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Editorial

The Use of Normothermic Regional Perfusion Increases The Burden of Organ Donation After Euthanasia

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Abstract

Post-mortem introduction of normothermic regional perfusion (NRP) is increasingly used in organ donation. In this procedure, after declaration of death according to circulatory-criteria and "no touch" time of 5 minutes, organ donors' circulation is restored by extracorporeal membrane oxygenation (ECMO). Before restoring circulation during NRP, surgeons block arteries perfusing brain to avoid regaining of brain functions. The mechanics and set-up of NRP may difficult understand for patients and medical professionals alike. In addition, restoration of circulation puts permanence of circulatory death into question, especially in the context where blocking of arteries perfusing brain after death is already declared is considered necessary. An outsized role that autonomy and consent play for a growing number of euthanasia patients considering organ donation exacerbates these already significant issues. A planned nature of euthanasia donors' death invites numerous perimortem interventions to optimize organ quality, with NRP joining an already long list of premortal donor interventions. The growing burden of donation in euthanasia patients increases the odds that patients and their families do not fully understand this process, and raises significant ethical and potentially legal questions.

Keywords: organ donation, euthanasia, normothermic regional perfusion, donation after circulatory death, organ donation euthanasia, heart donation after euthanasia

Introduction

Post-mortem introduction of normothermic regional perfusion (NRP) is increasingly used in organ donation [1,2] and already celebrated as a "cost-effective alternative in donation after circulatory death in heart transplantation" [1]. In this procedure, after declaration of death according to circulatory-criteria and "no touch" time of usually 5 minutes (to avoid spontaneous resuscitation), organ donors' circulation is restored by extracorporeal membrane oxygenation (ECMO). ECMO may reduce ischemic injury of organs to be transplanted and may enable heart transplantation, which is often not possible after circulatory determination of death. During one such recent heart transplantation from euthanised donor in Belgium, that followed NRP protocol, regular heartbeat during NRP was also restored. Diagnosis of amyotrophic lateral sclerosis was basis for euthanasia in this patient [3]. Importantly, during NRP, surgeons block the blood flow to the brain through clamping, intravascular balloons or ligation of arteries perfusing brain, thus ensuring brain death.

ECMO is not a new procedure-it has been routinely used during open heart surgery for decades (for short time during surgery or, in a modified version, for days and weeks to support lung and heart function and give them time to heal). However, it has now been repurposed from saving the life of the patient, to procuring their organs more efficiently while pursuing additional steps (i.e. blocking the blood flow to the brain) to make sure that an already dead patient (by determination of circulatory death) remains dead after circulation is restored, and that their brain function is not regained.

Bioethicists have already brought attention to some of the difficulties that this procedure presents for organ donation [4] and interventional research [5]. As Moorlock and Draper explain, "because the organ donation process has become more complex, it is not obvious that a willingness to donate some or all of one's organs necessarily translates into a willingness to undergo… all of the steps and interventions that may be… a part of donation". Meanwhile, a 2015 study [6] that randomly assigned brain dead to be organ donors to normothermia (standard approach) vs. hypothermia has faced challenges from a US-based consumer group, despite getting the approval of an Institutional Review Board [5] (the complaint argued that kidney graft recipients were involved in the trial without being with aware of it). Here we want to draw attention to ethical problems that the use of NRP may pose for organ donation in

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euthanasia patients, including informed consent of to-be-donors and recipients.

The Burden of Organ Donation In Euthanasia Patients Keeps Growing

Euthanized patients are increasingly important source of organs in countries where euthanasia is legal (The Netherlands, Belgium, Luxemburg, Canada and Spain), representing 14% of recipients from donors after circulatory determination of death as of 2021 [7]. This was entirely predictable and, in fact, predicted [8,9]. Early on, beneficence was the prime justification for euthanasia and euthanasia laws were meant to be limited to terminal somatic patients experiencing unbearable suffering. Majority of these patients were cancer patients whose organs are not suitable for transplantation, nor are organs from other terminally ill patients or old patients. However, since then, autonomy became the overriding motivation for euthanasia and criteria for euthanasia have expanded, to non-terminal somatic patients, psychiatric patients, people with disabilities [10] and children. As a result, growing proportion of a growing number of euthanasia patients have or may become organ donor candidates in future. From the perspective or organ quality and a potential for a transplanted organ longevity, physically healthy and relatively young donors (for example those euthanized for psychiatric disorder) are likely to become the preferred donors to transplant physicians and recipients. According to recent report about the experience of organ donation from psychiatric patients (2012-2022, the Netherlands) patients euthanized for psychiatric disorder represented 28.9% of all organ donors after euthanasia in this period, the youngest being 21 years old [11].

Due to the planned and intentional nature of their deaths, organ donation in euthanasia patients (whose life is terminated in a hospital a by a lethal injection), invites numerous interventions aimed at increasing quailty of organs, effectively combining the burden of premortal and postmortal interventions. Thus, according to a recent review of the practices, "depending on which premortal interventions the country's legislation permits"… premortal donor interventions…" may include imaging, blood tests, invasive arterial blood pressure monitoring, heparin administration, and changing the setting where death takes place" ([7]. Relatively new are postmortal regional perfusion procedures.

Heart transplantation from euthanized donors

The heart is the organ that is most sensitive to ischemia, so until recently, heart transplantation from euthanized persons was not possible. To override this problem, some have suggested abandoning dead donor rule and performing heart procurement while the euthanasia candidate is still alive (with other organs procured while heart still beating) [12,13,14]. However, respectting the dead donor rule is crucial to preserving trust in transplantation medicine and physicians. This makes the procurement of a heart from euthanized patients using NRP, which was recently reported in Belgium [3] a preferable alternative. In the recent report on 10-year experience transplanting organs from donors euthanized from psychiatric disorders in the Netherlands, two hearts were donated, without details on procurement technique reported [11].

All these procedures make it difficult for patients (and their families) to understand the burden they will be carrying when donating their organs. These problems are already apparent with the "change of setting where death takes place" referenced above. Euthanasia patients typically want to die at home which complicates organ donation. For this reason, this preference is not commonly satisfied. Since a certain vision of death experience, including the setting, is one of the main motivators for euthanasia, efforts have been made to combine the two. This has been accomplished by having patients (to-be-donors) deeply sedated at home surrounded by their loved ones for a farewell, and then driven to the hospital accompanied by an anaesthesiologist, where termination of patient's life still takes place [15]. This is a convoluted series of steps with a potential for being misunderstood and the possibility of the need for donor to be resuscitated on their way to the hospital.

Finally, although procedural guidelines make sure that euthanasia patients are faced with organ donation decisions only after they have decided to pursue euthanasia, increasing number of patients may already be familiar with the possibility of organ donation combined with euthanasia and may request both. However, they are likely to be making this request without fully realizing the specifics of the burden of donation, possibly including NRP. Only when they are well on their way to donation are they likely to fully understand what that entails, making potential reversal of their decisions difficult, as lives of others are now dependent on them [9]. It has already been published that some patients who requested euthanasia admitted to their physicians that they still wanted to live, however, they were now afraid to confess this to their families [15]. In directed organ donation (to a specific person) after (or even before) euthanasia the risk of coercion may be difficult to avoid [16].

Increasingly complex transplant procedures and convoluted definitions of death may pose risk of invalid informed consent

NRP (which includes blocking of arteries perfusing brain despite death is already declared) is a complex procedure which is not easy to understand. A regular person is unlikely to be familiar with the finer details of regular transplant protocols, much less with NRP. Yet,

full understanding of "what one has signed-up for" is not only a prerequisite for the patient's exercise of autonomy but also for legal procedures involving informed consent.

Large numbers of people are prone to "routinize" consent, i.e. sign consent forms without reading them [17]. Yet, informed consent hinges on the patient's capacity and willingness to understand what he is consenting to. More complex the procedures necessitate longer explanations, increasing the risk that patients are singing them without reading them. It has already been argued that, despite opt-out system of organ donation, citizens of the UK should fill out a separate form to spell-out their wishes in respect to increasingly complex procedures (such as NRP), that are involved in the actual process of organ donation [4].

Finally, already convoluted definitions of death are further strained by NRP. A common-sense understanding of death implies irreversibility and permanence. However, the mechanics of NRP-the fact that blood flow to the brain needs to be blocked although the person is already declared to be dead (by circulatory criteria)-implies that irreversibility and permanency of the initial circulatory death was not complete. In the US, Uniform Determination of Death Act laws demand that, to be consider dead, either circulation or all functions of the entire brain function must have ceased irreversibly; NRP violates this condition [18]. It is therefore not surprising that American College of Physicians urged a pause in use of NRP to allow further study before wide adoption [19].

Discussion

The slippery slope in organ donation after euthanasia is a reality. Euthanasia was primarily introduced to end suffering of "the sickest of the sick". This was, and still is, the basis for its public support. However, at this point the practice has shown that, once introduced, euthanasia laws tend to expand, to include individuals experiencing mental pain, to children, and to organ procurement. Patients euthanized for neuromuscular disease, and those for euthanized for psychiatric disorders and mental suffering are the main candidates for organ donation. Abandoning the dead donor rule as a way to enable heart transplantation from euthanasia candidates acting as living donors is explored (and endorsed) in top scientific journals [12,13,15].

However, more attention needs to be paid to the often invisible costs carried by donors and their families. Euthanasia patients interested in organ donation will only learn about the details of the compromises they will have to undertake to optimize quality of organs for recipients when they are already deeply involved in the process, making it difficult to reverse their decision because they feel that "I'd better do this because people are waiting for my organs". Initial cause to request euthanasia for suffering may be overshadowed by the need to save lives. Vulnerable individuals prone to entertaining such thoughts should not face additional donation burdens. These suggestions may be all the more relevant for heart donation after euthanasia requiring NRP.

Transplantation medicine is one of the major achievements of the 20th century. Together with artificial organs, it was involved in the birth of modern bioethics. Transplantation medicine is deeply involved in our understanding and definition of death. However, alongside predominant triumphalist reports in scientific journals [20], ethical concerns about organ donation related practices should continuously be explored. As suggested by anesthesiologist Claire Middletone, euthanasia combined with organ donation »must surely give everyone in the transplant community (including organ recipients) a pause for thought« [21].

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Rewiev

Markers of Cardiovascular Toxicity in CKD Patients-where we are now?

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Abstract

Numerous diseases such as heart failure, ischemic heart disease and sudden cardiac death are one of the most common reasons of increased morbidity and mortality in CKD through all stages. In view of such devastating epidemiology prediction of CV morbidity and mortality in the population especially those with creatinine clearance (e GFR) below 60 ml/min/1.73m^2, is becoming more and more important. Old, biomarkers such as troponin and braine natriuretic peptides and novel superfamilies of membrane receptors and their ligands, among which we single out Soluble ST2 (sST2) and Growth differentiation factor-15 (GDF-15) as markers that were examined in groups of patients with CKD. The body's defense against increased oxidative stress passes through several lines of defense, and in the first line are enzymes and enzyme systems than "collectors" of pro-oxidants *de novo* enzymes who are responsible for repairing and eliminating the damage caused by free oxygen radicals. Considering the importance of inflammation as a risk factor for atherosclerosis, a large number of studies examined a different biomarkers that were shown to be indicators of inflammation including adiponectin, leptin, interleukin-1, interleukin-6, interleukin-18, C-reactive protein and tumor necrosis factor. There is an increasing number of investigated biomarkers of acute kidney injury, which have even been examined in terms of predicting the progression of CKD and the occurrence of CV events. A lot of research studies have examined the impact of a large number of different miRNAs on the increase in CV morbidity and mortality in the population of CKD patients. Despite the great possibilities and far more modern applied diagnostic and therapeutic procedures, there is still a very high general and CV morbidity and mortality in the population of CKD patients, which is partly a consequence of the lack of application of newer CV biomarkers for prognosis and early prediction of events. Insufficiently defined newer cut of values regarding the application of classic, old biomarkers need attention.

Keywords: Cardiovascular morbidity, cardiovascular mortality, old and novel biomarkers

Cardiovascular morbidity and mortality in CKD patients

Chronic kidney disease (CKD) contributes to an increase in overall morbidity and mortality in the group of non-communicable diseases, and is also recognized as a strong and independent risk factor that contributes to the development of cardiovascular diseases (CVD) [1,2]. A whole series of diseases such as heart failure, ischemic heart disease and sudden cardiac death are one of the most common reasons of increased morbidity and mortality in CKD, and severe cardiovascular (CV) events account for almost 50% of all deaths in the kidney patient population [3,4]. The risk of developing CVD in patients with CKD surpasses the risk of reaching end-stage chronic kidney disease, and therefore, CKD is considered as one of the strongest risk factors for the development, progression and complications of CVD [5]. Although the application of numerous diagnostic and therapeutic procedures is enabled for the treatment of CKD, patients with CKD still have a dramatically reduced life expectancy, with a loss of 25 years of life at advanced stages compared with individuals in general population without kidney disease [6,7]. CV mortality accounts for 40% to 50% of all deaths in patients with advanced CKD (stage 4) as well as end-stage kidney disease (stage 5), compared with 26% in controls group with normal kidney function [8,9]. In more than 70 studies in nondialyzed patients with diagnosis of CKD, correction for CV risk factors, such as arterial hypertension, diabetes mellitus, and dyslipidemia, did not neutralize the impact of CKD on cardiovascular risk [10]. Large number of diverse cardiovascular risk factors can be classified into the group of traditional (arterial hypertension, hyperlipidemia, diabetes mellitus, age, smoking) and non-traditional factors risks (inflammation, oxidative stress, malnutrition,

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anemia, mineral metabolism disorder, proteinuria, hypervolemia) [11-13].
In view of such devastating epidemiology data the investigation of biomarkers for the prediction of CV morbidity and mortality in the population of patients with CKD, especially those with creatinine clearance (estemited glomerular filtration rate; GFR) below 60 ml/min/1.73m^2, is becoming more and more important.

Old and novel cardiospecific biomarkers

Increasingly, combinations of old, traditional biomarkers such as troponin and braine natriuretic peptides (BNP), and newer ones, are used more often in the prediction of CV morbidity and mortality in CKD patients. There are groups of novel superfamilies of membrane receptors and their ligands, among which we single out Soluble ST2 (sST2) and Growth differentiation factor-15 (GDF-15) as markers that were examined in groups of patients with CKD.
Troponin, as a well-known biomarker of acute myocardial infarction, can also be seen in other conditions and diseases such as myocarditis, cardiac decompensation, pulmonary embolism. Also, it proved to be more of a predictive biomarker than a diagnostic one in the group of CKD patients, especially those on chronic dialysis [14]. In addition, there are a number of different doubts regarding its use in patients with CKD, given the fact that its values increase with a decrease in GFR due to a probable decrease in its clearance and even "leakage" of troponin through the cell membrane of cardiomyocytes due to uremic cardiomyopathy. Therefore, true cut-off values of this biomarker in patients with CKD arises and some centers accept the limit of normal values that is up to ten times higher than in non-CKD patients [15]. The same story applies to BNP as a marker of cardiac decompensation, either new-onset, acute, or exacerbation of existing chronic heart failure. Its elevated values have been observed in various variants of cardiomyophaties, chronic constrictive pericarditis and acute coronary syndrome, as well as in pulmonary diseases. Different authors and hospital centers have different ranges of normal values of BNP biomarkers in patients diagnosed with CKD, considering its altered metabolism and clearance, so its cut off values are also subject to discussion [15].
Legitimate question arises as to how sensitive and specific the standard diagnostic biomarkers in the general population are for the diagnosis and prediction of new CV events in CKD patients. There are more and more investigations about newer biomarkers in order to obtain the most useful tool in risk stratification, diagnosis and prediction of CV events, especially decompensated heart failure and acute myocardial infarction [16].
sST2 is a relatively new biomarker of CV events that belongs to the IL-1 receptor superfamily, and the natural ligand of the said receptor family is IL-33 [17]. It is believed that sST2 by neutralizing the effect of IL-33, acts as a promoter of hypertrophy and fibrosis of the heart and inflammation itself with the progression of atherosclerosis [18]. Association of sST2 with heart failure as well as with other major CV events has been confirmed, but its association with the progression of renal failure is less well known. It is considered that the alteration in the examined values is a consequence of the parallel process of the progression of heart failure and CKD or simply occurs to changes in the cardiorenal syndrome [19,20]. Its potential as a cardiobiomarker in the group of predialysis patients was also confirmed in the CRIC study mentioned below.
GDF-15 belongs to the TGF-beta cytokine family, also known as macrophage inhibitor of cytokine 1, whose elevated values are observed in damage, repair and stress of a large number of tissues, including cardiovascular system [21,22]. In a study by Ho *et al*. that examined 85 CV markers for CV risk assessment in 3523 subjects with a median follow-up of 14.3 years, only GDF-15 was associated with all possible outcomes (overall and CV mortality, heart failure, atherosclerotic CVD) [23]. The study by Pareek *et al.* also indicated the superior capabilities of GDF-15 for predicting composite adverse CV outcomes, independently of certain older cardiac biomarkers [24]. It was observed that the mentioned biomarker has the ability to predict the progression of CKD and further reduction of GFR independent of the effect of a wide range of different risk factors [25]. A slightly smaller number of studies, but with a large number of subjects, investigated the association of GDF-15 with CV events and the progression of renal failure. CANVAS study came out with the conclusion that patients with DM type 2 and with a initial high CV risk had high values GDF-15 levels which were associated with onset of heart failure and progression of renal failure. In this study, patients with GFR over 30 ml/min were included, and therefore, the group of hemodialysis patients was not examined, which is also the case for the CRIC study [26].
A large, multicenter, prospective study (CRIC) including 3939 patients with a calculated GFR between 20 and 70 ml/min was aimed to examine the association of CV events with combination of old and new cardiac biomarkers including high-sensitivity cardiac troponin T, N-terminal pro-B-type natriuretic peptide, GDF-15, and sST2. The results of the aforementioned study support the fact that elevated baseline values of all four investigated biomarkers were associated with increased total and cardiac mortality in CKD patients [27].

Biomarkers of oxidative stress

The body's defense against increased oxidative stress passes through several lines of defense, and in the first line are enzymes and enzyme systems (superoxide dismutase, catalase and glutathione peroxidase). In the

second line are the elements that act as "collectors" of pro-oxidants; by binding them they become less harmful or are completely neutralized (ubiquinol, ascorbic acid, alpha tocopherol, glutathione). In the third line of defense, *de novo* enzymes are responsible for repairing and eliminating the damage caused by free oxygen radicals (enzymes for repairing damaged DNA molecules). In the last, fourth line of defense, we classify adaptation mechanisms that enable a faster and more efficient fight against free oxygen radicals. It is possible to conclude that examination of those enzymes (as potential biomarkers of oxidative stres), among other markers, could predict CV events [28].

Superoxide-dismutase (SOD) is the most effective intracellular enzyme in the first line of defense against free oxygen radicals [29]. In some studies, the association of SOD with certain cardiovascular diseases such as coronary artery disease (CAD) was observed, and of all isoenzymes, the SOD 2 variant proved to be the most promising biomarker for predicting the occurrence CAD [30,31].

Ischemia-modified albumin (IMA) is considered a newer biomarker that can be an indirect indicator of increased inflammation, ischemia, tissue hypoxia and, at the same time, increased oxidative stress [32]. Changes in IMA values were observed in patients with ischemic heart disease as well as in patients with acute myocardial infarction, after performed percutaneous coronary interventions, which actually reflects the ischemia-reperfusion model with increased oxidative stress [33,34]. Many authors find IMA a more sensitive indicator of acute coronary syndrom than troponin, myoglobin, and creatin kinase-MB. Evaluation of serum IMA is recommended not only for early detection of myocardial ischemia but also as a prognostic indicator of disease severity. People with higher IMA showed longer hospitalization days and had more readmissions as compared to patients with high troponin [35]. IMA has been shown to be a predictor of mortality in end-stage CKD patients in some studies [36]. By some authors IMA may serve as a useful biomarker in determining oxidative stress, and it should be kept in mind that the changes in anemia and albumin values are likely to have an impact on IMA [37].

Biomarkers of inflammation

Considering the importance of inflammation as a risk factor for atherosclerosis, a large number of studies examined a different biomarkers that were shown to be indicators of inflammation. The most frequently investigated biomarkers were adiponectin, leptin, interleukin-1, interleukin-6, interleukin-18, C-reactive protein and tumor necrosis factor [38-40].

Visceral adipose tissue is considered an important for the regulation of inflammation as well for the production of a large number of different adipocytokines [40]. For example, adiponectin (ADP) is an extremely important polypeptide that has a strong anti-inflammatory and anti-atherogenic effect, while enhancing the action of insulin at the level of insulin-dependent peripheral tissues [41]. It exerts its effect by suppressing the production of inflammatory cytokines Il-6 and tumor necrosis factor alpha [42]. ADP values are significantly increased in the population of patients with CKD, most likely due to reduced clearance or increased catabolism, while reduced values were registered in patients with metabolic syndrome, obesity, ischemic heart disease and other CV diseases [43,44]. A large number of observational studies confirmed the association of the lowest ADP values with the occurrence of adverse CV events in the population of patients with CKD [40,45,46]. The confirmation of ADP as a CV biomarker requires additional research and monitoring, given that its values depend on many other parameters in CKD patients (nutritional status, catabolism, inflammation of any other reason).

Leptin, is a neurotransmitter that is exclusively produced within adipocytes, and has a role in the regulation of metabolism, appetite and calorie consumption [40]. It is interesting that leptin is also a pro-inflammatory cytokine and elevated value of leptin was observed in patients with metabolic syndrome, which represents a state of inflammation and CV risk factor [40]. Elevated leptin values were observed in patients with CKD, which is a consequence of multiple factors such as reduced clearance, metabolic acidosis and the effect of uremic toxins [47,48]. Leptin has a proatherogenic effect, leads to strong stimulation of the sympathetic nervous system and is considered one of the early markers of atherosclerosis [49,50]. Hence, the value of leptin as a marker of risk remains unclear in CKD, although there are studies that have confirmed reduced leptin values in CKD patients with adverse CV events [51].

Interleukin 18 (IL-18) as a biomarker of inflammation has been considered as a potential biomarker of a large number of diseases including peripheral vascular disease, cerebrovascular and ischemic heart disease [52]. In high concentrations, it is registered in unstable atherosclerotic plaques and can lead to plaque destabilization and occurrence of an arterial ischemic event [53]. Based on previous studies, increased IL-18 serum concentration values are an important indicator of CV mortality in patients with CKD [54].

Biomarkers of acute kidney injury

There is an increasing number of investigated biomarkers of acute kidney injury, which have even been examined in terms of predicting the progression of CKD and the occurrence of CV events. Among others we single out Fatty acid-binding protein liver, the previously mentioned IL-18, which is also a marker of inflammation, and finally perhaps the most studied Neutrophil

Gelatinase Associated Lipocalin (NGAL) and Kidney Injury Molecule-1 (KIM-1).

NGAL is a small protein that is released from kidney tubulocytes under the action of various harmful agents and conditions, most often in conditions of hypoperfusion, ischemia and the action of nephrotoxins. Although investigated as an early indicator of acute kidney injury, the latest studies talk about its role in the progression of CKD. In the CRIC cohort study of 3386 patients suffering from CKD 2-4 stages, NGAL was an independent predictor of worsening of CKD [55,56]. With an increase in the value of NGAL in the urine, there is an increase in the incidence of ischemic atherosclerotic events in patients with CKD, independently of GFR, albuminuria and other comorbidities [57]. It is often compared to BNP in terms of prognosis and prediction in patients with the onset of heart failure where proved to be a more potent marker [58]. It has also been described that an elevated value of NGAL in the plasma of patients with CKD can be an independent predictor of future CV events [59]. Finally, there is a limited number of results that would confirm the possibility of applying NGAL markers in the prediction of CV events in daily clinical work.

Likewise, KIM-1 is released during the ischemic and nephrotoxic action of agents on the proximal tubules of the kidney, initially presented as a urinary biomarker of acute kidney injury, but its association with the progression of CKD has been proven [60]. It is also a marker of reparation and proliferation of proximal tubulocytes. Recent studies indicate that elevated values of urinary KIM-1 may be associated with a higher risk of ischemic heart disease, heart failure and overall mortality in patients with CKD [61]. Like with NGAL, there is a limited number of results that would confirm the possibility of applying KIM-1 markers in the prediction of CV events in daily clinical work.

Micro RNA family

The micro RNA (MiRNA) family represents a wide and heterogeneous group of newer biomarkers that actually represent short segment non-coding RNA molecules, and participate in the regulation of protein translation in a direct or indirect way, and over 2000 different miRNAs have been described so far [62]. The studies done so far have not yet clarified the participation of this group of biomarkers in the development and progression of CKD, although animal models confirm this [63]. A lot of research studies have examined the impact of a large number of different miRNAs on the increase in CV morbidity and mortality in the population of CKD patients, so some studies state, for example, that miRNA-223 and miRNA-126 did not prove to be prognostic markers, markers of all-cause mortality, cardiovascular events or renal events, but on the other hand, Fourdinier *et al.* for the previous two molecules, they state that there is a connection in the change of their expresion with mortality, CV events that are most probably GFR dependent [63,64]. This is the case with almost the largest number of miRNAs, whose values and results differ from author to author, as well as from study design, and require a lot of additional research.

Despite the insufficient results, a large number of researchers believe that biomarkers of miRNA origin, which are measured in hundreds, are the future in terms of their use as prognostic, diagnostic and markers for monitoring the effectiveness of therapeutic procedures, which is of particular importance for the population of kidney patients.

Despite the great possibilities and far more modern applied diagnostic and therapeutic procedures, there is still a very high general and CV morbidity and mortality in the population of CKD patients, which is partly a consequence of the lack of application of newer CV biomarkers for prognosis and early prediction of events. Insufficiently defined newer cut of values regarding the application of classic, old biomarkers need attention. In meantime, it is crucial to follow up the parameters and indicators of common risk factors (malnutrition, anemia, left ventricular hypertrophy), while it is necessary to continue further investigations of novel biomarkers during a longer period of follow-up on a larger number of patients, and especially vulnerable patient groups whose GFR is below 60 ml/min [15].

Conflict of interest statement. None declared.

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Original article

Oral Health Status in Diabetic and Non-Diabetic Patients on Maintenance Hemodialysis Treatment

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Abstract

Introduction. Uremic toxins and inflammation influence the oral health in patients on maintenance hemodialysis treatment. The presence of diabetes additionally aggravates the oral status. The aim of the study was to compare the oral health status in diabetic and non-diabetic patients on chronic hemodialysis program.

Methods. Observational, cross-section, monocentric study was conducted in 72 hemodialysis (HD) patients divided into two groups regarding the presence of Diabetes mellitus (DM). Demographic characteristics as patients age, dialysis vintage, laboratory inflammatory markers as C-reactive protein (CRP), albumin and Interleukin 6 (IL-6) were measured at the start of the study. Also, uremic small and middle molecules as blood urea nitrogen (BUN), creatinine, β2-microglobilin (β2M), myoglobin, albumin, free light chains kappa (FLC-k), and free light chains lambda (FLC-λ) were analyzed. Patients were examined by a dentist specialist scoring the oral hygiene index (OHI) by Greene Vermillion as good, fair and poor. Presence of hyperkeratosis, periodontal disease, erosions, ulceration, erythema, pigmentations, tongue coating and uremic fetor were notified. Gingival hyperplasia (GH) was scored (1-3) with 3 for the worst score. Data was presented as mean and standard deviation for continuous and percentages for nominal values. X squared Fisher exact and Mann-Whitney test were used for statistical analysis. $P<0.05$ was considered as significant.

Results. The patients from group 1-with DM (N=26) didn't differ from the non-diabetic group (N=46) in respect of gender, age but had significantly shorter dialysis vintage (48.68±37.45 vs. 88.13±63.29, p=0.02, respectively). From the inflammatory markers only Il-6 was significantly higher in DM patients (p=0.03). All the analyzed uremic toxins-small and middle molecules also didn't differ between the two groups. Diabetic patients were at 3 fold risk for manifestation of fissure, 4 fold risk for pigmentations and 7 fold risk for erythema (OR 3.58; CI:1.017-12.380, p= 0.003; OR 4.12; CI:0.684-22.870; p=0.02, OR 4.84; CI:1.343-17.498, p=0.000), (OR 7.25; CI:1.123-46.880, p=0.000), respectively. GH was more likely to be present in diabetic patients (35%, 54%, 11% vs 83%, 15, 0%, p=0.000, respectively). The presence of hyperkeratosis, periodontal disease, erosions, didn't differ between the groups. Patients with DM were found with higher percentage of bad oral hygiene index (38% vs 20%), but the overall comparison of OHI showed no significant difference.

Conclusion. Oral health is significantly deteriorated in dialysis patients, especially in those with inflammation. Diabetic patients are at higher risk of developing changes in the oral health status.

Keywords: hemodialysis, oral health, diabetes

Introduction

Chronic kidney disease (CKD) is the global health burden and one of the most frequent causes of morbidity and mortality in the 21st century. The dominant risk factors are still hypertension, obesity and diabetes mellitus. According the published reports more than 850 million individuals worldwide have some degree of CKD, and most of them have been diagnosed in the later stage, even in terminal stage of the disease. About 4 million people require kidney replacement therapy (KRT) [1,2]. According to the Kidney Disease: Im-

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proving Global Outcomes (KDIGO) CKD Work Group chronic kidney disease is defined as a persistent abnormality in kidney structure or function (e.g. glomerular filtration rate [GFR] <60 mL/min/1.73 m^2 or albuminuria ≥30 mg per 24 hours) for more than 3 months [3]. There are 5 stages of CKD, where the fifth stage with GFR below 15 ml/min/1,75m2 requires renal replacement therapy (RRT). For patients who do reach terminal stage of CKD, there are several modalities of RRT. The mostly frequent is intermittent hemodyalisis treatment (in centre or home HD). The other modalities are peritoneal dialysis and transplantation [4]. Dialysis treatment are associated with systemic changes in this group of patients, including cardiovascular disease, mineral bone disease, anemia, lower health-related quality of life compared with the general population as well as oral health complications [5,6]. Incidences of diabetes mellitus (DM) have increased rapidly in the past 2 decades as a result of the lifestyle changes, behavioral and human environmental changes. In situation when a patient has been diagnosed with both CKD and DM, the two diseases intensify each other with consequence of difficult-to-treat clinical manifestations. Chronic hyperglycemia, microvascular damage, hypoproteinemia and dyslipidemia make patients with DM prone to many systemic alterations [7].
Oral health in patients on hemodialysis needs more attention and multidisciplinary approach. Published data reports oral manifestations as present in almost 90% of dialysis patients, affecting the soft or hard tissues of the oral cavity [8,9]. It is evidently a great decline of periodontal health among dialysis patients, very low level of awareness regarding the dental care [10]. According to the small number of published studies, there are diversity of oral manifestations in chronic dialysis patients. Oral manifestations include mucosal tissues, the gingival and the periodontal apparatus, and also the dental status [11,12].
The most common mucosal oral finding is xerostomia, which means the subjective sensation of dry mouth. Characteristic halitosis called "uremic fetor" and a metallic taste are frequently described in hemodialysis patients. Other uremic manifestation reported in the literature include covered tongue, mucosal inflammation and petechiae, oral ulceration. A high incidence of periodontitis was also reported in the published literature [13,14].

Material and methods

This observational, cross-section, monocentric study was conducted in 72 hemodialysis (HD) patients from different hemodialysis units in western region of N. Macedonia, divided into two groups regarding the presence of Diabetes mellitus (DM). We have evaluated demographic characteristics as gender, patients age, dialysis vintage, as well as laboratory inflammatory markers as C-reactive protein (CRP), albumin and Interleukin 6 (IL-6) and those were measured at the start of the study. Also, uremic small and middle molecules as blood urea nitrogen (BUN), creatinine, β2-microglobilin (β2M), myoglobin, albumin, free light chains kappa (FLC-k), and free light chains lambda (FLC-λ) were analyzed. Patients were examined by a dentist specialist scoring the oral hygiene index (OHI) being good, insufficient and bad. Hyperkeratosis, periodontal disease, erosions, fissure, erythema, pigmentations, covered tongue and oral fetor were notified. Gingival hyperplasia (GH) was scored (1-3) with 3 as the worst score. Data was presented as mean and standard deviation for continuous and percentages for nominal values. X squared Fisher exact and Mann-Whitney test were used for statistical analysis. P<0.05 was considered as significant.

Results

The patients from group 1-with DM (N=26) didn't differ from the non-diabetic group (N=46) with respect of gender, age but had significantly shorter dialysis vintage (48.68±37.45 vs. 88.13±63.29, p=0.02, respectively).

Table 1. Demographic, clinical and biochemical characteristics comparison regarding the presence of diabetes

N=72	DM N=26	non DM N=46	*P*
Men	14(54%)	33(72%)	0.197
Hemodialysis	19(73%)	23(50%)	0.08
Age (years)	58.34±12.24	53.13±10.39	0.074
Dialysis vintage (months)	48.68±37.45	88.13±63.29	0.02
Albumin (g/L)	37.30±3.67	37.04±3.55	0.774
CRP (mg/L)	0.79±1.07	0.49±0.53	0.195
Glycemia (mmol/L)	8.59±3.01	5.92±1.43	0.0001
Hemoglobin (g/L)	116.92±117.75	115.08±12.43	0.541
Urea (mmo/L)	18.49±4.86	19.33±4.44	0.476
Interleukin 6 (pg/mL)	137.29±497.673	51.21±200.67	0.03

When analyzing the inflammatory markers only Il-6 was significantly higher in diabetic patients (p=0.03). All the analysed uremic toxins-small and middle molecules also didn't differ between the two groups.

Diabetic patients were at 3 fold risk for manifestation of fissure, 4 fold risk for pigmentations or xerostomia or oral pigmentations or covered tongue and 7 fold risk for erythema (OR 3.58; CI:1.017-12.380, p=0.003; OR 4.12;

Table 2. Middle molecules comparison between patients with and without diabetes

N=72	DM (N=26)	non DM (N=46)	*P*
β - 2M (mg/L) Median (IQR)	12.6 (9.5;15.5)	13.10 (8.57;16.40)	0.778
Myoglobin (ng/ml) Median (IQR)	253.84 (199.22; 294.41)	238.52 (151.32; 317.80)	0.650
FLC-k (mg/ml) Median (IQR)	109.00 (91.40; 147.00)	109.00 (70.50; 149.00)	0.650
FLC-λ (mg/ml) Median (IQR)	97.40 (69.30; 133.00)	102.50 (55.70; 136.00)	0.422
IL-6 (pg/mL) Median (IQR)	8.16 (5.66; 10.23)	4.84 (2.49; 8.84)	0.003

CI:0.684-22.870; p=0.02, OR 4.21; CI:1.134-34.77, p= 0.006),OR 4.84; CI:1.343-17.498, p=0.000), (OR 7.25; CI:1.123-46.880, p=0.000), respectively. GH was more likely to be present in diabetic patients (35%, 54%, 11% vs 83%, 15, 0%, p=0.000, respectively). The presence of hyperkeratosis, uremic fetor, periodontal disease and erosions didn't differ between the groups. DM patients were found with higher percentage of bad oral hygiene index (38% vs 20%), but the overall comparison of OHI showed no significant difference.

Table 3. Oral and dental changes in patients with and without Diabetes

N=72	DM N=26	non DM N=46	X^2 *test*	Risk Odds ratio	95% CI lower	95% CI upper
Hyperkeratosis	3(11%)	1(2.2%)	p=0.131			
Periodontal disease	2(8%)	12(27%)	p=0.071			
Uremic fetor	20(77%)	40(86%)	P=0.130			
Erosions	3(12%)	4(8.7%)	p=0691			
Pigmentations	5(20%)	1(2.2%)	p=0.02	4.12	0.684	22.87
Xerostomia	18(69%)	4(9%)	P=0.006	4.21	1.134	34.77
Fissure	8(31%)	2(4.3%)	p=0.003	3.58	1.017	12.38
Covered tongue	11(42%)	2(4.3%)	p=0.000	4.84	1.343	17.498
Mucosal erythema	9(35%)	1(2.2%)	p=0.000	7.25	1.123	46.889
Oral hygiene score						
1 - good	7(26%)	16(35%)				
2 - unsatisfied	9(34%)	20(44%)				
3 - bad	10(38%)	9(20%)	p=0.238			
Gingival hyperplasia						
0	9(35%)	38(83%)				
1	14(54%)	8(17%)				
2	3(11%)	0(0%)	p=0.000			

Discussion

End stage renal disease (ESRD) affect every system in humans including the oral cavity, in a clinical condition defined as uremic syndrome presented with fluid overload, electrolyte disturbance, deterioration in acid-base homeostasis, and uremic toxins retention, normally eliminated through urine output [15].

New dialysis techniques have been developed for better removal of uremic toxins. Advances in understanding of uremic retention solutes and their role in development of clinical symptoms and outcomes, facilitate personalized and targeted dialysis treatment, and may improve quality of life and decreased morbidity and mortality. In the classic taxonomy, uremic retention molecules are divided into 3 categories: small solutes, middle molecules, and protein-bound toxins [16]. In 2021 a consensus conference was held to develop recommendations for an updated definition and classification scheme on the basis of a holistic approach that incorporates physicochemical characteristics and dialytic removal techniques of uremic retention solutes and their association to clinical symptoms and outcomes [17].

Standard High Flux (HF) membranes are effective in the removal of uremic toxins in a range of small solutes (urea) and middle molecules (β2-microglobulin). But the efficient removal of middle molecules (MM) uremic toxins, in a molecular range of 15-50 KDa, is currently limited. Increased concentration of uremic toxins in ESRD patients, leads to pathophysiological process including anorexia, chronic inflammation, calcification, and cardiovascular morbidity and mortality [18].

We have evaluated 5 middle molecules: β2-microglobilin (β2M), myoglobin, free light chain kappa (FLC-k), and free light chain lambda (FLC-λ) and interleukin-6 (IL6). For all middle molecules we have found increased

values in both groups, but significant difference was found for IL 6. According to the new classification of uremic toxins IL6 with MW >15-25 kDa belongs to the group of Medium-middle molecules and the group of Uremic toxins with the highest toxicity evidence score [17]. Interleukin-6 is a proinflammatory cytokine that play a role in development of insulin resistance and overt type 2 diabetes mellitus (T2DM) through the generation of inflammation, differentiation, proliferation, and cell apoptosis [19]. In our study patients with DM had a shorter dialysis vintage. The published data presents no significant difference between diabetic and nondiabetic patients at 1-year survival (87.1% versus 89.7%, P=.66). but, 3- and 5-year survival were significantly lower in patients with DM (52.2% versus 73.8%, P=.04; zero versus 56.9%, P<.001; respectively) [19]. In another retrospective study of 897 patients the 5-year survival rates after censoring were 20.7 and 38.2% for diabetic and non-diabetic patients, respectively (P <0.001) [20].

In the group of DM patients there was significantly higher blood glucose level. Our study confirmed hyperkeratotic lesion as part of the uremic stomatitis in 11% of DM patients and 2% in non DM patients without statistical difference. Uremic stomatitis is an uncommon complication of uremia in advanced renal failure patients. Since it was first reported by Lancereaux in 1887 and described by Barie in 1889, a few affected patients have been presented in the literature [21]. The etiology is still unclear, but a hypothesis for an increased levels of ammonia complexes produced by the action of bacterial ureases that modify salivary urea has been postulated [22]. Four forms of uremic stomatitis have been described in the literature: Ulcerative form, Hemorrhagic form, Nonulcerative, pseudomembranous form, and Hyperkeratotic form. The last two forms appear as white lesions. The hyperkeratotic form presents as multiple, painful, white hyperkeratotic lesions. This hyperkeratotic lesion can be also due to the effects of chemical substances on the oral mucosa [23]. We have reported 35% DM patients with mucosal erythema and only 2.2% in other group of patients (p=0.000).

We have found oral pigmentation in 20% on patients with DM, and 2% in non DM group (p<0.02). Oral pigmented lesions are one of the most significant changes present in patients with end-stage renal disease. The case control study published by Hasan at al. described the most common oral pigmentation were abnormal lip pigmentation and petechiae. A possible mechanism for this pathological findings was attributed to an increased level of beta melanocyte-stimulating hormone (beta-MSH) as a result of an impaired elimination which resulted in continuous stimulation of melanocytes in oral epithelium [24], abnormal lip hyper pigmentation was the most frequently seen lesion in 90% of the CKD patients [25], whereas our study observed only 7.0% patients with pigmentation.

Chronic uremia state and many co-morbid conditions in end stage kidney patients can cause changes in the periodontium leading to an exacerbation of the inflammatory process in the gingival tissue. Poor oral hygiene and development of dental calculus are risk factors for periodontal disease. Prevalence of periodontitis is significantly higher among middle-aged patients with diabetes than among the similarly aged individuals without diabetes [26,27]. In our study periodontal disease was present in 8% and 27% of patients with DM and non DM patients, but without statistical signifycance. The most important is certainly, the patient's education and prevention, as well as frequent clinical check-ups to rule out oral lesions. High prevalence of dental calculus in CKD patients is a common finding. A possible relationship between the amount of biofilm, gingivitis and the amount of dental calculus may be as an additional risk factor for severe destruction of the periodontium. Additionally, hemodialysis patients have faster dental calculus formation as a result of secondary hyperparathyroidism and therapy with calcium-based phosphate binders [28].

Covered tongue (CT) and fissured-dry lips are other frequent oral lesions in hemodialysis patients. Yellowish-white plaque on tongue dorsum, can be seen on the dental examination. Covered tongue is caused by retention of desquamated epithelial cells and leucocytes, and bacterial accumulation on slightly elongated filiform papillae. Reported prevalence are 12.2% to 47.1% in CKD patients, respectively [15,29]. We have found covered tongue in 43% in DM group, and 4% in non DM patients (p<0,000).

In chronic hemodialysis patients, gingival hyperplasia has multiple etiologies among which drug-induced enlargement is a common reason that is related to long term effect of calcium channel blockers, mostly Nifedipine [30]. Overproduction of gum tissue by fibroblasts is the main mechanism of gingival hyperplasia. Poor plaque control, dysregulation of vitamin D metabolism and calcium level acts as a predisposing factor for nifedipine induced gingival enlargement. Neither the dosage, nor the duration of treatment is related to the prevalence of gingival enlargement [30]. In patients with kidney transplants, it is mostly due to the immunosuppressive therapy with cyclosporine [32]. Severe gingival hyperplasia has negative impact to esthetics and function as well as to the overall oral health-related quality of life. Treatment of these conditions requires comprehensive periodontal management by a dentist. In our study, the group of patient with DM have had significantly greater gingival enlargement than non DM group.

There are several risk factors for the prevalence of xerostomia (dry mouth) in chronic hemodialysis patients. The decreased salivary flow may be caused by a direct uremic toxins effect on salivary glands, chemical inflammation, decreased water consumption and chronic de-

hydration and mouth breathing. The study of Swapna *et al*. reported xerostomia presence was seen both in diabetic and nondiabetic patients, with no significant statistical difference. It was opposite of the previous literature reports which showed dry mouth was more severe in the diabetic group compared to the nondiabetics [33-35]. In our study DM patients have signifycantly higher xerostomia compared to the other group of patients.

In association with xerostomia, one third of hemodyalisis patients present a characteristic halitosis called "uremic fetor" and a metallic taste due to the high urea content in saliva and its breakdown in ammonia [36]. In our study majority of patients have bad oral hygiene. According to the literature, less than 45% of hemodialysis patients visited a dentist. Dental care utilization among these patients is very low and this trend can be partly explained by the reason that a greater importance is given to the treatment of systemic diseases rather than dental problems. An another reason can be lack of awareness, physical barriers, because part of these patients come from areas where dental services are rarely available [37]. In a study conducted by Klassen and Krasko in 2002, dental care in dialysis patients was found to be almost completely neglected [38].

Conclusion

Knowledge of oral health among chronic hemodyalisis patients in general is poor and there is an obvious need for an appropriate oral health education. Patients with DM are at increased risk for more severe oral manifestations and complications when compared with non diabetic HD patients due to the chronic inflammatory state.

Supportive dental programs must be established for these patients in order to rise awareness of an urgent need for prevention of dental disease. These patients should be well educated about the significance of oral health on systemic health and should be motivated to have regular dental checkup while undergoing the treatment for kidney disease. Multidisciplinary approach of dental specialist and nephrologist can be crucial in improvement of oral health in chronic hemodialysis patients.

Conflict of interest statement. None declared.

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Original article

Muco-Cutaneous Changes/Symptoms in Patients with Stage 5 Chronic Kidney Disease on Haemodialysis

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Abstract

Introduction. Chronic Kidney Disease (CKD) is defined when the glomerular filtration rate is reduced (GFR) <60 mL/min per 1.73 m2 (GFR categories G3a-G5) for more than 3 months and with the presence of albuminuria: the albumin-creatinine ratio (ACR) is ≥30 mg/g and the albumin excretion rate is (AER) ≥30 mg/d. Stage 5 of Chronic Kidney Disease (Stage 5 CKD) is the stage with a need for renal replacement therapy or kidney transplantation. CKD is characterized by numerous muco-cutaneous manifestations. Our aim was to determine the frequency of non-specific and specific muco-cutaneous changes in patients with Stage 5 CKD on haemodialysis.

Methods. We conducted a cross-sectional study at the University Clinic for Nephrology, Skopje and PHI Special Hospital for Nephrology with Dialysis DIAMED, Skopje from March to June 2022. The study involved 42 patients from both dialysis centers. A detailed medical history for each patient was used to obtain data on demographic characteristics, the cause of the renal insufficiency, dialysis duration, and the latest findings of routine laboratory parameters.

Results. The most common muco-cutaneous changes/symptoms were xerosis 88.1%, pruritus 73.81%, hyperpigmentation 45.24%, echymosis in 42.86%, onychomycosis 40.47%, absence of lunula 23.81%, "longitudinal ridge" 21.43%, sparse hair 21.43%, "Half-and-half nails" 19.05%, brittle nails 19.05%, subungval hyperkeratosis 19.05%, photosensitivity 19.05%, etc.

Conclusion. Muco-cutaneous changes/symptoms were a common finding in patients with Stage 5 CKD on haemodialysis. All respondents were diagnosed with at least one muco-cutaneous change/symptom. Interdisciplinary management involving dermatologists is essential.

Keywords: chronic kidney disease, Stage 5 CKD, haemodialysis, muco-cutaneous changes/symptoms

Introduction

Chronic kidney disease (CKD) is in fact a progressive irreversible loss of kidney function syndrome. It is defined when the glomerular filtration rate (GFR) is reduced below 60 mL/min per 1.73 m2 (GFR categories G3a–G5) for more than 3 months alongside with the presence of albuminuria: the albumin-creatinine ratio (ACR) is ≥30 mg/g and the albumin excretion rate is (AER) ≥30 mg/d [1]. The End Stage Renal Disease (ESRD) or the Stage 5 CKD is the point at which life can no longer be sustained without renal replacement therapy or kidney transplantation.

The muco-cutaneous changes are with high prevalence in Stage 5 CKD patients who undergo dialysis [2]. Affecting the skin, its adnexa and mucosa can be rather extensive and that impacts the quality of life in these patients. In a study by Pico *et al*., it was found that all 102 patients had at least one noted skin change [3]. It is very difficult to deduce whether a certain muco-cutaneous manifestation is affected by the CKD itself or only by the hemodialysis process with all its distinctive features, as many of them can be related to both factors [4].

In all publications pertaining to this subject matter, the muco-cutaneous changes are classified into two groups-non-specific and specific, with pre-defined terms. The entities of interest in terms of certain cutaneous changes "having preference for" Stage 5 CKD patients who undergo chronic dialysis program allude to the specific, and the other ones, non-specific, are found amongst the rest of the population, in which case these patients are also not spared [5].

The muco-cutaneous changes are in actual fact a clinical tool for evaluation of patients' life quality and are a reflection of the general health condition of this population.

This study has the purpose of measuring the frequency of the non-specific and specific muco-cutaneous changes (pre-defined terms) in 42 ESRD patients undergoing dialysis from two dialysis centers in Skopje: University

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Materials and methods

Study design, duration and location: the research was a cross-sectional study conducted in two already mentioned dialysis centers (UC of Nephrology, Skopje and PHI Special Hospital for Nephrology with Dialysis, DIAMED) in Skopje. The research was carried out in the period between March and June 2022. The research sample was comprised by patients on hemodialysis. Inclusion criteria: patients with glomerular filtration <15 ml/min/1.73m2 undergoing chronic hemodialysis program for more than 3 months; age: ≥18; irrespective of sex, ethnic or religious background; and willingness to participate in the study with given informed consent.
Exclusion criteria: patients with chronic dermatological diseases history prior to the commencement of dialysis. The selection of respondents was made in accordance with the inclusion and exclusion criteria by the method of random choice, i.e. the first 42 patients examined from the two previously mentioned dialysis centers (21 patients from each center). All patients were on hemodialysis program, three times weekly, four-hour sessions.
The muco-cutaneous changes/symptoms were classified in two groups, i.e. non-specific and specific with the goal of pre-defining those who were about to be monitored. Non-specific muco-cutaneous changes and symptoms: photosensitivity, paleness, xerosis, hypo and hyperpigmentation, ecchymosis, various changes in nails (Half-and-half nails, Terry's nails, absence of lanula, onycholysis, Beau's lines, clubbing, longitudinal ridging, onychomycosis, subungual hyperkeratosis, koilonychia, total leukonychia, nail pitting, splinter hemorrhage, pincer nail deformity etc.), various changes in hair (brittleness, lack of shine, thinning hair, effluvium, alopecia etc.), mucosal changes (gingivitis, stomatitis, dryness, angulus infectiosis oris etc.), cutaneous infections (bacterial, viral, fungal, parasitic), neck elastosis, cutaneous carcinoma (basocellular, squamocellular, melanoma, etc.), venous dilation near the fistula, eczematous changes around the fistula, etc. Specific muco-cutaneous changes and symptoms: CKD-associated pruritus, pseudoporphyria, porphyria cutanea tarda, acquired perforated dermatosis, calciphylaxis, nephrogenic systemic fibrosis.
The detailed medical history of each patient was reviewed to obtain data on the demographic features (sex, age, nationality, socio-economic background), the original kidney disease, the duration of dialysis and the latest findings from the routine biochemical and hematological analyses (hemoglobin, ferritin, calcium, urea, phosphates creatinine, albumins, parathormone, HCV, HBV, HIV). All patients were subjected to a clinical dermatological exam to record changes in the skin, hair, nails and the accessible mucous membranes. The patients were examined by a dermatovenerologist during the course of one dialysis session. Each patient was informed about the study, and was granted a guarantee for anonymity, as well as a guarantee for use of their personal data for scientific purposes only. Each participant signed an informed consent form. The diagnosis of dermatological changes was made according to the good clinical practice criteria and the directions for evidence-based medical practice. For the purpose of evaluation of the itching intensity, a horizontal visual analog scale (VAS) was used, from 0-no itching, to 10 -severe/unbearable itching. The responses on the VAS were grouped into: mild itching ≥0, but <3, medium itching (≥3, but <7), severe itching (≥7, but <9), and very severe (≥9) [39]. In order to assess the weight of xerosis, a xerosimeter was used: 0-no xerosis, 1-mild xerosis, 2-medium xerosis, 3-severe xerosis, 4-very severe xerosis [38]. The duration of the clinical exam lasted between 20 to 40 minutes per patient.
The analysis was made by utilizing a SPSS software package, version 20.0 for Windows (SPSS, Chicago, IL, USA). The data was presented by means of simple measurements such as frequency, percentage points, average, standard deviation and rank (minimum-maximum values).
The study was approved by the Ethics Committee at the Faculty of Medicine at the Ss. Cyril and Methodius University, Skopje, N. Macedonia.

Table 1. Basic sample data

Parameters		n - number	%
Age	Average value in years ± SD (range)		63.8±13.3 (34-90)
Sex	Male	31	73.81
	Female	11	26.19
Nationality	Macedonian	36	85.71
	Albanian	2	4.76
	Roma	2	4.76
	Serbian	1	2.38
	Turkish	1	2.38
Socio-economic background	poor	1	2.38
	medium	9	21.43
	good	23	54.76
	excellent	9	21.43
Hemodialysis duration	Average value in years ± SD (range)		9.26±8.36 (0.3-36)

Results

Basic sample data are presented in Table 1.
The most frequent muco-cutaneous changes/symptoms included xerosis-88.1% (n=37), followed by pruritus-73.81% (n=31). Any changes in nails were identified in 66.67% (n=28), out of which the most common place was onychomycosis 40.47% (n=17), followed by absence in the nail lunula 23.81% (n=10), after that the longitudinal ridging 21.43% (n=9), half-and-half nails 19.05% (n=8), brittle nails 19.05% (n=8) and subungual hyperkeratosis 19.05% (n=8). Hyperpigmentations were identified in 45.24% (n=19), ecchymoses in 42.86% (n=18), and paleness in 28.57% (n=12). Changes in hair were observed in 23.81% (n=10), out of which the most common finding was thinning hair 21.43% (n=9). Photosensitivity was reported in 19.05% (n=8) of the patients. In 16.67% (n=7) other muco-cutaneous changes were found, which include: Poikiloderma Civatte 7.14% (n=3), prurig simplex 2.38% (n=1), dermatitis around the fistula 2.38% (n=1), xanthoma 2.38% (n=1), Raynaud syndrome 2.38% (n=1). Mucous membrane changes were found in 7.14% (n=3) of the patients. Only in 2.38% (n=1) of the patients who undergo hemodialysis Morbus Kyrle (acquired perforating dermatosis) was diagnosed. All sample patients had a varying degree of dilatation of the arterio-venous fistula (Table 2).

Table 2. Frequency of muco-cutaneous changes in Stage 5 CKD on hemodialysis in this sample

Muco-cutaneous changes/symptoms	%	n=42
Photosensitivity	19.05%	n=8
Xerosis	88.10%	n=37
Changes in nails (one or more than one could be observed in a single patient)	66.67%	n=28
Half-and-half nails	19.05%	n=8
Terry's nails	7.14%	n=3
Absence of lunula	23.81%	n=10
Onycholysis	14.28%	n=6
Brittle nails	19.05%	n=8
Beau's lines	4.76%	n=2
Clubbing	4.76%	n=2
Longitudinal ridging	21.43%	n=9
Onychomycosis	40.47%	n=17
Subungual hyperkeratosis	19.05%	n=8
Koilonychia	7.14%	n=3
Total leukonychia	11.90%	n=5
Pincer nail deformity	2.38%	n=1
Hyperpigmentations	45.24%	n=19
Paleness	28.57%	n=12
Hypopigmentations	11.90%	n=5
Ecchymoses	42.86%	n=18
Skin infections	4.76%	n=2
Bacterial skin infections	2.38%	n=1
Fungal skin infections	2.38%	n=1
Changes in hair (one or more than one could be observed in a single patient)	23.81%	n=10
Effluvium	4.76%	n=2
Alopecia	4.76%	n=2
Thinning hair	21.43%	n=9
No shine	7.14%	n=3
Brittle hair	7.14%	n=3
Changes in the oral mucosa (one or more than one could be observed in a single patient)	7.14%	n=3
Dryness of mucous membranes	2.38%	n=1
Stomatitis	4.76%	n=2
Gingivitis	2.38%	n=1
Neck elastosis	9.52%	n=4
Other skin diseases	16.67%	n=7
Poikiloderma civatte	7.14%	n=3
Dermatitis around the fistula	2.38%	n=1
Dilatation of the arteriovenous fistula	100%	n=42
Prurigo	2.38%	n=1
Xanthomas	2.38%	n=1
Raynaud syndrome	2.38%	n=1
CKD associated pruritus	73.81%	n=31
Acquired perforating dermatosis (Morbus Kyrle)	2.38%	n=1

None of the patients was diagnosed with skin and mucous membranes cancer, pseudoporphyria, calciphylaxis and nephrogenic systemic fibrosis.
The most common cause of renal insufficiency was nephro-arteriosclerosis (NAS) found in 28.57% (n=12) of the respondents (Table 3).

Table 3. Etiology of CKD

Reasons for CKD	%	n=42
Diabetes	7.14	3
NAS	28.57	12
Glomerulonephritis	11.90	5
Infectious and obstructive nephropathy	9.52	4
Hereditary nephropathy	23.81	10
Other	7.14	3
Unknown	11.90	5

The analysis of the lab parameters are expressed as follows with mean ±SD and range: a) elevated values of parathyroid hormone in 97.62% (n=41) of patients -375.9±240.39 pg/mL (52.3-1034); b) elevated values of ferritin in 42.86% (n=18)-329.9±206.58 ng/mL (31-841.25); c) hypercalcemia was noted in 9.52% (n=4), and hypocalcemia in 11.9% (n=5)-2.28±0.24 mmol/L (1.91-2.9); d) only 9.52% (n=4) of patients had hemoglobin reference values, whereas in other patients anemia was observed at the rate of 90.48% (n=38)-113.40± 11.97 g/l (78-135); e) elevated inorganic phosphate levels were noted in 50% (n=21) of patients-1.54±0.53 mmol/L (0.7-3.9); f) the urea levels were elevated in all patients-18.18±4.28 mmol/L (10.2-29); g) elevated creatinine levels were observed in all patients-688.25 ±173.85 μmol/L (273.2-1092); h) 11.9% (n=5) of patients were HBV positive, 9.52% (n=4) tested positive for HCV, and only one patient tested positive for HIV (2.38%). Albumin values in all patents from this sample were within reference values.
Xerosis was found in 88.1% (n=37) of patients. In 30.95% (n=13) of the respondents there was mild xerosis, in 33.33% (n=14) medium xerosis, in 16.67% there was severe xerosis (n=7) and in 7.14% very severe xerosis was found (n=3). In 46.15% (n=6) there was a mild localized xerosis, 53.85% (n=7) with mild generalized xerosis. Medium generalized xerosis was found in 64.29% (n=9), while severe generalized xerosis was found in 70% (n=7) of the respondents (Table 4).
Pruritus was found, irrespective of whether it was localized or generalized, in 73.81% (n=31) of patients. Localized pruritus was observed in 47.62% (n=20), whereas 26.19% (n=11) of patients had generalized pruritus. Mild pruritus had 30.95% (n=13) of patients, 26.19% (n=11) of the patients had medium pruritus and 16.67% (n=7)-severe pruritus. Neverheless, none of patients referred to severe pruritus (Table 4).

Table 4. Intensity and distribution of pruritus and xerosis in Stage 5 CKD on hemodialysis

Intensity / Distribution	Severe						Medium						Mild						Total	
	Total		Local		General		Total		Local		General		Total		Local		General			
	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n
Pruritus	22.58	7	28.57	2	71.43	5	35.48	11	54.55	6	45.45	5	41.94	13	92.31	12	7.69	1	73.81	31
Xerosis	23.81	10	30	3	70	7	37.84	14	35.71	5	64.29	9	30.95	13	46.15	6	53.85	7	88.10	37

Table 5. Distribution of pruritus with varying intensity in cases of severe xerosis in Stage 5 CKD on hemodialysis patients

	Severe xerosis		total
	%	n	
Mild pruritus	7.69	1	13
Medium pruritus	45.45	5	11
Severe pruritus	57.14	4	7

Distribution of pruritus with varying intensity in cases of severe xerosis in Stage 5 CKD on hemodialysis patients is presented in Table 5.Xerosis was found in all patients diagnosed with diabetes (n=3), infectious and obstructive nephropathy (n=4), as well as the other pa-

Table 6. Distribution of selected muco-cutaneous changes/symptoms according to the causes of CKD

Muco-cutaneous changes/symptoms	Causes of CKD													
	HTN		Diabetes		Hereditary nephropathy		Glomerulo-nephritis		Infectious and obstructive nephropathy		Other		Unknown	
	%	n	%	n	%	n	%	n	%	N	%	n	%	n
Xerosis	83.33	10	100	3	90	9	80	4	100	4	100	3	80	4
Changes in nails	66.67	8	100	3	60	6	40	2	75	3	100	3	60	3
Half-and-half nails	8.33	1	66.67	2	20	2	0	0	0	0	33.33	1	40	2
Absence of lunula	25	3	0	0	20	2	0	0	25	1	33.33	1	60	3
Longitudinal ridging	16.67	2	66.67	2	30	3	0	0	25	1	0	0	20	1
Onychomycosis	41.67	5	33.33	1	50	5	0	0	50	2	33.33	1	60	3
Hyperpigmentations	50	6	66.67	2	20	2	20	1	75	3	66.67	2	60	3
Changes in hair	33.33	4	33.33	1	20	2	40	2	0	0	0	0	20	1
CKD associated pruritus	91.67	11	0	0	60	6	100	5	50	2	100	3	80	4
Acquired perforating dermatosis	0	0	0	0	0	0	0	0	0	0	0	0	20	1

tients from the category-other causes of CKD (chronic pyelonephritis, cancer, reflux nephropathy) (n=3). Pruritus was found in all patients from the glomerulonephritis categories (n=5) and the other etiologies (n=3) (Table 6).

Table 7. Duration of hemodialysis in patients with pruritus, xerosis and hyperpigmentations

Cutaneous changes/symptoms	Hemodialysis duration average±SD (rank)
No pruritus	5.97±6.31 (0.3-18)
With pruritus	10.43±8.77 (0.3-36)
Generalized pruritus	10.36±8.99 (3-30)
Localized pruritus	10.46±8.89 (0.3-36)
Severe pruritus	12.86±10.25 (4-30)
Medium pruritus	7.3±5.17 (0.3-20)
Mild pruritus	11.76±10.16 (1-36)
Severe xerosis	6.28±2.21 (3-9)
Very severe xerosis	18±8.18 (9-25)
Severe and very severe xerosis together	9.8±7.08 (3-25)
With hyperpigmentations	10.59±10.6 (0.3-36)
No hyperpigmentations	8.51±5.85 (0.33-22)

The longest duration of hemodialysis was registered in patients with severe pruritus 12.86±10.25 years (4-30). The patients with no pruritus were undergoing hemodialysis by a half duration compared to the hemodialysis duration in patients where pruritus was actually found, 5.97±6.31 years (0.3-18) and 10.43±8.77 years (0.3-36), respectively (Table 7).

In patients with very severe xerosis, the average duration of hemodialysis in years has shown the highest values 18±8.18 years (9-25) (Table 7).

The duration of hemodialysis in patients with hyperpigmentations was on average 10.59±10.6 years (0.3-36) and has shown higher values compared to the patients with no hyperpigmentations, i.e. 8.51±5.85 years (0.33-22) (Table 7).

Anemia was found in all patients where paleness was noted 100% (n=12), especially in the group with hemoglobin values ranging 78-108 g/l. Pruritus was found in 90.32% (n=28) of the patients with anemia. Xerosis was found in 89.19% (n=17) of the patients with anemia. In 89.48% (n=17) of the respondents, both anemia and hyperpigmentations were found. Absence of lunula and anemia was found in 80% (n=8) of patients with anemia. Changes in nails, the likes of which include half-and-half nails (Lindsay's nails) and anemia was found in 75% (n=6) of patients (Table 8).

Table 8. Distribution of selected cutaneous changes/symptoms according to hemoglobin values

Cutaneous changes/symptoms	Hemoglobin (78-108 g/l)		Hemoglobin >108 (<120 female and <130 male)		Total		
	%	n	%	n		%	n
Paleness	100	12	0	0	12	100	12
Xerosis	29.73	11	59.46	22	37	89.19	33
Hyperpigmentations	26.32	5	63.16	12	19	89.48	17
Pruritus	35.48	11	54.84	17	31	90.32	28
Absence of lunula	30	3	50	5	10	80	8
Half-and-half nails	37.5	3	37.5	3	8	75	6

Patients with pruritus also had hyperphosphatemia 85.71% (n=18), hypercalcemia 100% (n=4) and hypocalcemia 60% (n=3). Hyperphosphatemia 61.11% (n=11) and hypercalcemia 50% (n=2) were noted in patients with localized pruritus, whereas hypocalcemia 60% (n=3) was noted in patients with generalized pruritus. The patients with severe generalized pruritus 50% (n=2) had hypercalcemia (Table 9).

Table 9. Pruritus distribution according to calcium and inorganic phosphates values in Stage 5 CKD patients on hemodialysis

	Calcium				Inorganic phosphates	
	Hypocalcemia		Hypercalcemia		Hyperphosphatemia	
	%	n	%	n	%	n
Pruritus	60	3	100	4	85.71	18
Mild pruritus	20	1	25	1	38.89	7
Mediam pruritus	40	2	25	1	33.33	6
Severe pruritus	0	0	50	2	27.78	5
Localized pruritus	0	0	50	2	61.11	11
Generalized pruritus	60	3	50	2	38.89	7
Mild localized pruritus	0	0	25	1	33.33	6
Medium localized pruritus	0	0	25	1	16.67	3
Severe localized pruritus	0	0	0	0	11.11	2
Mild generalized pruritus	20	1	0	0	5.55	1
Medium generalized pruritus	40	2	0	0	16.67	3
Severe generalized pruritus	0	0	50	2	16.67	3

Table 10. Distribution of Half-and-half nails (Lindsay's nails) according to anaemia and hyperparathyroidism findings in Stage 5 CKD patients on hemodialysis

Cutaneous change	Anemia		Hyperparathyroidism		Total
	%	n	%	n	n
Half-and-half nails	75	6	100	8	8

Nail changes such as half-and-half nails (Lindsay's nails) were found in all patients (100%, n=8) with hyperparathyroidism, and 75% (n=8) of them were also anemic. (Table 10).

Discussion

In the latest ERA-EDTA (the European Renal Association-European Dialysis and Transplantation Association) report from 2019, the register reported 1853 patients with Stage 5 CKD on a chronic dialysis program from N. Macedonia. The Prevalence per million population (Pmp) for N. Macedonia for the year of 2019 was 893 Pmp [25].

The dialysis centers in N. Macedonia do not have data registry for muco-cutaneous manifestations in patients with ESRD on dialysis. The prevalence of muco-cutaneous changes in patients with Stage 5 CKD on dialysis has been determined in several studies of respondents who are not European residents. The occurrence and development of muco-cutaneous changes in patients with ESRD largely depend on the regional climate factors, the race and socio-economic status of the patients, as well as on the accuracy of the diagnosis according to the study from Iran [24].

In our study, 100% of the patients had at least one muco-cutaneous change/symptom, and xerosis was the most common finding, i.e. 88.10%. In many other studies, xerosis is described as the most prevalent cutaneous change in patients with Stage 5 CKD on hemodialysis [26,27]. In the Anees *et al*. study, xerosis 83% ranks second in prevalence behind pigmentations 86% [6]. In the Adégbidi H. *et al.* study from 2020, xerosis has lower prevalence 48% [28]. In the Böhme et al. study, the xerosis frequency is 90% [15]. A study that examined the relation of xerosis and pruritus reported the intensity of the pruritus grows dramatically alongside with the severity of the xerosis [34]. In our study, in the patients with very severe xerosis, the average of the dialysis duration showed the highest values 18± 8.18 (9-25) with the prevalence of 89.19% (n=17) in patients with anemia.

The second ranking in frequency in our sample was the pruritus 73.81%. The pruritus varies in its prevalence in different studies, i.e. ranging between 50-90% [11]. In the study from 2019 published by Rehman I. U. *et al.*, the prevalence of pruritus was 61.4% [29], whereas in a study from 2021 by Tajalli F. *et al*. it showed prevalence of 57.14% and it was not yet considered as the most common cutaneous manifestations [30]. The CKD associated pruritus causes anxiety, depression and sleep disturbance, whereas the severe pruritus is described as an independent risk factor for an increased mortality and poor prognosis in this population [12]. Clinically speaking, it can be divided into localized and generalized pruritus. In our study 47.62% of the patients had localized pruritus and 26.19% of the patients had generalized pruritus. The intensity/severity of the pruritus was assessed according to the visual analog scale -VAS [31,39]. In 30.95% of the patients, mild pruritus was recorded (ranged between VAS ≥ 0, but <3), in 26.19% of the patients-medium pruritus (ranged between VAS ≥3, but <7), and in 16.67% of the patients-severe pruritus (ranged between VAS ≥7, but <9). In patients where no pruritus was found, the duration of their hemodialysis in years was only a half compared to the patients with pruritus 5.97±6.31 (0.3-18) and 10.43±8.77 (0.3-36), respectively. The longest duration of hemodialysis was registered in patients with severe pruritus 12.86±10.25 (4-30). Pruritus was found in 90.32% of the patients with anemia. Patients with pruritus had hyperphosphatemia 85.71%, hypercalcemia 100% and hypocalcemia 60%. In one study, the hyperphosphatemia is more frequent in patients with severe pruritus [32]. Pruritus is commonly a prolonged condition and deteriorates by heat exposure, sweating and xerosis. The cause of its occurrence may be multifactorial. The risk factors include male sex, elevated levels of uremic nitrogen, calcium, phosphorus, ß2 microglobulin, magnesium, aluminum, Vitamin A, histamine and fats. It is considered to be a manifestation of a chronic inflammatory condition which includes the TNF, IFN-γ, and IL2 cytokines, as well as CRP (C-reactive protein). Additional possible mechanisms for pruritus have suggested abnormal innervations, nerve damage and central sensitization, as well as a genetic predisposition associated with HLA B35 [13,14].

The hypercalcemia and hypocalcemia can cause certain cutaneous manifestations in this population. A study from 2018 has shown that 20.6% of the patients with Stage 5 CKD on hemodialysis suffer from hypocalcemia, compared to patients on hemodialysis with reference values of calcium in serum, which can be considered as an important complication in this population. The hypocalcemia and hypercalcemia can be detected via certain cutaneous manifestations [33]. In our study, hypercalcemia was noted in 9.52%, whereas hypocalcemia in 11.9% of patients.

The most common cause of renal insufficiency in this sample is NAS with 28.57% prevalence, followed by hereditary nephropathy with 23.81%, glomerulonephritis and unknown etiology with the same percentage point

prevalence 11.91%, infectious and obstructive nephropathy with 9.52%, and diabetes with 7.14%, the same as the other etiology group with 7.14%. On the other hand, in the Anees *et al*. study, the most common causes for CKD include: diabetes mellitus 41.5%, hypertension 40%, nephrolithiasis 7.5% and chronic glomerulonephritis 2.5% [6].

In our study, the hyperpigmentation was diagnosed in 45.24% of patients. Hyperpigmentation as a finding has varying prevalence percentage points in various studies amongst this population, from 40% to 80% [7-10]. In majority of studies, it was shown that the degree of pigmentation is in direct correlation with the duration of dialysis. Our results have shown a double duration of dialysis in terms of years in patients with hyperpigmentations, compared to those with no such findings. Hyperpigmentation is the result of high levels of the melanocyte-stimulating hormone (MSH) which causes elevated levels of melanin and it deteriorates by photo exposure [18].

Paleness is a result of chronic disease associated anemia and the erythropoietin deficiency, noted in 40% of the patients [18]. In our study, paleness was noted in 28.57% of the respondents, with 100% prevalence in patients with anemia. Paleness can be corrected by administering erythropoietin and correction of the anemia. Ecchymoses are extremely common and occur due to the platelet dysfunction, secondary to elevated urea and creatinine levels. In our study, ecchymoses were found in 42.86% of the respondents. There are several studies focusing on hemosiderin deposits treatment, reporting treatment success rate by means of Q-Switched (QS) 650-nm Nd:YAG laser, 50-ns QS 755-nm alexandrite laser, QS ruby laser and 700-picosecond alexandrite laser [19-21].

Half-and-half nails (Lindsay's nails) are characterized by whitening of the proximal half up to two-thirds of the nail, and the distal part is either pinkish or brownish in color. Such nail changes were found in approximately 20% of the patients with Stage 5 CKD on chronic dialysis program [22]. The precise mechanism remains unknown, however one hypothesis maintains that it is due to the increased concentration of MSH (melanocyte stimulating hormone), while another hypothesis claims that this occurs due to an edema on the nail bed [23]. In our sample "Half-and-half nails" were diagnosed in 19.05% of the respondents. All patients with hyperparathyroidism had these changes in their nails, and 75% of them were also anemic. These findings are similar to those of the a Dyachenko *et al*. (study from 2007), that showed the prevalence of nails changes in patients with CKD are not significantly dependent on the age, sex, the CKD duration, medications or the primary disease that is the cause of CKD. In this study, a significant correlation is established between changes in nails and the levels of PTH >220 pq/ml (p=0.03) [40]. PTH is the major uremic toxin responsible for the long-term consequences, such as renal osteodystrophy, vascular calcification, alterations in the cardiovascular structure and function, immune system dysfunction and anemia. These side effects contribute to an increased mortality and morbidity caused by cardiovascular disease in Stage 5 CKD patients. PTH has a vasorelaxant effect on cells of the smooth muscles of blood vessels and is in fact a potent synthesis activator of the endothelial nitric oxide [36], leading to vasodilatation of the small blood vessels.

Absence of lunula on the nail was recorded in 23.81% of the respondents. 80% of the patients with anemia had this finding. Onychomycosis was found in 40.47% in this sample and is one of the most common cutaneous changes in patients with Stage 5 CKD on hemodialysis. Thinning hair was found in 21.43% of the respondents, a similar finding as in numerous other studies [6, 7].

Morbus Kyrle was diagnosed in 2.38% of the respondents. Some authors consider it as a serious disorder of keratinization, while the majority believes that Morbus Kyrle belongs in the acquired perforating dermatitis (APD) category with prevalence of 11% in patients with Stage 5 CKD on chronic dialysis program. APD is characterized by disseminated papules, plaques and nodules with a hyperkeratotic plug on spots susceptible to pressure or manipulation. The lesions can be linearly arranged secondary to kebnerization. This should be clinically differentiated from prurigo nodularis, arthropod bites, multiple keratoacanthomas, psoriasis and lichen planus. The APD's pathophysiology is not entirely clarified. It could be due to: a) slow healing of the skin in diabetes-induced microangiopathy; b) local trauma caused by itching or dermal necrosis, microangiopathy, which results in extrusion of dermal material through the epidermis; c) foreign body reaction to altered dermal collagen and deposition of calcium salts [17].

None of the respondents was diagnosed with skin and mucous membranes cancer, pseudoporphyria, calciphylaxis and nephrogenic systemic fibrosis.

Conclusion

The muco-cutaneous changes/symptoms were a common finding in patients with Stage 5 CKD on hemodialysis. In all patients, at least one muco-cutaneous change/symptom was diagnosed. The causes of CKD do vary, however in this sample the most common cause was the NAS associated nephropathy. The most common muco-cutaneous changes/symptoms were xerosis, pruritis and hyperpigmentations.

Many of the skin changes/symptoms, its adnexa and mucosa are benign and do not affect the course of CKD. However, some of them may be considered as serious systemic disorders in the patients. Studies investigating the muco-cutaneous changes/symptoms in patients with Stage 5 CKD on hemodialysis are quite necessary so that the quality of life in these patients can be impro-

ved. Interdisciplinary management that involves dermatologists is of vital importance.

Conflict of interest statement. None declared.

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Original article

Dermatologic Problems During COVID-19 Pandemics in Kidney Transplant Recipients

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Abstract

Introduction. Our stady aimed toinvestigate the rate of dermatologic disorders in kidney transplant recipients during the COVID-19 pandemic.
Methods. We performed a retrospective observational single-center cohort study including all adult renal transplant recipients with a functioning kidney allograft and who have recovered from the SARS CoV-2 infection. The study was conducted at a tertiary center in Croatia from March 2020 to August 2022. The study included 321 patients (57% were male). Data were obtained retrospectively from hospital charts and records, while self-reported cosmetic problems were reported prospectively.
Results. The study included 321 patients (57% were male). Overall, 59 patients (18%) reported or were diagnosed with dermatological conditions. Eleven patients presented with facial eruptions that were most pronounced in the area covered by the mask, one patient developed similar changes in the skin of her hands. Hair loss was reported by twenty female patients, with the hair loss persisting in three patients. Six patients were diagnosed with skin cancer in the areas covered by facial masks. Three had squamous cell skin cancer, two were diagnosed with basal cell skin cancer, and one had a neuroendocrine skin tumor on the chin.
Conclusion. Dermatologic problems are frequent in kidney transplant recipients recovered from acute COVID-19. Besides cosmetic problems, skin malignancies may be diagnosed with a delay. Kidney transplant recipients should be advised to regularly self-examine their skin for potential skin cancer with dermatologic evaluation when necessary.

Key words: dermatological disorders, skin cancer, facial mask, covid-19, kidney transplant recipients

Introduction

Many skin manifestations associated with SARS-CoV-2 infection have been reported, including chilblain-like lesions, maculopapular lesions, urticarial lesions, vesicular lesions, and livedoid lesions. Erythema multiforme-like lesions and multisystem inflammatory syndrome are rare but may occur in association with COVID-19 [1,2]. Besides the virus itself, COVID-19 vaccines can cause a variety of skin reactions. The most common are unspecific injection-site reactions, different hypersensitivity reactions, autoimmune-mediated skin findings, and less frequently, functional angiopathies [3]. Much less is known about dermatologic problems arising during the COVID-19 pandemic which are not a direct consequence of the virus.
Kidney transplant recipients (KTRs) are a specific group of patients with unique problems during the COVID-19 pandemics. They have an increased risk for the development of skin cancer and require regular dermatologic follow-up. Data on dermatologic problems in KTR during the COVID-19 pandemics are lacking. Therefore, we investigated the rate of dermatologic complications and their outcome in this group of patients.

Material and methods

The study was designed as a retrospective observational single-center cohort study, participants were recruited from tertiary center in Croatia to estimate dermatologic complications and their outcomes. Data were retrospectively obtained from hospital charts and records, while self-reported cosmetic problems were reported prospectively. The study included all adult renal transplant recipients with a functioning kidney allograft between March 2020 and August 2022, who have recovered from COVID-19. SARS-CoV-2 infection was proven by a positive SARS-CoV-2 real-time reverse transcripttase-polymerase chain reaction (RT-PCR) test. We have no data on the type of SARS-CoV-2 that caused the infection.
To assess clinical complications, patients were interviewed by a standardized survey by trained transplant nephrologists to recount their symptoms during acute

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Table 1. Patients' characteristics. ADPKD, autosomal dominant polycystic kidney disease; CMV, cytomegalovirus; EBV, Epstein-Barr virus; BKV, BK virus; MMF, mycophenolate mofetil; Aza, azathioprine; CyA, cyclosporine; Tac, tacrolimus

Characteristics	Number (%) of patients	Range
Sex		
Male	183 (57)	
Age (years) [Median (IQR)]	55 (44 - 64)	22 - 81
Primary kidney disease		
Glomerulonephritis	98 (30,6)	
Diabetic nephropathy	12 (3,8)	
ADPKD	48 (15)	
Pyelonephritis	26 (8,1)	
Nephroangiosclerosis	26 (8,1)	
Other	110 (34,4)	
Time from transplantation (months) [Median (IQR)]	94,5 (52 - 135,8)	1 - 368
Height (cm) [Median (IQR)]	171 (163 - 180)	124 - 199
Body weight (kg) [Median (IQR)]	79 (67 - 92)	42 - 150
BMI [Median (IQR)]	26,5 (23,9 - 29,2)	17,36 - 45,79
Nutritional status		
Underweight (BMI< 18,5)	4 (1,3)	
Normal weight	105 (32,8)	
Pre-obesity (25 – 29,9)	144 (45)	
Obese (≥ 30)	67 (20,9)	
Previous thrombosis	30 (9,4)	
Previous myocardial infarction or stroke	32 (10)	
Previous CMV infection	36 (11,3)	
Previous BK infection	68 (21,3)	
Previous EBV infection	28 (8,8)	
Allograft rejection	46 (14,4)	
Creatinine value [Median (IQR)]	129 (98 - 165,8)	45 - 430
CKD EPI [Median (IQR)]	49 (35 - 64)	0,23 - 133
Biuret [Median (IQR)]	0,2 (0,1 - 0,5)	0 - 79
Vaccinated against COVID-19	246 (76,9)	
Before COVID-19 infection	149 (46,6)	
After COVID-19 infection	97 (30,3)	
Number of vaccine doses [Median (IQR)]	2 (2 – 3)	1 - 4
Number of vaccine doses (n = 246)		
One	21 (8,5)	
Two	138 (56,1)	
Three	83 (33,7)	
Four	4 (1,6)	
COVID-19 initial symptoms		
Febrility	245 (76,6)	
Diarrhea	39 (12,2)	
Respiratory	230 (71,9)	
Asymptomatic	21 (6,6)	
COVID-19 initial complications		
Hospitalisation	125 (39,1)	
Pneumonia	141 (44,1)	
Mechanical ventilation	4 (1,3)	
Other	66 (20,6)	
Initial immunosuppression		
Tacrolimus	222 (69,4)	
Cyclosporin A	70 (21,9)	
Mycophenolate	280 (87,5)	
Azathioprine	12 (3,8)	
Everolimus	48 (15)	
Prednisolone (dose) [Median (IQR)]	5 (5 - 5)	0 - 30
Acute COVID-19 treatment		
Cessation of MMF/Aza	133 (41,6)	
Decreasing MMF/Aza	102 (31,9)	
Cessation of Tac / CyA	1 (0,3)	
Decreasing Tac / CyA	29 (9,1)	
Hyperimmune anti-CMV globulin	30 (9,4)	
Intravenous immunoglobulin	13 (4,4)	

COVID-19, and additionally questioned on the persistence or new onset of any symptoms. After the interview, the patients underwent a detailed physical examination. Additional diagnostic methods were used per clinician judgment (laboratory, radiologic). Data on the immunosuppressive regimen and acute COVID-19 characteristics were recorded.
The study was approved by the University Hospital Center Zagreb Ethics committee.

Results

Study population

From March 2020 to August 2022, 408 out of the initial cohort of 1432 patients who received a renal allograft at our institution developed COVID-19 disease proven by a positive SARS-CoV-2 RT-PCR of a nasopharyngeal swab and were potentially eligible for study participation. Twenty-five patients died in the period during or after the infection and 62 patients have not been assessed in our clinic and were therefore excluded from the study population. Overall, 321 patients were included, 57% males with data presented in the table 1. One-hundred-and-fifty patients (46,7%) received at least one dose of the anti-SARS-CoV-2 vaccine before the infection. One hundred twenty-five (39,1%) patients required hospitalization, 141(44,1%) developed pneumonia and 4 patients (1,3%) required mechanical ventilation. Treatment included immunosuppression modification in 233 patients (77,1%) and remdesivir in 53 patients (16,6%), along with other supportive measures.

Self-reported cosmetic skin problems

Twenty-one patients (6.5%) reported severe acne, two of them on the chin, cheeks, and forehead, while four had acne on the back. They all received steroids for treatment of acute COVID-19.
Eleven patients presented with new onset facial eruptions that were most pronounced in the area covered by the mask. Six were diagnosed with contact dermatitis or "rosacea" and five as "seborrheic dermatitis" (Figures 1, 2, 3). Additionally, one patient developed similar changes in the skin of her hands due to excessive hygiene.

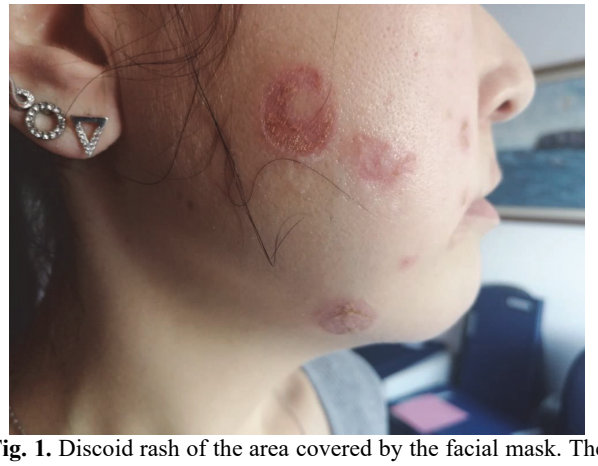

Fig. 1. Discoid rash of the area covered by the facial mask. The patient was diagnosed with contact dermatitis

Fig. 2. Local status 2 months later. The patient did not use her facial mask for two months

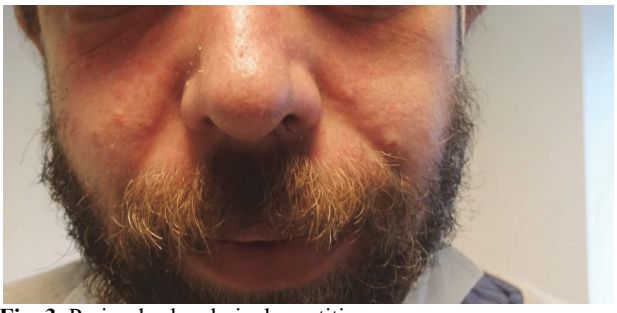

Fig. 3. Perioral seborrhoic dermatitis

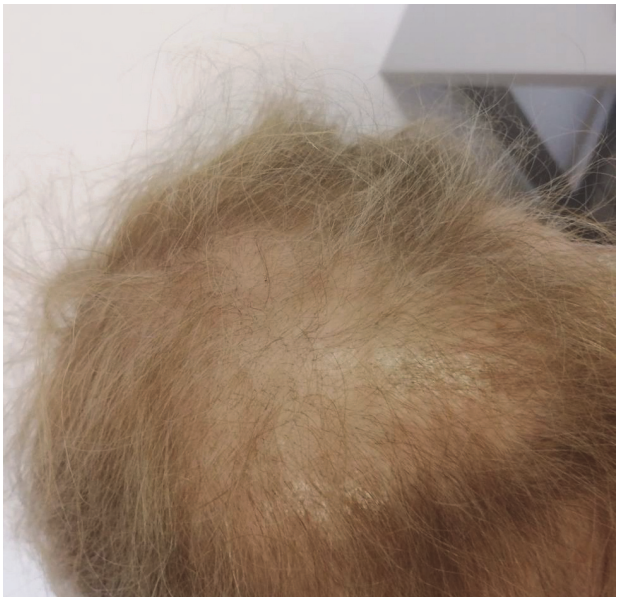

Fig. 4. Telogen effluvium in a 46-year-old female

Hair loss was reported by twenty female patients. The problem was severe in 8 patients (Figure 4), while 12 reported moderate hair loss. The symptom of hair loss developed three to six weeks after the clinical manifestation of COVID-19. Increased shedding was most pronounced during the first three months, with gradual improvement over the next six months. Although most patients recover, three of them have persistent hair loss.
Twelve patients received topical treatment with minoxidil solution as per dermatologist recommendations.
Six patients were diagnosed with skin cancer in the areas covered by facial masks. They were all diagnosed when asked by the attending nephrologist to remove the mask and none of them reported the changes. Three had squamous cell skin carcinoma, two were diagnosed with basal cell skin cancer, and one had a neuroendocrine skin tumor on the chin (Figures 5 and 6).

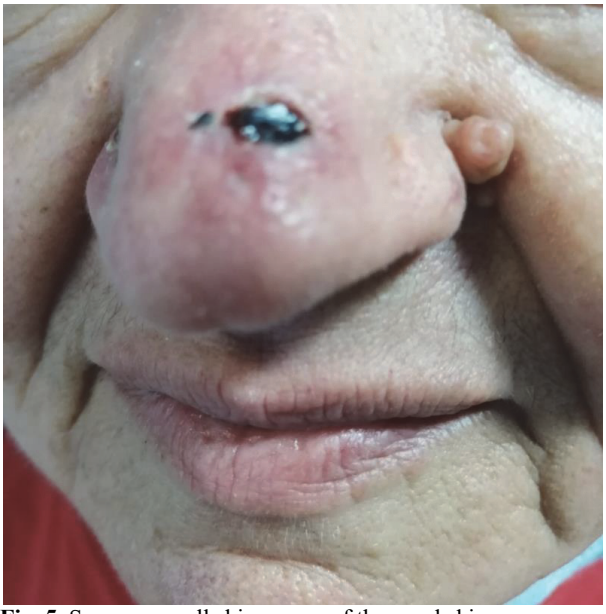

Fig. 5. Squamous cell skin cancer of the nasal skin

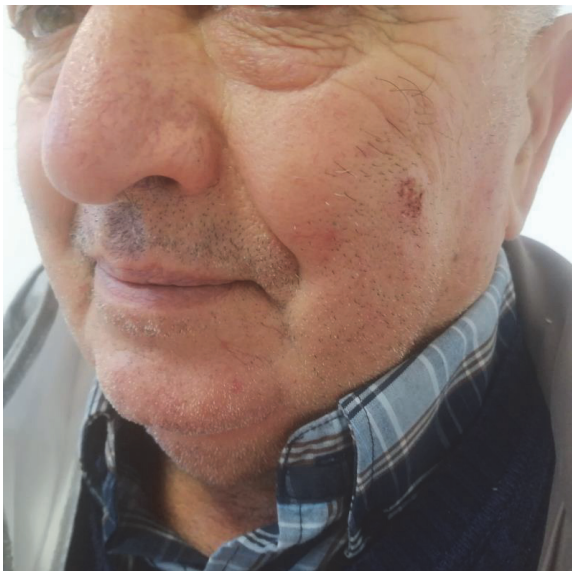

Fig. 6. Basocellular skin cancer of the cheek

Discussion

In our study, nearly every fifth patient (18%) was diagnosed with a dermatological condition following recovery from acute COVID-19, regardless of symptom severity. Most frequently diagnosed were acne, hair loss, seborrheic dermatitis, rosacea, and skin cancer. Most importantly, the discovered skin cancers were all in the areas covered by face masks, no patient self-reported the skin change, and the changes would not have been discovered had the clinician not asked specific questions and asked for the mask to be removed.
Since the outbreak of the COVID pandemic, outpatient visits are performed with strict epidemiological measures enforcing the use of facial masks. However, masks covering parts of the face during the examination and the lack of patients self-reporting skin changes may be associated with unrecognized problems beneath the mask [4]. Upon learning that patients don't report skin changes, our team introduced obligatory mask removal for a few seconds. This approach resulted in the discovery of different skin problems including malignancies. Several studies have recognized the problem of diagnostic delay of skin cancers during the COVID-19 pandemic [5-7], with postponed surgical excisions resulting in an increased incidence of advanced skin cancers [7)]. This problem might be additionally emphasized in kidney transplant recipients with skin cancers being more aggressive than in the general population due to the immunosuppressive drugs used for the prevention of rejections [8].
Our study's most common self-reported dermatologic problem was hair loss (telogen effluvium). Telogen effluvium is a well-known COVID-associated problem [9-11]. The most common triggers for telogen effluvium are severe infections and nutritional deficiencies, especially vitamin D, ferritin, and zinc deficiencies [12,13]. In COVID-19, both triggers are present, together with higher levels of inflammatory cytokines and microthrombi formation which may obstruct hair follicle blood supply [14].
The association between acne and prolonged use of protective face masks has been observed even before the onset of the COVID-19 pandemic [15]. Adverse skin reactions caused by prolonged wearing of masks include pressure injury, urticaria, contact dermatitis, skin dryness, and aggravation of preexisting skin diseases [16]. A greater number of individuals who suffered from acne associated with the prolonged wearing of protectt ive masks during COVID-19 pandemic have been referred to dermatologists, leading to the introduction of a new term for this variant of acne mechanica-"maskne" [17]. Possible mechanisms of acne pathogenesis include mechanical stress through pressure and friction caused by the mask [18], microbiome dysbiosis caused by heat, alteration of skin pH and humidity [19] which is stimulative for bacterial proliferation [20], but also increased

steroid dosages during acute COVID-19 [21]. Steroids may explain the occurrence of acne on the back and not only on the face of our patients. In the study by Ozkesici *et al* the course of acne during the COVID-19 pandemic in healthcare professionals was monitored. Almost half of the participants reported an increase in pre-existing acne, while more than one-third of the participants reported first occurrence or had a relapse. Among many factors, surgical masks have been found to be responsible for the development of acne [22].
KTRs are known to have a significantly increased risk of developing skin malignancy secondary to chronic immunosuppression [23]. In the study by Keeling *et al.* a slight increase in the number of diagnosed skin cancer during COVID-19 pandemic was observed in KTRs and finally, the importance of face-to-face outpatient examinations was emphasized [24].

Conclusion

In conclusion, dermatologic problems are frequent in kidney transplant recipients recovered from acute COVID-19. Besides cosmetic problems, skin malignancies may be diagnosed with a delay. Kidney transplant recipients should be advised to regularly self-examine their skin for potential skin cancer, report any changes to their physicians and to seek a dermatologic evaluation when necessary.

Conflict of interest statement. None declared.

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Original article

Anti-Cytomegalovirus Hyperimmune Immunoglobulins as Adjunctive Therapy during Acute COVID-19 in Kidney Transplant Recipients: A Single-Center Retrospective Cohort Study

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Abstract

Introduction. Anti-CMV immunoglobulin (CMV-HIG) contains antibodies against various infective pathogens and not only against the cytomegalovirus (CMV), thus possibly mimicking the convalescent plasma.
Methods. A retrospective analysis concerning the practice of CMV-HIG off-label use during acute COVID-19 in kidney transplant recipients (KTR).
Results. From March 2020 to August 2022, 403 KTR (59.8% male) who developed COVID-19 were eligible for investigation. 151(44.4%) patients required hospitalization, and eighteen (5,6%) mechanical ventilation. Thirty-four (8.4%) patients received CMVHIG. Two patients had CMV reactivation and received CMVHIG 2 ml/kg in five doses. Others had hypogammaglobulinemia which was an additional off-label indication for using CMVHIG during acute COVID-19. 22 patients (6.5%) died, 4 of them from the CMVHIG group.
Conclusion. A correction of hypogammaglobulinemia, potential remodeling of the immunological response, and CMV reactivation during acute infection, may justify the use of CMVHIG during acute COVID-19.

Keywords: COVID-19, SARS-CoV-2, CMV, Hyperimmune anti-CMV globulin

Introduction

The pandemic of the coronavirus disease 2019 (COVID-19) has resulted in more than 690 million infections and almost 7 million deaths by July 2023 [1]. Advanced age, obesity, hypertension, diabetes, immunosuppression, and other chronic diseases have all been associated with increased severity of COVID-19 [2-4]. However, almost 50% of severe cases occur without obvious pre-existing conditions [4]. Cytomegalovirus (CMV) is a widely prevalent herpes virus. CMV-induced immune system remodeling was suggested in the pathogenesis of SARS-CoV-2 infection, and may be associated with more severe COVID-19 forms [5,6].
Treatment of acute COVID-19 remains challenging, while even vaccination failed to protect immunocompromised patients due to frequent breakthrough infections [7]. COVID-19 convalescent plasma (CP) contains neutralizing SARS-CoV-2 antibodies obtained from patients who recovered from acute COVID-19 but has been used with inconsistent results [8,9]. However, it had an important role in the early era of COVID-19 treatment when neither effective vaccines nor monoclonal antibodies were available on the market. Although the role of CP remains controversial, it may remain an important tool for the treatment of immunocompromised patients [10]. Intravenous immunoglobulins (IVIG) are widely used as additional treatment for patients with severe COVID-19 due to their immunomodulatory actions which can be potentially useful [11-14]. Hyperimmune anti-CMV immunoglobulin (CMV-HIG) has been approved as an adjuvant treatment for patients with CMV infection]15]. The product contains antibodies against various infective pathogens and not only against the CMV [16], thus possibly mimicking the convalescent plasma. At the beginning of the COVID-19 pandemic, with no available treatment or vaccine, we were the first to hypothesize that CMV-HIG might provide passive protection against SARS-CoV-2 [17].
Herein, we report our experience in off-label treatment of kidney transplant recipients with CMVIG in the context of SARS-CoV-2 infection.

Material and methods

This retrospective observational study comprised kidney transplant recipients with acute SARS-CoV-2 infection who received off-label CMV-HIG during acute SARS-CoV-2 infection. Treatment was individually chosen according to the attending transplant nephrologist.

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It was based on patients' history, severity of acute COVID-19 and laboratory findings (hypogammaglobulinemia, positive CMV DNA). The study was approved by the Ethics Committee with the informed consent from the patients.

Primary outcomes of the study were indications for the use of CMV-HIG (prevention or therapy of CMV infection), CMV-HIG protocol and dosages. Secondary outcomes included outcome of treatment, percentage of patients who reactivate CMV, adverse events, rehospitalizations after acute COVID-19, and follow-up results from the start of treatment until 6 months after the end of treatment with CMVHIG.

To assess clinical complications, patients were interviewed by a standardized survey by trained transplant nephrologists to recount symptoms during the acute illness and whether they persisted or some new occurred to assess clinical complications. Patients also underwent a detailed physical examination. Additional diagnostic methods were used individually (laboratory, radiologic). Data on immunosuppressive regimen and acute COVID-19 characteristics were recorded. Venous blood samples were collected for complete blood count, biochemistry, coagulation examinations (prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen), D-dimers, C3, C4, total complement, platelet aggregation with ADP (adenosine 5′-diphosphate), serum electrophoresis, donor-specific antibodies, and virology (molecular diagnostic detection for cytomegalovirus (CMV), Epstein-Barr virus (EBV) and BK virus (BKV)). Donor specific antibodies were determined by Luminex bead-based technology (One lambda). Results were compared with historical values. We had no data regarding the SARS-CoV-2 serology.

Patients have been in continuous follow-up, with reassessment at six months after acute SARS-CoV-2 infection.

Categorical data were presented by absolute and relative frequencies. The normality of the distribution of continuous variables was tested by the Shapiro-Wilk test. Continuous data were described by the median and the limits of the interquartile range (IQR). The Mann-Whitney U test was used to compare the median between two groups, while Fisher's exact test was used to analyze the differences between proportions. Logistic regression analysis was used to analyze the independent factors associated with the development of clinical complications or laboratory abnormalities. A stepwise multivariable logistic regression was used to assess the association between potential risk factors and development of laboratory or clinical complications, adjusting for known confounders. Variables assessed included demographic characteristics (ie, age, gender, primary kidney disease), clinical characteristics (ie, different comorbidities), acute COVID-19 characteristics (ie, presentation, need for hospitalization). Parameters with statistical significance in the univariate analysis were incorporated into the multivariate logistic regression model for in-depth analysis. The level of significance was set at an Alpha of 0.05. Considering the relatively small sample size and the possibility of overfitting in the multivariate logistic regression model, we adopted a forward stepwise method (probability for stepwise: entry $P<0.05$, removal $P>0.1$) for logistic regression analysis to reduce the number of independent variables entering the model. There was no substitution of the missing data. The statistical analysis was performed using MedCalc® Statistical Software version 19.6 (MedCalc Software Ltd, Ostend, Belgium; https://www. medcalc.org; 2020) and the IBM SPSS Stat. 23 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.).

Results

From March 2020 to August 2022, 403 patients (59.8 % male) who received renal allograft at our institution developed COVID-19 and were eligible for investigation. The most common primary kidney diseases were glomerulonephritis (28%) and autosomal dominant polycystic kidney disease (15.3%).

Patients' characteristics are presented in Table 1. Hospitalization during acute SARS-CoV-2 infection was necessary for 151(44,4%) patients. Eighteen (5,6%) patients required mechanical ventilation. Thirty-four (8.4%) patients received CMVHIG during acute COVID-19. Eleven patients (33%) from the CMVHIG group and forty-six (20%) from the non-CMVHIG group received at least one dose of vaccine before developing acute

Table 1. Patients' characteristics. TX, transplantation; BMI, body mass index; No, number, CKD-EPI eGFR, Chronic Kidney Disease Epidemiology Collaboration estimated glomerular filtration rate; IQR, interquartile range

	Median (IQR)			P*
	CMVHIG	Other	Total	
Age (years)	52.5(45-66.5)	57(46-64.75)	57(46-65)	0.57
TX vintage (months)	85.5(59-138.75)	96.5(53-138)	95.5(53-137.75)	0.88
BMI (kg/m^2)	25.14(23.4-28.48)	26.6(23.98-29.37)	26.5(23.92-29.32)	0.17
No of AHT drugs	3(1-4)	2(1-3)	2(1-4)	0.04
Steroid dose	5(5-7.5)	5(5-5)	5(5-5)	0.11
CKD-EPI eGFR	42.5 (32-53.5)	49(35-63)	48(35-62)	0.13
Proteinuria	0.32 (0.17-0.91)	0.23(0.12-0.49)	0.23(0.13-0.51)	0.03
*Mann Whitney U test				

COVID-19.
Patients who received CMVHIG more frequently had diabetes (38.3% vs.22.6%, p=0,04), a history of CMV infection after the transplantation (24.2% vs. 10.1%, p=0.01), needed more antihypertensive drugs and more frequently had a history of acute rejection (26.5 vs. 13.1 %, p=0.03).
During acute COVID-19, patients treated with CMVHIG more frequently had pneumonia that required hospitalization and mechanical ventilation (Table 2).

Table 2. Clinical presentation during acute COVID-19 in patients treated with CMVHIG and patients wo did not receive CMVHIG. Other symptoms included chest pain, abdominal pain, and loss of smell and taste

Acute COVID	Number (%) patients			P*
	CMVHIG	Other	Total	
Febrility	29(85.3)	252(80.3)	281(80.7)	0.48
Diarrhoea	9(26.5)	46(14.7)	55(15.9)	0.08
Respiratory	27(79.4)	226(72.7)	253(73.3)	0.40
No symptoms	1(3)	24(7.8)	25(7.3)	0.49†
Pneumonia	30(90.9)	135(45)	165(49.5)	<0.001
Other symptoms	15(44.1)	61(16.5)	76(18.9)	<0.001
Hospitalization	31(93.9)	120(39.1)	151(44.4)	<0.001
Mechanical ventilation	5(17.9)	13(4.4)	18(5.6)	0.01†

*χ^2 test;†Fisher exact test

Treatment during acute SARS-CoV-2 infection included immunosuppression modification in 261 patients (64.7%) (Table 3), remdesivir (61 patients (52,1%)), hydroxychloroquine (12 patients (2.9%), prophylactic use of low-molecular-weight heparin, glucocorticoids and antibiotics. Additionally, besides the patients who were treated with CMVHIG (34 patients, 8.4%), 17 patients (5.5%) received intravenous immunoglobulins, and four (1%) received convalescent plasma. Four patients (1%) were treated with tocilizumab. Other patients did not receive specific treatment because they either had a mild disease or did not inform us timely about the infection.

Table 3. Immunosuppressive therapy modification during acute COVID-19

	Number (%) of patients			P*
	CMVHIG	Other	Total	
MMF/Aza cessation	26(78.8)	122(41,8)	148(45.5)	<0.001
Decreased MMF/Aza	7(21.9)	106(36,3)	113(34.9)	0.12
Tac/CyA cessation	4(12.9)	1(0.3)	5(1.6)	<0.001
Decrease Tac/Cya	0	26(9)	26(8.9)	>0.99

*Fisher exact test"

Two patients had positive CMV DNA during acute COVID-19. Besides the ganciclovir, they both received CMVHIG 2 ml/kg in five doses. Hypogammaglobulinemia was an additional off-label indication for using CMVHIG during acute COVID-19. Hyperimmune anti-CMV globulin was applied in the dose of 1 ml/kg in 1 to 3 doses depending on the condition of patients, but also on the length of hospitalization.
Twenty-two patients (6.5%) died during acute COVID-19 (18 from multiorgan failure, three from myocardial infarction, and one from resistant CMV infection). Four of them were from the CMVHIG group.
Patients who survived acute COVID-19 underwent post-COVID-19 follow-up at the ambulatory visit 6-8 weeks after the infection. CMV reactivation was recorded in 21.7% of patients after recovery from acute COVID-19, with no statistically significant difference between patients who received CMVHIG during acute COVID-19 (30.4%) and patients who did not receive CMVHIG (20.7%). There were no significant differences in post-COVID complications between groups (Table 4).

Table 4. Post-COVID complications in patients treated with CMVHIG and patients who did not receive CMVHIG during acute COVID-19. CMV, cytomegalovirus; EBV, Epstein Barr virus; BKV, BK virus.

	Number (%) of patients			P*
	CMVHIG	Other	Total	
Kidney biopsy	4(16.7)	12(5.2)	16(6.3)	0.05
Neuropathy	0	10(4.3)	10(3.9)	0.61
CMV	7(30.4)	45(20.7)	52(21.7)	0.29
BKV	6(27.3)	54(25)	60(25.2)	0.80
EBV	12(54.5)	77(35.8)	89(37.6)	0.11
Hypogammaglobulinemia	10(45.5)	62(30.7)	72(32.1)	0.31

*Fisher exact test

Table 5. Post-COVID-19 laboratory analysis. CKD-EPI eGFR, Chronic Kidney Disease Epidemiology Collaboration estimated glomerular filtration rate; APTT, activated partial thromboplastin time

	Median (IQR)			P*
	CMVHIG	Other	Total	
Proteinuria	0.25(0.13-1.1)	0.26(0.14-0.59)	0.26(0.13-0.59)	0.70
CKD-EPI eGFR	44(28-58)	50 (35,25 - 69)	49 (35 - 69)	0.15
D-dimers	1.31(0.55-1.96)	0.56(0.34-1.06)	0.57(0.35-1.26)	0.01
Prothrombin time	1.17(1.08-1.27)	1.13(1.02-1.23)	1.13(1.04-1.24)	0.31
APTT	21.75(20.08-23.7)	22.2(20.8-23.4)	22.2(20.8-23.4)	0.52
Fibrinogen	4.6(3.3-6,45)	3.4 (2.93-4.4)	3.5(3-4.55)	0.01
Plattelet aggregation	71(62-78.5)	78(72-85)	78(72-84)	0.004
C3	1.21(0.99-1.46)	1.23(1.06-1.47)	1.22(1.06-1.46)	0.63
C4	0.30(0.23-0.36)	0.26(0.21-0.33)	0.26(0.21-0.33)	0.19
CH50	105(94-122)	105.5(94-113)	105(94-114)	0.51
*Mann Whitney U test				

Laboratory analysis performed after acute COVID-19 revealed increased D-dimers, fibrinogen, and platelet aggregation in patients treated with CMVHIG compared to patients who did not receive CMVHIG (Table 5).
Laboratory analysis performed after acute COVID-19 revealed increased D-dimers, fibrinogen, and platelet aggregation in patients treated with CMVHIG compared to patients who did not receive CMVHIG (Table 5).

In bivariate logistic regression analysis, diabetes mellitus, the severity of acute COVID-19, and kidney allograft dysfunction during acute infection were identified as significant predictors for CMV reactivation after recovery from SARS-CoV-2 infection (Table 6) however, the number of cases needed to be bigger for multivariate analysis.

Table 6. Bivariate logistic regression analysis for prediction of CMV reactivation after recovery from acute COVID-19. MMF, mycophenolate mofetil; Aza, azathioprin; CMVHIG, hyperimmune antiCMV globulin; IvIG, intravenous immunoglobulin

Bivariate analysis	ß	Wald	OR (95% CI)	P
Therapy (CMVHIG vs. other)	0.51	1.13	1.67(0,64-4.31)	0.29
Diabetes	0.68	3.87	1.98(1.01-3.9)	0.04
Febrility	1.06	4.48	2.89(1.08-7.72)	0.03
Respiratory symptoms	1.35	8.49	3.8(1.56-9.5)	0.004
Pneumonia	1.15	11.5	3.15(1.62-6.09)	<0.001
Other complications during COVID-19	0.80	4.74	2.22(1.08-4.56)	0.03
Allograft dysfunction	0.23	3.91	10.2(1.02-101.5)	0.04
MMF/Aza cessation	0.74	5.25	2.09(1.11-3.94)	0.02
CMVHIG	0.53	1.18	1.69(0.65-4.40)	0.28
IvIg	1.62	5.47	5.06(1.3-19.7)	0.02

ß-regression coeficient

Within six months after acute COVID-19, 40% of patients from the CMVHIG group required hospitalization, compared to 17.3% of patients not treated with CMVHIG (p=0.01). The most common indications in both groups were pneumonia and urinary tract infections.
COVID-19 reinfection was recorded in one patient from the CMVHIG and three patients from the group not treated with CMVHIG.

Discussion

In our retrospective analysis concerning the practice of CMV-HIG off-label use during acute COVID-19, we assessed two main indications: the application of CMV-HIG as adjunctive treatment of acute COVID-19 and CMVHIG as adjunctive treatment of CMV reactivation to the antivirals during the SARS-CoV-2 infection.
Out of 34 patients treated with CMVHIG, two had concomitant SARS-CoV-2 and CMV infection, while others received CMVHIG as adjunctive therapy for COVID-19. Administration of CMVHIG was well tolerated without any side effects. Compared to the rest of our cohort, patients treated with CMVHIG had more severe acute COVID-19, as indicated by the need for mechanical ventilation.
Cytomegalovirus is one of the most significant non-genetic determinants of the immune system with its pronounced immunomodulatory effects. It has the strong potential to shape the course of SARS-CoV-2 infection, either because of CMV reactivation or due to the reshaping of immune response to SARS-CoV-2. However, it remains unclear whether CMV reactivation is a direct consequence of SARS-CoV-2 infection, or results from COVID-19 immunomodulatory therapies [18], but was found to be associated with an increased risk of COVID-19-related hospitalizations [19]. Osawa *et al.* reported that in the population of patients hospitalized in intensive care units, steroid administration, pro-

longed mechanical ventilation, and sepsis have all been recognized as risk factors for CMV reactivation [20].

Prevention and treatment of acute COVID-19 are still not optimal. Vaccination of immunocompromised patients results with frequent breakthrough infections [7], and antivirals are of limited efficacy. Convalescent plasma has been used for treatment of SARS-CoV-2 infection from the beginning of the pandemic with controversial results [21-24]. Convalescent plasma seems to exert its therapeutic potential through direct viral neutralization, antibody-dependent cellular cytotoxicity, complement system activation, and phagocytosis. A cardinal factor in its efficacy is the high level of antibodies administered [25] what was not uniqe and not evaluated in majority of published studies. Dulipsingh *et al*. have shown that subjects with a single infection with SARS-CoV-2 did not have the same levels of neutralizing antibodies that we observed in subjects either in the convalescent or the naive vaccinated groups. Neutralizing antibodies were significantly higher in vaccinated patients than in the convalescent unvaccinated group [26]. As the pandemic evolved, antibody treatment transitioned from convalescent plasma (CP) to monoclonal antibody preparations [27,28]. However, convalescent plasma should still be considered for immunosuppressed COVID-19 patients.

The benefit of IVIG therapy for acute COVID-19 is also controversial [29-31]. As well as with CP, the insufficient effects of IVIG therapy may be the results of dosage, administration timing, and disease severity at the time of administration [32]. Unfortunately, IVIG was usually used for severe or critically ill patients due to the high price and possible side effects. Also, a high number of patients with severe and critical COVID-19 resulted in frequent shortages of IVIG during the pandemic. We used CMVHIG for treatment of CMV infection or for correction of hypogammaglobulinemia in hospitalized patients with moderate to severe acute COVID-19. SARS-CoV-2 infection is often associated with secondary hypogammaglobulinemia, which correlates with the risk of infection and is often treated with immune globulins to support humoral immune responses [33].

In our cohort, patients treated with CMVHIG all had moderate or severe acute COVID-19, and four patients died (11.7%). Reported mortality rates from the literature approaches up to 28% [34-36], suggesting the possible efficacy of CMVHIG as adjunctive therapy for hypogammaglobulinemic immunocompromised kidney transplant recipients and patients with CMV reactivation for treatment of CMV recativation during acute COVID-19.

Data on the use of CMV antivirals in COVID-19 patients is scarce. Interestingly, Schoninger *et al*. failed to find any clear clinical benefit to treating CMV reactivation in the COVID-19 patients in intensive care unit [37]. The ganciclovir-treated subgroup did not display an increased morality rate in Italian study [38]. Our patients responded well to tretament with antivirals and CMVHIG.

While data on tretament of CMV reactivations during acute COVID-19 is limited, even less is known on CMV reactivations post-COVID. In our study, fifty-two patients (12.9%) reactivated CMV infection after recovery from acute SARS-CoV-2 infection. In bivariate logistic regression analysis, diabetes mellitus, the severity of acute COVID-19, and kidney allograft dysfunction during acute infection were identified as significant predictors for CMV reactivation. There was no correlation between the treatment with CMVHIG during acute COVID-19 and CMV reactivation in the post COVID follow up.

Rehospitalizations were frequent. In out previous, multicentre study, the most common indications for hospitalization after acute COVID-19 were pneumonia (24.5%) and renal allograft dysfunction (22.4%), followed by sepsis (14.3%) and thrombotic events (10.2%). The strongest predictor for hospitalization after recovert from SARS-CoV-2 infection in this study was hospitalization for acute COVID-19, while better allograft function decreased the probability of hospitalization [39,40].

During the post-COVID-19 follow up, patients from the CMVHIG group had significantly higher D-dimers, fibrinogen and platelet aggregation. These findings may indicate pro-inflammatory and hypercoagulable state, increasing the likelihood for induction of thromboembolism or stroke [41]. However, only one patient from the CMVHIG group had developed thromboembolic complication (embolization of the ulnarv artery). Reported incidence of IVIG-induced thrombotic complications ranges from 3 to 13% [42]. Risk factors for IVIG-induced thrombosis include male gender, older age, renal insufficiency, diabetes, dyslipidemia, hypertension; immobility; coronary heart disease, history of vascular diseases, family history of thromboembolic diseases, atrial fibrillation, high-dose and high-speed IVIG infusions [43]. In our previous study, the most common laboratory abnormalities after recovery from acute COVID-19 were shortened activated partial thromboplastin time (50%), elevated D-dimers (36.5%), elevated fibrinogen (30.16%), and hypogammaglobulinemia (24%) [39].

Limitations of this study are the retrospective single-centre design, lack of randomization, and the heterogeneity of the available data. The number of patients who received CMVHIG was too small for multivariate analysis. Also, we had no detailed laboratory data during acute COVID-19 for all patients. Additionally, the use of CMVHIG was limited by the length of hospitalization, which was often determined by the pressure of the huge number of infected patients requiring hospital treatment for SARS-CoV-2 infection. However, this is the first study on the use of CMVHIG during acute COVID-19. It indicates a potential benefit of CMVHIG during acute COVID-19 in immunocompro-

mized, hypogammaglobulinemic kidney transplant recipients. Additionaly, based on our experience, it seems that CMVHIG may provide a certain level of antibodies against some other pathogens in the future.

Conclusion

In conclusion, a correction of hypogammaglobulinemia, potential remodeling of the immunological response, and CMV reactivation during acute infection, which adversely affect outcomes in infected individuals, may justify the use of CMV-HIG during acute COVID-19. Its role in protection from SARS-CoV-2 reinfection sholud be investigated. Given the limited therapeutic options and COVID-19 mortality rate, CMVHIG is worth considering. However, prospective randomized trials on the use of CMV-HIG under regimens for dosage, mode, and time of administration are urgently needed to obtain better efficacy and safety data.

Conflict of interest statement. None declared.

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