“OVIDIUS” UNIVERSITY OF CONSTANTA

DOCTORAL SCHOOL OF MEDICINE

MEDICINE

DOCTORAL THESIS

BENEFITS AND RISKS OF ALLOGENEIC TRANSFUSION THERAPY IN PATIENTS WITH CHRONIC KIDNEY DISEASE

SUMMARY

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CONSTANȚA 2024

CONTENTS

Keywords: chronic kidney disease, anemia, thrombocytopenia, transfusion.

GENERAL PART

INTRODUCTION

Chronic kidney disease (CKD) has become in the last decade a major public health problem with a demonstrated continuous increase in both incidence and prevalence, and is considered one of the most costly pathologies, with an increase in high healthcare costs, especially related to renal replacement therapy through dialysis or kidney transplantation. The course of CKD is variable, depending on the primary kidney disease. The management of RCB is challenging and often requires inter- and multidisciplinary collaboration between nephrologists and physicians from other specialties (cardiologists, hematologists, endocrinologists, diabetologists, family physicians, laboratory and medical microbiology) due to multiple and severe complications.

Blood transfusion is an important part of modern clinical services. The correct use of transfusion can save the patient in imminent danger, rapidly improving the patient's health status and the risk of major cardiovascular complications through decompensation of heart failure, for example, in severe anemia or hemorrhagic stroke in thrombocytopenia. Due to limited resources of blood products, especially those that cannot be stored for a long time (platelets), it is sometimes difficult to get them to the patient in time and in the required quantity. At the same time, it is of paramount importance that the blood is 'safe and of good quality'.

1. CURRENT STATE OF KNOWLEDGE

1.1. Chronic kidney disease

Chronic kidney disease is defined by the presence of kidney damage or decreased kidney function for at least three months, regardless of cause. Kidney involvement generally refers to pathologic abnormalities in the native or transplanted kidney, established by imaging, biopsy, or deduced from clinical markers such as increased albuminuria - i.e. urinary albumin-to-creatinine ratio >30 mg/g creatinine (3.4 mMol/g) - or changes in urine sediment. Decreased renal function refers to a reduction in glomerular filtration. According to the National Kidney Foundation, the name and definition of Chronic Kidney Disease (CKD) which is a kidney disease of at least 3 months duration that may present with functional and structural abnormalities of the kidney with morpho pathologic lesions and altered blood, imaging and urine investigations, glomerular filtration rate being less than 60 ml/min/1.73 m2 over a 3-month period.

In adults, the MDRD equation and CKD-EPI (2021) are the most widely used formulas for estimating serum creatinine and glomerular filtration rate, with CKD-EPI currently the most widely used for the progression of renal function.
"Variables are entered in the CKD EPI or MDRD formulas with four variables without taking into account the patient's weight, because the result is related to an average body surface area of 1.73mp (unanimously accepted): eGFR = 141 × min (Cr /κ, 1)α × max(Cr /κ, 1)-1.209 × 0.993age × 1.018 [for female] × 1.159 [for black race], Where: Cr = serum creatinine (mg/dL), κ = 0.7 pt females and 0.9 pt males, α = -0.329 pt females and -0.411 pt males".

1.2. Hematologic complications of chronic kidney disease

Anemia in chronic kidney disease

Anemia is the most important hematologic complication in patients with chronic kidney disease (CKD), especially in advanced stages of the disease. Although it may be absent in the early stages, influenced by many factors such as underlying renal disease, diet, associated diseases or microhemorrhages, in the uremic stage anemia becomes almost constant. This usually

occurs when creatinine clearance falls below 25 ml/min or when serum creatinine exceeds 4 mg/dl.

Anemia associated with chronic kidney disease is usually hypochromic or normochromic, normocytic and hyporegenerative, with a multifactorial etiology. Understanding the mechanisms of erythropoiesis and its relationship with erythropoietin (EPO) and iron contributes to a better understanding of the pathophysiological processes involved in the development of anemia in CKD. Decreased renal function impairs the ability of the kidneys to produce erythropoietin, leading to the onset of anemia. Other factors that may contribute to its worsening include: shortened red blood cell lifespan; blood loss (especially in dialysis patients); impaired absorption of dietary iron; accumulation of uremic toxins, which inhibit erythropoietin synthesis in the kidney and interfere with the growth and differentiation of erythrocyte cells in the bone marrow. Thus, reduced erythrocyte production contributes to BCR-associated anemia. The platelet abnormalities in BCR are the consequence of complex mechanisms, driven by factors such as uremic toxins, altered platelet signaling, endothelial dysfunction and chronic inflammation. These factors contribute to the dual risk of bleeding and thrombosis in patients with CRB, requiring a nuanced approach to therapeutic interventions and management strategies to optimize hemostatic balance.

1.3. Use of blood component transfusion in patients with CKD

Blood components for therapeutic use in Romania

According to Romanian legislation, the official list of blood products for therapeutic use is clearly defined. The "National Nomenclature of Blood Components" includes all blood components that are harvested, prepared, preserved and validated according to regulated good transfusion practices. These blood components are of human origin and can be from allogeneic (homologous) or autologous donation. Homologous donation involves the collection of blood from one person (donor) for use in another person (recipient), whereas autologous donation involves the collection of one's own blood, as part of a scheduled transfusion program, for exclusively personal use.

Erythrocyte phenotyping

The term "phenotyped" refers to a unit of blood containing erythrocytes for which the Rh minor antigens C, c, E, e, and K (Kell 1) antigen have been determined. If necessary, phenotyping may be extended to other erythrocyte antigen systems. All antigens determined and their results are labeled on the label of the erythrocyte concentrate unit.

1.4. Erythrocyte concentrate transfusion

The decision for transfusion in patients with chronic anemia and chronic kidney disease (CKD) should not be guided by the achievement of a fixed hemoglobin (Hb) threshold but should be influenced by the clinical manifestations and symptoms of anemia. Symptoms such as dyspnea and fatigue are nonspecific and may occur at different Hb levels depending on the patient.

A lower transfusion threshold, present when transfusion is reserved for cases with severe symptoms or very low Hb, may reduce the risks associated with transfusion. Although there is no clear consensus on the optimal time for transfusion, clinical practice suggests that it may be necessary when Hb falls below 10 g/dL, even when the need is not certain. Regarding the risk of sensitization to transfusion of leukocyte-depleted blood, although leukocyte removal reduces some immune adverse reactions, there remains a significant risk of allosensitization. This sensitization may complicate future transfusions by making it difficult to find compatible blood and may reduce the chances of a successful kidney transplant by decreasing the availability of compatible organs and shortening graft survival.

1.5. Platelet transfusion

Several studies have documented decreased platelet counts in patients with chronic renal failure, although this is not as well researched as anemia, reduced erythrocyte count or mean red cell volume. To prevent the risk of bleeding, regular monitoring of platelet counts in these patients is recommended. Thrombocytopenia associated with renal failure has been confirmed in multiple studies and plays an important role in the excessive clotting and bleeding commonly seen in chronic kidney disease (CKD). The mechanisms of clotting and bleeding are closely related to platelet function. A low platelet count has also been identified as an unfavorable

prognostic indicator in patients with sepsis. In advanced stages of CKD, platelet function undergoes important changes. Platelet hypoactivity, caused by the accumulation of uremic toxins and anemia associated with CKD, contributes to an increased risk of bleeding. At the same time, platelet activation, increased platelet-leukocyte conjugate formation and platelet-derived microparticles play a central role in the pathogenesis of thrombosis in patients with CKD.

1.6. Plasma and cryoprecipitate transfusion

Plasma transfusions are usually used to stop or prevent bleeding due to blood clotting disorders, major surgery, burns and severe liver disease. A plasma transfusion can also help increase blood volume and prevent shock. Plasma units are generally 200 to 250 ml and the recommended dose is 15 to 20 ml/kg. Plasma transfusions are generally safe with a low risk of complications. Adverse reactions may include infections, allergic reactions, transfusion-associated circulatory overload, transfusion-related acute lung injury. Other medical conditions, such as cardiopulmonary disease and systemic inflammation, increase the risk of side effects. The use plasma and cryoprecipitate is essential in the management of bleeding and complex coagulopathies such as disseminated intravascular coagulation (DIC), liver disease or chronic kidney disease, where multiple plasma proteins are affected and therapeutic options are limited.

PERSONAL CONTRIBUTION

2. General methodology

2.1 OBJECTIVES AND AIM OF THE WORK

The overall objective of this work was to evaluate the effectiveness of allogeneic transfusion therapy in non-dialyzed and dialyzed patients with chronic kidney disease and the possibilities of reducing immunization by determining Rh phenotype and extended phenotype, investigating the occurrence of irregular antibodies, which can be reduced by administering leukocyte-depleted or leukocyte-depleted isophenotype units.

Determination of Rh phenotype and administration of ABO isogroup and Rh iso-phenotype erythrocyte concentrate were aimed to reduce the degree of immunization and increase transfusion efficiency. Alloimmunization was demonstrated by the detection and identification of irregular antibodies.

In terms of how chronic kidney disease acts on platelets, mainly due to the effect of uremic toxins, both decreased platelet counts but especially decreased platelet function in patients with CKD are reported, although thrombocytopenia is not as well documented as anemia of renal cause, both as a diagnostic and therapeutic approach. An important issue has been to determine the threshold for prophylactic platelet administration in patients with moderate to severe chronic kidney disease and associated thrombocytopenia, related to their higher risk of bleeding .

2.2. Material and method

The study was carried out at " St. Apostol Andree " Emergency County Hospital, Constanta, within the Nephrology Clinical Department and the Blood Transfusion Unit (BTU 1), where we monitored and entered in the database patients with chronic kidney disease in different stages of evolution, who were transfused.

The research was conducted in three study groups. Study 1 a group of 1,127 patients with chronic kidney disease with secondary anemia, who were hospitalized in the nephrology

clinical nephrology ward of the and who received transfusion of erythrocyte concentrate. The follow-up period was 4 years (January 2019-December 2022).

Study 2: a group of 104 patients, diagnosed with chronic kidney disease and thrombocytopenia, who were hospitalized in the nephrology ward of the County Emergency Hospital Constanța "Sf. Apostol Andrei" and who received thrombocyte concentrate. The study period was from January 2015 to December 2021.

Study 3: We conducted a retrospective observational study on a population of patients with varying degrees of chronic kidney disease who required blood and blood derivative transfusions during their hospitalization in our county clinical emergency hospital. In the pandemic years 2019-2022, 1,590 patients with chronic kidney disease were transfused at Constanta County Emergency Clinical Hospital.

The inclusion criteria for inclusion in the study were: patients with CKD hospitalized in the nephrology ward and having indication for transfusion of blood products: erythrocyte concentrate, thrombocyte concentrate, plasma products; signed informed consent; ability to travel to the St. Apostol Andree County Emergency Hospital for periodic monitoring.

Exclusion criteria consisted of: incomplete data in the patient's observation sheets, anemia other than renal anemia such as: active gastrointestinal bleeding (hematemesis, melena, rectorrhagia, hematochezia), hemoptysis, macroscopic/microscopic hematuria.

3. Study 1- Red blood cell concentrate transfusion in renal anemia therapy

3.1. Objectives

In the first study of my PhD research we aimed to evaluate the benefits of allogeneic transfusion therapy in non-dialyzed and dialyzed patients, as well as the possibilities of reducing immunization, by determining Rh phenotype and extended phenotype, investigating the occurrence of dysregulatory antibodies and administering isophenotype, leukocyte-depleted or leukocyte-depleted erythrocyte concentrate units.

3.2. Material and method

The study included 1,127 patients with chronic kidney disease with secondary anemia, who were hospitalized in the nephrology ward of the "St. Apostol Andree" County Emergency Hospital Constanta, and who received transfusion of red blood cell concentrate. Patients included in the study were clinically and biologically investigated to establish the diagnosis. Complete blood count was performed on Hycount 5 XL analyzer (flow cytometry with hydrodynamic focusing). Biochemistry tests were worked on Beckmann AU480 automatic analyzer. Pre-transfection assays were performed by gel column agglutination using the DiaMed line. Alloimmunization was tested by detection of dysregulated antibodies (IAT) using LISS/Coombs and Enzyme cards, which can detect IgG class antibodies anti Rh, Kell, Kidd. The data were obtained using the medical computer system "Hippocrates" owned by " St. Apostol Andree " Emergency County Hospital, Constanta and the observation sheets of patients with chronic kidney disease with secondary anemia on the nephrology and hemodialysis ward.

3.3. Statistical data analysis

Statistical processing was performed using data and number processing program in a Microsoft Excel Microsoft database. For numerical variables, statistical differences were processed using Student's t- test and ANOVA. The CHI-squared test was used to compare nominal variables. A statistical difference was considered when the p-value was less than 0.05%.

3.4. Results for Study I

During the 4-year period in which we conducted this study, a total of 733 patients had hemoglobin values below 8 g/dL, categorized as severe grade of anemia requiring emergency transfusion. The rest of the patients had moderate anemia - 259 patients - or even mild anemia in 101 patients, with transfusion being liberal but required by the symptoms of anemia or other symptoms, at the discretion of the attending physician, as well as the presence of co-morbidities (table 1).

Anemia/ Hb values (g/dl)	Absolute frequencies	Relative frequencies
Hb ≥11	34	3%
Mild Anemia/Hb 10-11	101	9%
Moderate Anemia/ Hb 8-10	259	24%
Severe Anemia/ ≤8	733	64%

Table 1. Pre-transfusion hemoglobin analysis between 2019-2022.

It was observed that the majority of the patients were severely anemic, a number of 733 patients, in whom transfusion was necessary and urgent, even using the restrictive, strict criteria of blood administration (table 2).

Categories	**Absolute frequencies**	**Relative frequencies**
Dialysis patients	665	59%
Non-dialysis patients	462	41%

Table 2. Frequency of dialysis patients transfused with CER in the study group.

As noticed in Table 2, the need for red cell concentrate transfusion was higher in dialysis patients. The liberal transfusion strategy, above a hemoglobin threshold of 11 g/dl was used only in a number of 34 patients, who according to the new who classification of anemia are mild anemia cases, while transfusion guidelines discourage the administration of PRBC at Hb values above 10g/dl (table 3).

Categories	Mean Hb (g/dl)
Average Dialysis patients before transfusion	7.412
Average Dialysis patients after transfusion	9.027
Mean Undialyzed patients before transfusion	8.107
Mean Undialyzed patients after transfusion	9.585

Table 3. Mean Hb values before and after transfusion in dialysis and non-dialysis patients.

Medical practice findings continue to support anemia as one of the main complications of chronic kidney disease. This is also illustrated in Table 3, where the mean Hb values expressed in g/dl in patients at different stages of chronic kidney disease are shown. Thus, a

patient in a more advanced stage of CKD with dialysis requirements has a much lower Hb value than a patient without dialysis requirements. The mean Hb in dialysis patients is 7.412 g/dl, 0.695 g/dl lower than the mean Hb in non-dialysis patients (8.107 g/dl). Note however that both mean values presented fall into the class of moderate-severe anemia. Through the process of CER administration, Hb is increased in patients with anemia. We observe from the data present in the above table a 1.6g/dl increase in mean Hb in dialyzed patients respectively 1.4g/dl in non-dialyzed patients, relatively similar values post-transfusion.

The mean value of hemoglobin in BCR patients dialyzed pre-transfusion was 7.412 g/dl and after transfusion with erythrocyte concentrate a value of 9.027 g/dl was obtained. In the case of undialyzed patients the mean value of hemoglobin pre-transfusion was 8.107 g/dl and post-transfusion with erythrocyte concentrate a value of 9.585 g/dl was obtained.

3.5. Discussion and conclusions for Study I

Although a large number of patients with CKD were transfused during the study period and most of them were polytransfused, post-transfusion adverse reactions were extremely rare and we did not record any case of severe reaction. Regarding blood-borne infections, during the 4-year period of the study, there were 2 suspected cases of hepatitis B viruses, hepatitis C virus and hepatitis B virus transmission. In both cases, transmission of viruses through transfused blood products was ruled out. Following the retrospective survey conducted by the Regional Blood Transfusion Centre Constanta, the suspicion of post-transfusion hepatitis C virus infection was not confirmed. Also, no other severe reactions and severe incidents in transfused patients in nephrology and hemodialysis wards were reported in 2019-2022.

Although 73% of the patients were polytransfused during the study period, the presence of unregulated antibodies signalling alloimmunization was identified in 1.45% of the cases, a lower percentage than reported in the last decades. This was largely due to the testing of patients for Rh and Kell phenotype and the administration of Rh and Kell isophenotype blood, not just ABO isograft and Rh D. The administration of Rh and Kell isophenotype- isotype leukocyte-depleted blood was not limited to possible renal transplant patients, but was practiced in the majority of patients with chronic kidney disease.

4. Study II- Thrombocyte concentrate transfusion in patients with moderate-to-severe chronic kidney disease

4.1. Objectives

Research was performed on a second study group, which aimed to determine the threshold for prophylactic platelet administration in patients with moderate to severe chronic kidney disease and thrombocytopenia, related to their higher risk of bleeding. We performed a descriptive study of patients with chronic kidney disease and thrombocytopenia requiring platelet transfusions. The aim of this study was to evaluate the efficacy of platelet concentrate administration and how patient prognosis was influenced by platelet transfusion therapy.

4.2. Material and method

The total number of patients included in the study, diagnosed with chronic kidney disease and thrombocytopenia, was 104 who were hospitalized in the nephrology ward of Constanta County Emergency Hospital "St. Apostol Andree" and received thrombocyte concentrate. The study period was from January 2015 to December 2021. Patients were clinically and paraclinically evaluated. The number of platelet units transfused and platelet count results after transfusion were noted for each patient.

4.3. Statistical data analysis

Statistical analysis was performed using IBM SPSS statistical software, version 25. For hypothesis testing, the following parametric and non-parametric tests were used: t-tests for independent samples, one-way ANOVA tests, Wilcoxon signed, Wilcoxon tests, independent samples Kruskal-Wallis H tests, median independent samples t-tests, and Chi-square tests for association. The level of significance (α) was set at 0.05.

4.4. Results for Study II

The total number of patients included in the study diagnosed with chronic kidney disease and thrombocytopenia was 104, 55 males and 49 females. The mean age of patients with CKD and thrombocytopenia was 63.96 years. Associated diagnoses, which were seen as possible risk factors for thrombocytopenia in the CKD patients studied were: liver cirrhosis after hepatitis C virus , patients with urosepsis and recurrent urinary tract infections and renal abscesses.

Of the 104 patients with chronic kidney disease and thrombocytopenia, 21 were end-stage on hemodialysis - a known risk factor for thrombocytopenia.

No of transfused Platelet Units	No of patients	Percent
1 unit	56	53,85
2 units	40	38,46
3 units	8	7,69
Total	104	100,00

Table 4. Number of platelet units transfused**.**

Since both standard thrombocyte concentrate and apheresis thrombocyte concentrate units were administered, we equated one unit of apheresis thrombocyte concentrate with three units of standard thrombocyte concentrate, to have a unit approach to the amount transfused. The number of platelet units transfused in the study patients was summarized as follows: 56 patients received one unit of apheresis PLT, which is considered a low dose; 40 patients received two units of apheresis PLT, considered a medium dose; and 8 patients received three units of apheresis PLT, considered a high dose. An important issue was whether platelet administration should be prophylactic or therapeutic, as well as the platelet threshold for the transfusion indication. Prophylactic administration of platelets is reported to reduce the risk of bleeding and the chance of death in patients with hematologic malignancies, but there are only few studies in other pathologies. In all cases included in our study, platelet transfusions were prophylactic.

For each associated diagnosis and number of transfused platelet units a Wilcoxon signed-rank test elicit a statistically significant change in the number of platelets ($x10^3/\mu l$) between pre-transfusion or post-transfusion time points (see table below: z values and $p < \alpha = 0.05$). Indeed, the median of the number of platelets (x10^3/µl) increases from Pre- and Post- transfusion in each case (table 5).

No PLT	Categories of associates diagnosis		N	Mean	SD	Min	Max	P25	Median	P75
1 unit	CKD +LC	Pre-transfusion	10	53,70	23.86	13.00	83.00	37.25	53,50	80,50
		Post-transfusion	10	72.20	26.97	24.00	99.00	49,25	78,50	96,50
	CKD +S	Pre-transfusion	12	56,25	34,60	11.00	98.00	17.75	63.00	86,50
		Post-transfusion	12	97,83	47.13	20.00	151.00	47,50	102.00	139,25
	CKD	Pre-transfusion	34	64,26	28.35	12.00	99.00	35,75	73,50	91.00
		Post-transfusion	34	97,56	31.93	15.00	154.00	79.00	99.00	121.00
2 units	CKD +LC	Pre-transfusion	13	55,77	30.88	20.00	99.00	24.50	56.00	93.00
		Post-transfusion	13	87,46	34.23	43.00	143.00	61,50	74.00	126,50
	CKD +S	Pre-transfusion	7	23.00	7.46	9.00	31.00	20.00	25.00	29.00
		Post-transfusion	7	60,86	22.42	29.00	91.00	50.00	54.00	90.00
	CKD	Pre-transfusion	20	42,75	21.79	6.00	77.00	27.50	45.00	60,25
		Post-transfusion	20	93,30	24.73	29,00	125.00	79.00	100.00	111.50

Table 5: Number of transfused platelets units ($x10^3/\mu l$)

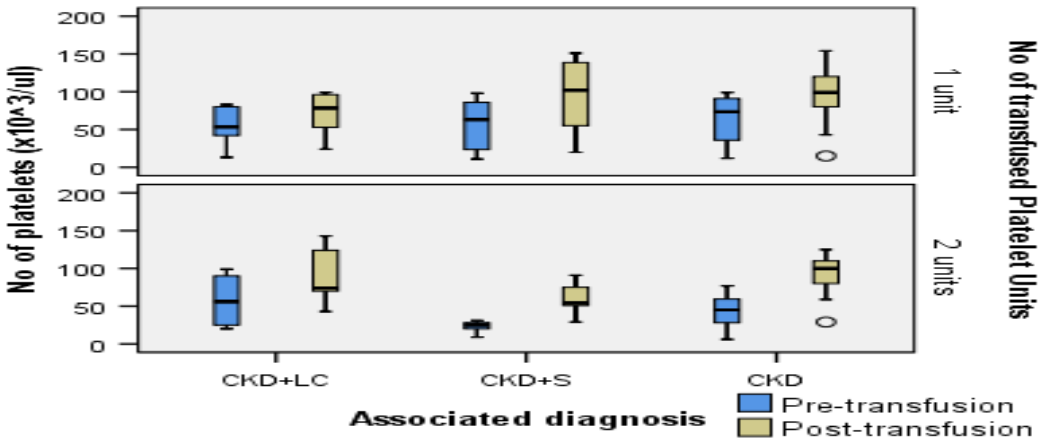

Figure 1. Box-Plot of no. of platelets (x10^3/ul) for each associated diagnostic category for both pre-transfusion or post-transfusion time points according to the number of transfused Platelet Units.

A Kruskal-Wallis H Test showed that: for 1 transfused Platelet Unit the distribution of no. of platelets (x10^3/ul) is the same across categories of Associated diagnostic for both Pre-transfusion ($H = 1.410$, $df = 2$, $p = 0.494 > \alpha = 0.05$) or Post-transfusion ($H = 4.521$, $df = 2$, $p = 0.104 > \alpha = 0.05$) time points. For 2 transfused Platelet Units the distribution of no. of platelets (x10^3/ul) is not the same across categories of associated diagnosis for both Pre-transfusion ($H = 6.836$, $df = 2$, $p = 0.032 < \alpha = 0.05$, with a mean rank no. of platelets score of 24.65 for CKD+LC, 10.50 for CKD+S and 21.30 for CKD) or Post-transfusion ($H = 6.819$, $df = 2$, $p = 0.033 < \alpha = 0.05$, with a mean rank no. of platelets score of 20.50 for CKD+LC, 10.57 for CKD+S and 23.98 for CKD) time points.(Table no 5, Figure1).

No of PLT	**≤20 x10^3 /** µl	**20-50 x10^3 /** µl	**50-100 x10^3 /** µl	**≥ 100 x10^3 /** µl
No of patients before transfusion	14	41	49	0
No of patients after transfusion	1	13	51	39
Total n=104				

Table 6. Results of platelet transfusion

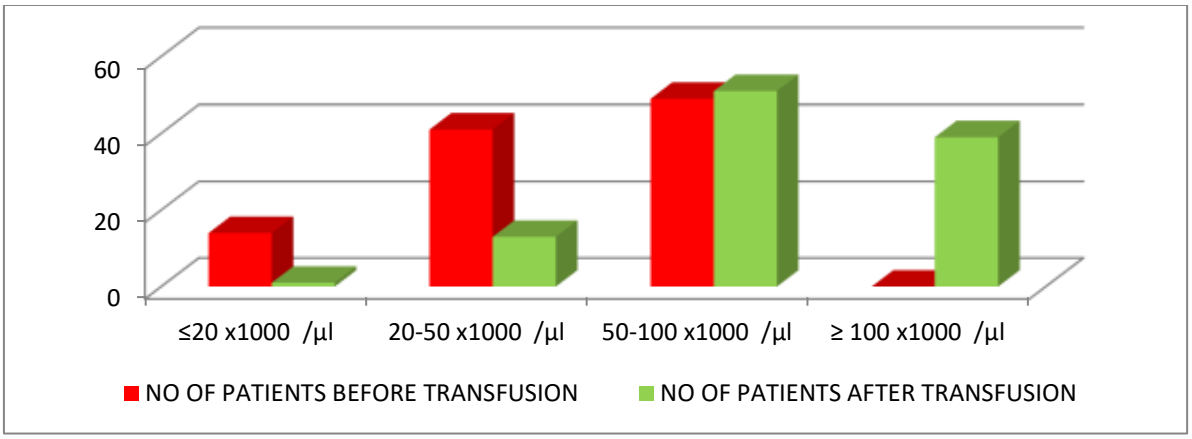

Figure 2. Results of platelets transfusion

The results of platelet transfusion were that all patients, with one exception, exceeded the critical threshold of 20x10^3/µl. In the majority of patients the platelet count rose above 50 x10^3/µl; there were 51 cases with values between 50-100 x10^3/µl and 39 cases above 100 x10^3/µl after transfusion treatment. Only 13 patients remained below 50 x10^3/µl.(Table no 6,Figure 6)

4.5. Discussion - Study II

After one transfusion (one thrombocyte unit of apheresis), platelet counts increased on average by 18.5 x10^3/µl for CKD + hepatitis cirrhosis, by 41.58 x10^3/µl the CKD + urosepsis and by 33.3 x10^3/µl for CKD. Platelets increased by a mean of 31.69 x10^3/µl in CKD +CH patients, 37.86 x10^3/µl in CKD +S patients, and 50.55 x10^3/µl in CKD patients without associated diagnoses who received two units of platelet apheresis. After transfusion of three platelet apheresis units, the mean platelet count increased by 42.75 x10^3/µl in CKD+S and by 105.5 x10^3/µl in CKD. No post-transfusion adverse reactions have been reported. This is an important result of the measures in place to avoid any risky transfusion. We used leukocyte-deleukocited blood products to avoid alloimmunization and tested for the presence of irregular antibodies on all polytransfused patients. Mortality during hospitalization in our study group was 8.65%, and in 4.83% (5/104) the cause was severe hemorrhage. In this particular category of patients with multiple comorbidities, severe, uncontrolled bleeding is reported, even if they received platelet transfusions. Patients with CKD develop severe thrombocytopenia as well as thrombocyte dysfunction, which causes bleeding. Most benefit from transfusions, but some cases are transfusion refractory. Some patients develop alloimmunization to platelet antigens.

5. Study III- Impact of the COVID-19 Pandemic on Blood Transfusion among Hospitalized Patients with Chronic Kidney Disease

5.1. Objectives

The aim of the 3rd study group was to analyse the impact of the COVID-19 pandemic on blood transfusion, for hospitalized patients and especially for those in the nephrology ward, as well as the measures adopted to ensure uninterrupted blood supply during this period.

5.2. Material and method

We conducted a retrospective observational study on a population of patients with varying degrees of chronic kidney disease who required transfusions of blood and blood derivatives during their hospitalization in our county clinical emergency hospital. In the pandemic years 2019-2022, 1,590 patients with chronic kidney disease were transfused at Constanta County Emergency Clinical Hospital. Our study group included adult patients (≥18 years of age) already diagnosed with CKD, in the pre-pandemic year 2019, but also between February 2020 (when the COVID-19 epidemic started in Romania) and December 2022. After who declared COVID-19 a public health emergency of international importance and then pandemic on March 11, 2020, patients were tested for viral infection. Inpatients in the study were followed up at the end of hospitalization (discharged or deceased). Clinical charts and hospital electronic records were used as data sources. We used the records of the blood transfusion service and the demand and release of blood at our blood transfusion unit of the Constanța County Emergency Hospital. The study was approved by local ethics committees and was in accordance with the Helsinki Declaration of 1976 and its subsequent amendments. The indications for red blood cell concentrate (PRBC) transfusion were in patients with active or acute bleeding and in patients with anemia-related symptoms such as tachycardia, dyspnea on exertion, precordial pain and hemoglobin less than 8 g/dL. The platelet concentrates have been used primarily for the treatment of severe thrombocytopenia, which can occur in CKD patients with drug-induced bone marrow failure and other immune and non-immune causes of platelet destruction, such as sepsis and disseminated intravascular coagulation. Fresh frozen plasma transfusion was used for CKD patients with urgent surgery or invasive procedure in the presence of abnormal coagulation tests, acenocoumarin reversal in the presence of active bleeding, cases

of thrombotic microangiopathy and congenital or acquired factor deficiency without alternative therapy.

5.3. Statistical data analysis

Categorical data were assigned to divide the enrolled population into groups according to the need for units of blood - erythrocyte concentrate (PRBC), platelet concentrate (PLT) or fresh frozen plasma. Variables included age, sex, chronic kidney disease, diabetes, hypertension, hyperlipidemia, cardiac disease, urea and creatinine levels and platelet count. Categorical data were collected and organized in a database using Microsoft Excel. Descriptive and inferential statistical analysis was performed using GraphPad Prism version 8.4.3. Categorical variables were illustrated in graphs and tables as absolute values or percentages and analysed using Fisher's exact test. Continuous data were described by mean and standard deviation. The Mann-Whitney U-test was used to compare differences in the total number of transfusions between the non-pandemic year 2019 and the years 2020, 2021 and 2022. We also compared the number of transfusions in patients with chronic kidney disease using the Mann-Whitney U test. A P value of < 0.05 was considered statistically significant.

5.4. Results for Study III

The study group was characterized according to the stages of CKD using the CKD-EPI 20221 estimated glomerular filtration rate (eGFR) CKD-EPI 20221. We included patients in stages G3, G4 and G5- pre-dialysis and with G3-5 end-stage kidney transplant or on dialysis (he-modial5-HD or 5-DP). It should be noted that p-values were not calculated for age, creatinine and eGFR, as these variables were already taken into account during eGFR measurement.

Demographic Features	
Age (mean ± standard deviation)	62.8± 18.7
Gender, *n* (%)	
Female	62.2
Male	37.8

Urban area *n* (%)	52.3
Clinical features	No of patients (%)
CKD stage (eGFR CKD-EPI)	
G3 (30-59 ml/min/1.73 m^2)	711 (44.7)
G4 (15-29 ml/min/1.73 m^2)	455 (28.6)
G5 (< 15 ml/min/1.73 m^2) - predialysis	249 (15.7)
G5-HD	296 (18.6)
G5- PD	15 (0.7)
G 3-5 T	24 (1.5)
Diabetes mellitus	762 (47.9)
Smokers	368 (23.1)
Arterial hypertension	1012 (63.6)
Hematological abnormalities (n)	
Anemia	1068
Thrombocytopenia	72
Clotting disturbances	509
Medication	821 (51.6)
Antiplatelets	216 (13.5)

Anticoagulants NSAIDs	402 (25.2)
SARS-CoV-2 positive	588 (36.9)
Admission Clinic/Department	
Nephrology	889 (55.9)
Urology	191 (12)
General Surgery	121 (7.6)
Cardiovascular surgery	119 (7.4)
Gynecology	107 (6.7)
Cardiology	91 (6.2)
Rheumatology	72 (4.5)

Table 7. Demographic and clinical characteristics of the study group (*n*= 1590). CKD- chronic kidney disease; HD- hemodialysis; PD- peritoneal dialysis; NSAIDs: non-steroidal anti-inflammatory drugs

YEAR	TOTAL NO OF PATIENTS (MEAN ± SD)	PATIENTS TRANSFUSED WITH PRBC	PATIENTS TRANSFUSED WITH PLASMA	PATIENTS TRANSFUSED WITH PLT
2019	414 (35±10)	283	107	24
2020	300 (25±7)	211	74	15
2021	355 (30±7,9)	254	84	17
2022	580 (48±4,8)	320	244	16

Table 8. The total number of CKD patients transfused with different blood products in 2019-2022 period.

In terms of the type of blood products administered, in the pre-pandemic year, 2019, 283 patients with chronic kidney disease received red cell concentrates, 107 patients received fresh frozen plasma and 24 patients were transfused with platelet concentrates. In 2020 there were fewer patients with chronic kidney disease transfused with erythrocyte concentrates, 211 patients compared to 283 in 2019. We also saw a decrease in patients who received fresh frozen plasma (74 patients) and the number of patients who received platelets was only 15. In 2021, we observed an increase in the number of transfused patients, i.e. 254 patients who received erythrocyte concentrates, 84 patients transfused with frozen plasma and a number of 17 patients who received platelet concentrates. In 2022, we observed an increase above pre-pandemic levels in the number of patients transfused with ERC and plasma respectively 320 patients with erythrocyte concentrate, 244 patients who received fresh frozen plasma while only 16 patients received platelet concentrates.

5.5. Discussions -Study III

In the pandemic years, as expected, under the strict and restrictive conditions imposed after the declaration of the pandemic, in patients with chronic kidney disease, the number of patients transfused and units of blood transfused decreased by 5% and 7%, respectively. Total demand for blood products, mainly red blood cell concentrate, frozen plasma and thrombocyte concentrate, decreased in 2020 due to reduced hospital admissions for patients without COVID infection and elective surgery. However, in 2021 and 2022, COVID wards were established in several hospital departments for patients with multiple comorbidities. The impact of the COVID-19 pandemic on the blood transfusion program in our county emergency blood transfusion unit was particularly significant, as evidenced by an assessment of the total number of blood products administered between 2019 and 2022, as well as an assessment of the number of patients transfused during this period. Patients with chronic kidney disease were found to be more susceptible to severe forms of COVID-19 in all studies conducted during the pandemic and after it officially ended. Dialysis patients and patients with end-stage chronic kidney disease were directly and indirectly affected by the COVID-19 pandemic.

6. GENERAL CONCLUSIONS

The conclusions of Study I are:

1. The Hb threshold used for restrictive transfusion in patients with CKD stages G4-5 was 8g/dL. The majority of patients (64%) were severely anemic, in whom transfusion was necessary and urgent, even using the strict restrictive criteria for blood administration.

2. The mean Hb values of patients transfused with PRBC increased from 7.69 g/dl to 9.25 g/dl this mean increase by 2.35 g/dl clearly demonstrating the effectiveness of the transfusion strategy used, in the particular conditions of patients with CKD.

3. Although 73% of the patients were polytransfused, the presence of unregulated antibodies signaling alloimmunization was identified in only 1.45% of cases. This was largely due to the administration of Rh and Kell isophenotype blood, not just ABO and Rh D isogroup, not only in possible transplant candidates, but in the majority of CKD patients.

4. The two main risks of PRBC transfusion (immunization and major immune and infectious adverse reactions) have been greatly reduced, below the level reported in recent years.

Regarding the conclusions of *Study II*, the important ones were:

1. The indication for prophylactic transfusion was considered, and the critical threshold was $20x10^3$ platelets /µl, being preferable to a threshold of $10x10^3$ platelets /µl.

2. Platelet transfusion clearly improved the prognosis in these patients, who exceeded the critical threshold and the majority exceeded $50x10^3$ platelets/ µl.

3. Severe hemorrhage was prevented in 95.17% of cases, even though the number of platelets available for administration was below the required number in many situations.

Considering the results of Study III, the conclusions were:

The COVID-19 pandemic accelerated renal pathology and worsened outcomes for patients with kidney disease. Patients with chronic kidney disease were found to be more susceptible to severe forms of COVID-19 in all studies conducted during the pandemic and after it officially

ended. Dialysis patients and patients with end-stage chronic kidney disease were directly and indirectly affected by the COVID-19 pandemic. The coping strategies implemented to ensure continuity and safety of blood transfusion during the COVID-19 pandemic mainly included restrictive transfusions and limiting elective surgical procedures.

This provided valuable information for the management of future similar situations, thus strengthening the resilience of the blood transfusion system to the challenges that healthcare professionals will face in future pandemics.

7. ELEMENTS OF ORIGINALITY OF THE THESIS

The correction of severe anemia in patients with CKD is mandatory for the control of acute cardiovascular events, also maintaining as low as possible a degree of immunization is very important, especially for patients on the waiting list for renal transplantation.

In patients with CKD, thrombocytopenia is under-documented compared to anemia of renal cause. The originality of the present work consisted in the detailed analysis of the efficacy of transfusion therapy in patients with CKD, the analysis of the restrictive versus liberal transfusion strategy in these patients, with the aim of optimal collaboration between nephrologists and transfusion units and the utilization of the invaluable resource of human blood with maximum benefit to the patient.

In the first study entitled "Transfusion of erythrocyte concentrates in the therapy of renal anaemia", the originality is given by the allogeneic transfusion of Rh and Kell isopheno-type deleukocyte isophenotype allogeneic blood transfusion performed in the majority of patients with CKD, with demonstration of reduction of immunization and adverse reactions, and reassessment of restrictive and liberal transfusion thresholds. It is the only study of its kind conducted in our region. The results of this study contribute to the establishment of an effective transfusion strategy for these patients with CKD pre-dialysis and dialysis stages, especially those on the waiting list for renal transplantation.

In the second study: "Thrombocyte concentrate transfusion in patients with moderate-severe chronic kidney disease" the originality is conferred by the analysis of platelet

requirements in patients with CKD and the main associated diseases, the use of the Kruskal-Wallis H test to analyse the distribution of platelet counts (x10^3/µl) in all associated diagnostic categories for each unit of platelets transfused, and the establishment of the threshold of 20x103 platelets/µl for prophylactic transfusion. There are few similar studies published in the literature related to the management of thrombocytopenia in chronic renal patients. The usefulness of this study in clinical practice allows to avoid any risky transfusion with maximum benefit for the patients and sets the stage for further investigation of the dynamic relationships between thrombosis, bleeding, and chronic inflammation associated with the progression of chronic kidney disease.

In the third study, entitled "Impact of the COVID-19 pandemic on blood transfusion among patients hospitalized in the County Emergency Hospital Constanța (2019-2022)", the originality is given by the comparative analysis of transfusion requirements in patients in a county emergency hospital practically serving the Dobrogea population of about 850,000 inhabitants. The study focused in particular on patients with CKD, both during the Covid -19 pandemic period compared to the pre-pandemic period, as well as the post-pandemic period. This study provides valuable information for the management of similar situations in the future, in particular for similarly vulnerable categories of patients, such as those with CKD on pre-dialysis or dialysis, while strengthening the resilience of the blood transfusion system to a potential future pandemic through evidence-based medicine.

8. Selective references

1. Covic A, Nefrologie. Principii teoretice si practice , Casa Editoriala Demiurg, Iasi, 2011
2. KDIGO Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney Int Suppl. 2024; 3: 1-150
3. Macdougall IC, Eckardt KU, Locatelli F. Latest US KDOQI Anaemia Guidelines update-what are the implications for Europe Nephrol Dial Transplant 2007; 22: 2738-2742
4. Dorgalaleh AM, Mohammad T, et al. Anemia and Thrombocytopenia in Acute and Chronic Renal Failure. International journal of hematology-oncology and stem cell research. 2013; 7: 34-9.
5. Karminder S. Gill, Paul Muntner, Richard A. Lafayette, Jeffrey Petersen, Jeffrey C. Fink, David T. Gilbertson, Brian D. Bradbury, Red blood cell transfusion use in

patients with chronic kidney disease, Nephrology Dialysis Transplantation, Volume 28, Issue 6, June 2013, Pages 1504–1515.

6. Rahman MA, Shanjana Y, Ahmed MS, et al. Hematological abnormalities and comorbidities are associated with the severity of kidney disease: a hospital-based cross-sectional study in Bangladesh. Clinical Pathology. 2022, 5: 1-10.
7. Arya RC, Wander GS, Gupta P. Blood component therapy: which, when and how much. J Anaesthesiol Clin Pharmacol. 2011; 27:278–284. [PMC free article]
8. Lise J Estcourt. Platelet transfusion guideline (BCHS platelets), 2017. https://b-s-h.org.uk/media/2641/bcsh_platelet_guideline_08_08_16_v2.pdf
9. Sorensen B, Bevan D. A critical evalution of cryoprecipitate for replacement of fibrinogen. Br J Haematol. 2010;149(6):934-943.
10. Arun, Funda. Cryoprecipitate. Transfusion Practice in Clinical Neurosciences. Singapore: Springer Nature Singapore, 2022. 293-300.
11. Budnick, Isadore M., et al. Transfusion with cryoprecipitate for very low fibrinogen levels does not affect bleeding or survival in critically ill cirrhosis patients. Thrombosis and Haemostasis. 2021; 121(10): 1317-1325.
12. Thomson, Candice, et al. "Extending the post-thaw viability of cryoprecipitate." Transfusion. 2021; 61 (5): 1578-1585.
13. Callum J.L., Karkouti K., Lin Y. Cryoprecipitate - the current state of knowledge. Transfus. Med. Rev. 2009;23(3):177-188.
14. Hippala ST, Myllyala GJ, Vahtera EM. Hemostatic factors and replacement of major blood loss with plasma-poor red cell concentrates. Anesth Analg 2006; 102:347-51.
15. Estcourt LJ, Birchall J, Allard S, Bassey SJ, Hersey P, Kerr JP, Mumford AD, Stanworth SJ, Tinegate H; British Committee for Standards in Haematology. Guidelines for the use of platelet transfusions. Br J Haematol. 2017 Feb;176(3):365-394. doi: 10.1111/bjh.14423. Epub 2016 Dec 23. Erratum in: Br J Haematol. 2017 Apr;177(1):157. PMID: 28009056.