to be infected with Corona virus.
Since the very beginning, Corona virus pandemic have great impact on the transplant program worldwide.
There are recommendations for kidney transplant professionals that suggest the prioritization of patients for kidney transplantation.
We present an expanded criteria donor, and recipient with multiple vascular access problems as an indication for kidney transplantation. In the early posttransplant period vascular problems with implication on the graft function were diagnosed and surgically treated, and cholecystectomy was performed due to an uncalculous cholecystitis. Unexpected Corona virus infection early post transplantation occurred as a devastating complication for our kidney transplant recipient.

Keywords: kidney transplantation, Covid 19 infection, hydrops of the gallbladder, lethal outcome

Introduction

COVID-19 disease caused by the new coronavirus (SARS-CoV-2) was first recorded in Wuhan China in December 2019, in cluster of patients with severe pneumonia. On 30 January 2020, the World Health Organization (WHO) declared the outbreak of a Public Health Emergency of International Concern (PHEIC). Later, on 11 February 2020, WHO named the disease "coronavirus disease 2019" (COVID-19), and the International Committee on Taxonomy of Viruses (ICTV) named the virus "severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). On 11 March 2020, COVID-19 was declared a pandemic by the General Secretary of WHO [1].
Older patients and also patients with chronic diseases are at highest risk of morbidity and mortality by COVID 19. Patients with Chronic kidney disease (CKD) have several comorbidities as diabetes mellitus, multiple cardiovascular diseases and hypertension. Considering these reasons, patients with CKD are highly susceptible to corona virus infection. Furthermore, CKD population undergoing chronic haemodialysis program and visiting dialysis unit three times per week are even at highest risk to be infected with Corona virus [2].
The fast spread of COVID-19 renders throughout the continents and countries significant restriction of the transplantation program in pandemic areas due to the high risk of infection in patients with immunosuppression, risk of transmission in health workers, and lack of medical resources especially in transplant units [3]. Such restrictions exclude transplanting only highly sensitized patients with negative cross match, eventually preemptive and those without dialysis access [4]. The American Society of Transplant Surgeons (ASTS), the American Society of Transplantation (AST), and The Transplantation Society (TTS) developed recommendations to promote donor and recipient safety [5-7].

Case report

A 68-years old female patient with CKD has been followed at the outpatient nephrology department with diagnosis of hypertension for 20 years and chronic pyelonephritis with frequent urinary tract infections. At the beginning of 2020 she experienced epistaxis and worsening of the CKD with increased blood urea nitrogen and serum creatinine (34 mmol/l and 840 µmol/L, respectively) and was hospitalized at the Department of Nephrology for dialysis initiation by femoral venous catheter on the right side.

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At the following months she consulted several times for vascular access problems and exchanging of vascular catheters and in the meanwhile had created 2 AV fistulas that were primarily not successful. Exhausting the vascular access possibilities has led to consideration of kidney transplantation as a possibility, with her husband as a potential and compatible donor with 2 HLA matched antigens.

The patient was transplanted with the left donor kidney in the right iliac fossa. Relatively easy transplantation due to the presence of pronounced lymphadenopathy around the right iliac vein, on which after lymphadenectomy an end to side external iliac with renal vein anastomosis was created. The renal artery that was a bit sclerosed and tortuous with plaques that were released was also anastomosed end to side to the iliac external artery. The ureter is fused to the bladder by the Leach-Gregoire method.

In the first postoperative days there was relatively satisfactory 1,5-2 L diuresis with a slow creatinine reduction remaining around 570-620 µmol/L at postoperative day (PO) 3 and 4. Doppler ultrasonography was performed at day 1 after transplantation and was with 67 cm/sec that felt down to 45 cm/sec at the fourth PO day. Thus, an indication for vascular revision was brought. ~~A k~~ink of the tortuous artery was found and the decision was taken not to re-anastomose de novo, but for reposition of the external iliac artery placing it in a Goretex prosthesis providing an adequate flow. The patient diuresis increased to 2-2,5 L based on an improved Doppler flow of 72 cm/sek and the creatinine slowly decreased to 440 µmol/L at the nineth PO day. However, the patient's clinical condition was not improved, she was passive in bed and begun to complain of a severe abdominal pain, especially positioned under the right costal arch and back. Laboratory examination was with elevated WBC and CRP. An ultrasonography and computed tomography (CT) was performed that revealed an enlarged gallbladder-hydrops vesicae fellae (13 x 6 cm) (Figure 1).

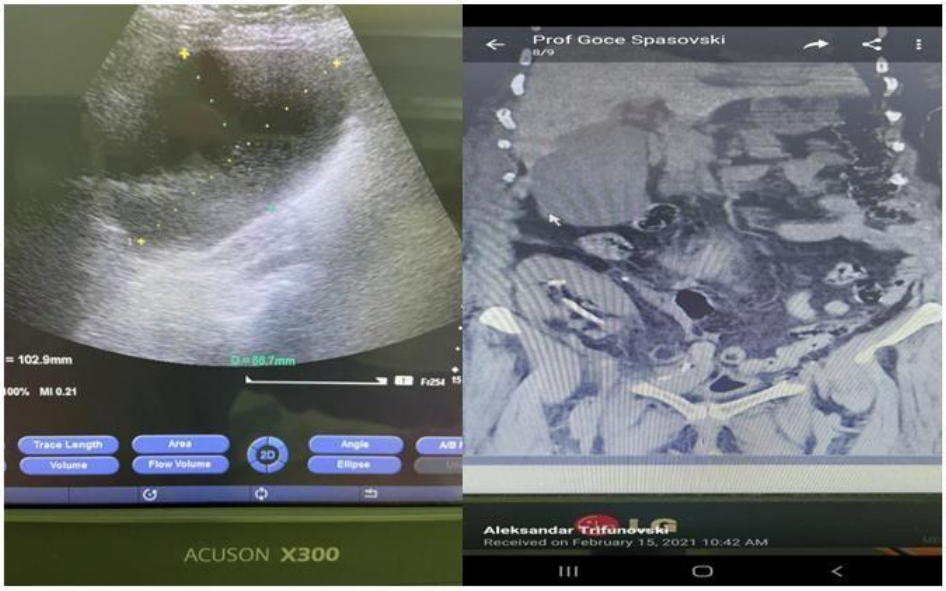

Fig. 1. Ultrasonography (left panel) and computed tomography of the enlarged gallbladder (right panel).

The next day cholecystectomy was performed and the samples taken for microbiological cultivation of strains of microorganisms revealed Pseudomonas and Enterococcus species that were treated with appropriate antibiotics.

~~P~~ostoperatively, there was a local bleeding from the operative wound of the performed cholecystectomy because of the infection and hemostatic disorder that also provoked bleeding from the wound of the transplanted kidney. Hence, for the regulation of hemostasis and anemia the patient was treated in the following 2 days with fresh frozen plasma and cryoprecipitates, as well as with filtrated erythrocytes transfusion and low molecular weight heparin (LMWH). Thus, the bleeding was successfully managed. The patient vital signs were stabile with good diuresis and subsequent decrease in serum creatinine.

Nevertheless, the patient developed fever and marked decrease in oxygen saturation. The pneumo-phtysiologist was consulted setting an indication for RTG of the lungs and a COVID test. RTG revealed an atypical interstitial pneumonia (Figure 2), and the COVID test resulted as positive. In the meantime, the graft function remained stable with normalized values of creatinine.

In the following days, the patient's condition worsened and she was transferred back to the intensive care unit and intubated with COVID protocol treatment, accordingly. All measures were taken for substitution, stimulation and support of the cardiorespiratory system but after 3 days patient died.

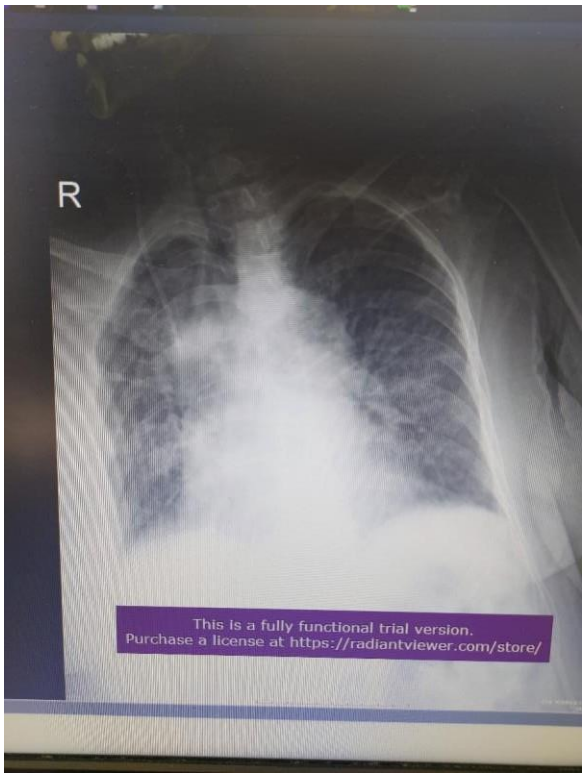

Fig. 2. Rtg of the lung with atypical interstitial Covid-associated pneumonia

There are two possibilities for positive Covid test in early posttransplant period, falls negative test or hospital-acquired infection due to multiple diagnostic and treatment procedure that were performed.

Discussion

Since the very beginning, Covid 19 pandemic have made great impact on the nephrology activity in general, especially on solid organ transplantation including kidney transplantation. In the end of March 2020, a survey of 88 US transplant institutions reported that 71.8% had completely suspended live donor kidney transplantation and 84% had implemented restrictions for deceased donor kidney transplantation [4].

The other study reported reduction in solid organ transplantation predominantly in kidney transplantation with 51% and 90% in the United States of America and France, respectively [8].

In our transplant center at the beginning of Covid pandemic from end of march 2020 complete kidney transplant program was stopped. However, some specific patients especially with vascular access problems were ranged as priority cases. The patient we presented was with multiple vascular access problems and inability to provide successful dialysis treatment. Because of these reasons we have completed all pretransplant investigations and taking in account the age of the donor and recipients we decided to perform kidney transplantation. We suggested two weeks stay at home period and pre-transplantation COVID 19 testing according to the TTS Guidance [7].

Our patient experienced early posttransplant vascular problem with impact on the graft function. Based on the literature, vascular complications were presented in about 1-5% of kidney transplant recipients, with possible increased morbidity and mortality risk. Immediate vascular complications occurred within few hours and days after transplantation procedure. The most prevalent complications are kinking of the renal artery or vein and torsion of the kidney allograft with stenosis or thrombosis of the vessels. In order to minimize morbidity and mortality caused by vascular complications, quick diagnosis and appropriated surgical treatment are necessary [9].

Posttransplant surgical complications in general are divided in three groups: Vascular complications, typical urological complications such as ureteric anastomosis complications and uretero- and lymphocele formation and surgical complication such as bleeding and/or infection. Different studies report different rate of vascular complications. The highest rate of posttransplant surgical complications was reported by Safa *et al.* (37%) but not specific reasons were explained [10]. The other studies reported relatively low rate of vascular complications (between 1.29% to 8,8%) [11,12]. In our report the vascular complication was associated with presence of CKD as well as age related blood vessels changes. Both, donor and recipient were in the group of expanded criteria donors and recipients. Additionally, vascular problems for dialysis access were the main reason for kidney transplantation. The problem with renal artery in our patient were timely diagnosed and promptly treated getting an optimal graft function in the follow up period.

In the postoperative period hydrops of the gallbladder was an unusual complication found. Our patient was surgically treated. This kind of complications were reported in Varga *et al*. study [13]. They have explained that acalculous cholecystitis is more frequent in transplant recipients than in the general population. Clinical presentation could have been milder when compared with severity of gallbladder affliction. Also, majority of patients require surgical treatment [13].

Unfortunately, when all actual problems in the recipient were solved and optimal graft function established, the most unexpected problem appeared. After prolonged episode of fever, positive COVID 19 test was obtained. Despite all preventive measurements overtaken from the medical stuff the transmission of corona virus was diagnosed in our kidney transplant recipient with a fatal outcome. Unfortunately, acquired COVID 19 infections in hospitals have been reported permanently. According to several reports, the SARS-CoV-2 hospital-acquired infection rate is 12-15% [14].

The article of Marago *et al*. analyzed the prevalence of hospital-acquired COVID-19 [15]. A retrospective case analysis presented a total of 239 patients tested positive for COVID-19, where the percentage of hospital-acquired

cases reached 16.2%. Patients with hospital-acquired infections underwent longer hospital stays [15].
Another study described a case series of 138 patients with COVID 19 [16]. Hospital-associated transmission was suspected in 12.3% of patients initially admitted for other health problems. Approximately 26% of the patients received intensive care treatment. Patient-to-patient transmission was considered the cause of infection in several cases [16].
A review and meta-analysis of cases in China-based databases of hospital-acquired infection in patients with COVID-19, SARS, and MERS. Among the confirmed patients, the proportions of nosocomial infections with early outbreaks of COVID-19, SARS, and MERS were 44.0%, 36.0%, and 56.0%, respectively. Of all confirmed cases, the medical staff and other hospital-acquired infections accounted for 33.0% and 2.0% of COVID-19 cases, 37.0% and 24.0% of SARS cases, and 19.0% and 36.0% of MERS cases, respectively. Nurses and doctors were the most affected among the infected medical staff [17].
Multiple aspects must be considered in order to understand whether the infection is a result of "malpractice" or may be considered as "inevitable" condition [14].
The other option is possible initial false negative test especially in hemodialysis patients. Studies of false-negative results from respiratory samples for SARS-CoV-2 are variable, and ranging from 1 to 30% [18]. There are multiple reasons for false negative results including suboptimal specimen collection, testing too early in the disease process, low sensitivity and low viral load [19].
Finding the reasons for false-negative tests until now had priority due to the devastating consequences of undetected cases in health-care and social care settings.
In systematic review involving 34 studies enrolling 12,057 COVID-19 confirmed cases, there was substantial and largely unexplained heterogeneity in the proportion of false-negative RT-PCR results. The findings reinforce the need for repeated testing in patients with suspicion of SARS-Cov-2 infection given that up to 54% of COVID-19 patients may have an initial false-negative RT-PCR [20].

Conclusion

During Covid 19 pandemia transplant team should carefully select the priority patients especially if resources of transplant center may be constrained. Priority cases are highly sensitized patients and without vascular access for dialysis. Besides all medical stuff efforts sometimes Covid 19 infection could occurred due to a nature of the disease and specificity of testing. Hospital-acquired COVID-19 is a serious public health issue. This problem may result in unwillingness of patients to seek hospital treatment for fear of becoming infected. Medical staff should undertake all necessary preventive measures to stop further spreading out of an in-hospital infection, especially COVID 19 transmission.
Early posttransplant vascular problems could be presented especially in expanded criteria donors and recipients.
Despite all mentioned possible complications transplant program should continue even in Covid 19 pandemic with careful pretransplant patient selection.

Conflict of interest statement. None declared.

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