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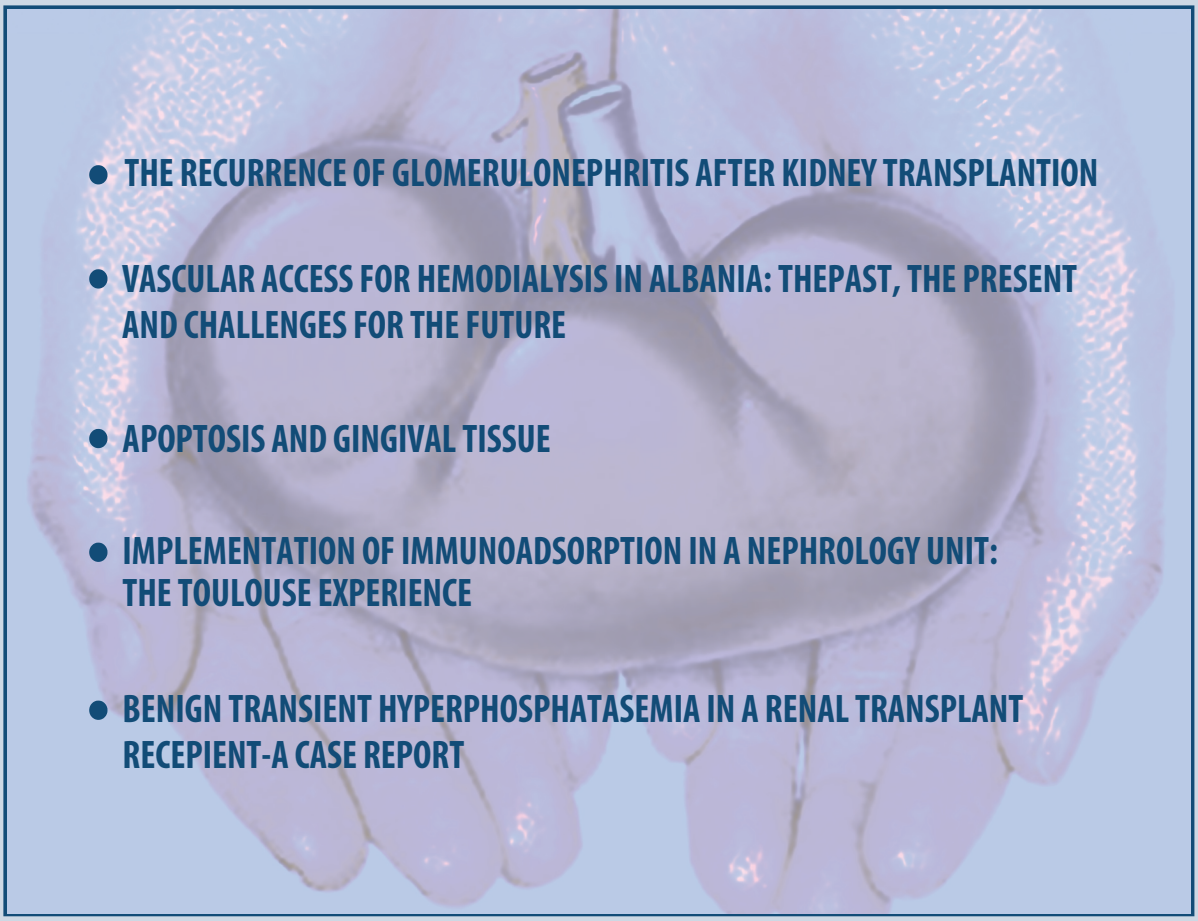

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Editorial Comment

Is it Possible to Maintain the Highest Number of Transplantations in Croatia on Long Term?

Nikolina Basic-Jukic

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Croatia is home to one of the most successful kidney transplant programs in the world. Since the beginning of the 1970s, more than 3500 kidney transplantations have been performed in one of four transplant centers in Croatia-University hospital centre Zagreb, University hospital centre Rijeka, University hospital Merkur and University hospital centre Osijek.

Based on progress in organ donation, Croatia was accepted as a full member of the Eurotransplant in 2007. This was an important moment in the development of transplantation medicine. Many patients who spent more than 20 years on the waiting list received kidney allograft. Kidney and patient survival results are better than in Eurotransplant, demonstrating good organization and well-educated personnel.

The success of our transplant program is based on factors including a thorough medical evaluation of each patient to determine eligibility and a full discussion of options for transplantation. Excellent organization, enthusiastic nephrologists and support from the media and society in general have made Croatia the world leader in organ donation and transplantation. However, it is much easier to reach the top than to stay there.

Many factors may influence future development of the transplant program. It is clear that organization should not be the problem-well-educated network of coordinators has proven its quality. Almost every hospital in Croatia has already referred at least one donor. Surgeons are well-experienced even for the most complicated surgical procedures. Nephrologists have evaluated more than 1500 potential renal transplant recipients and follow-up more than 2500 patients with functioning kidney allograft. Results are excellent.

While in many countries patients die needlessly whilst waiting for an organ transplant, average waiting time for renal transplant in Croatia was less than 2 years in the year 2013, mostly due to time necessary for pretransplant evaluation.

Support from the media is fantastic. There were no negative stories that would negatively impact on transplantation.

So, what is the problem? Do we have a problem at all? Is it possible to maintain the highest number of transplantation in Croatia on long term?

I believe it is possible to stay on the top. However, a different approach to transplant teams is necessary. Croatia is facing severe financial crisis as the rest of surrounding countries. Crisis inevitably reflects on the most consuming part of the budget-health service. Cuts in the costs are present everywhere. One could not expect transplant teams to come from home and work for nothing. Current financial situation in Croatia may influence organ donation and transplantation. Authorities should keep in mind that kidney transplantation is the cheapest method of renal replacement therapy. Enthusiasm of the transplant team members is melting.

Optimistically, I believe that our Ministry of health will recognize the problem and prevent destruction of our most successful health project.

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Editorial Comment

The South-eastern Europe Health Network (SEEHN) initiative on kidney transplant program-update for 2011/12

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The Regional Health Development Centre on Organ Donation and Transplant Medicine (RHDC-ODTM) located in Croatia was established in February 2011 with an aim to coordinate and strengthen collaboration and long-term cooperation within the South-eastern European countries of Albania, Bosnia and Herzegovina (BiH Federation and Republic of Srpska), Bulgaria, Croatia, Macedonia, Moldova, Montenegro, Romania and Serbia.

At the very first professional meeting in Macedonia (May 2011) country specific Action Plan and an implementing strategy on how to increase living and later-on deceased donation (DD) and transplantation activities through self-sufficiency and sustainable long-term models were defined [1]. The activities were overtaken in close collaboration with national health authorities under the umbrella of the South Eastern Europe Health Network (SEEHN), through the networking of regional professionals and transparent communication platform for intracountry data exchange and reporting among health authorities (e.g. MoHs), medical experts, and healthcare professionals.

Kidney transplantation (KTx) is considered as best treatment option in chronic kidney disease patients in terms of their long-term survival [2] and health-economic variables of the health care system [3]. However, the common problem for widespread KTx activities, especially in developing countries [4], is the comprehensive need of a multidisciplinary organisational infrastructure and professional approach raising possibilities for organ commercialism [5]. In addition to the professional care it is essential that healthcare system authorities are in favour and support the improvement in such developing transplant program.

After the first professional meeting in collaboration with professional societies (European Society of Organ Transplantation, European Transplant Coordinators Organization, The Transplantation Society, International Society of Organ Donation and Procurement), data on organ donation and transplantation activities for 2010 of each country within the SEE region were collected and published as first ever publication for a comparative transplant program SEE [6]. What are the results of the following two years since SEEHN (RHDC-ODTM) establishment?

We do have an editorial on how to maintain the highest number of transplantations in Croatia on long-term in the current issue of the Bantao journal [7]. Facing severe financial crisis as the rest of surrounding countries the professionals hope that the Croatian health care au-

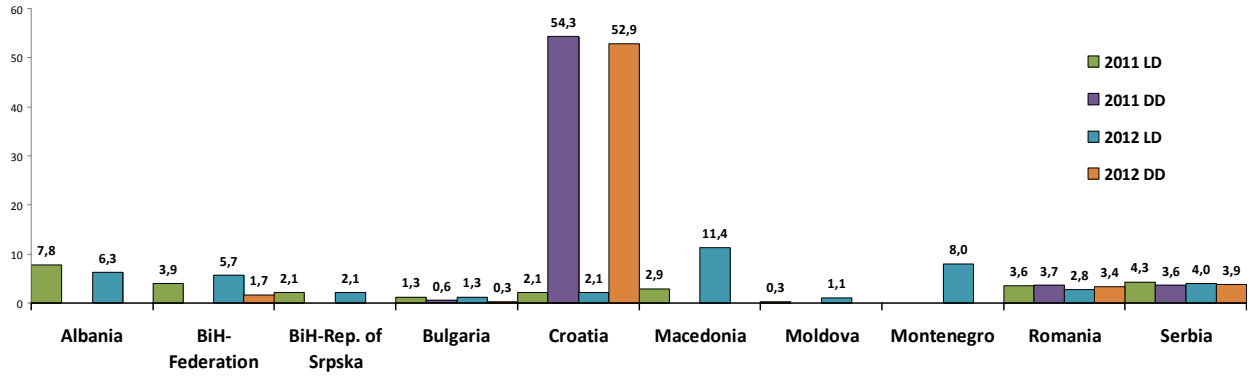

Fig. 1. Number of kidney transplants (PMP) per SEE country-year 2011/2012
Abbreviations: LD-Living Donor; DD-Deceased Donor

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thorities will recognize the problem and prevent destruction of the most successful project of organ transplantation in the country and the region as the most valuable pattern on transplantation.

Data on kidney transplantation are presented in Figure 1. We would like to emphasize that two countries have already advanced their KTx program in the year 2012, i.e. Montenegro with 8 KTx per million population (pmp) and Macedonia with 11,4 pmp. Namely, the KTx program in Montenegro has been supported by Croatian urologists and nephrologists for the very first procedures, hoping to have an independent program in the following period. The living donor kidney transplant (LDKTx) program in Macedonia was initiated in 1977 and between 1996-2011 an average of 13.5 LDKTx/year was performed in a single University center [8]. The SEEHN initiative and the support from the RHDC on Organ Donation and Transplant Medicine were shown as valuable for improvement in transplant program in Macedonia [9]. Importantly, the Ministries of Health (MoH) were closely coordinated within the SEEHN initiative and an interrelation with the professionals was established for implementation of the necessary actions for improvement in the kidney transplant practice. The small number of dedicated professionals and insufficient reimbursement per transplantation allocated according to the DRG code were instantly managed [10] and thus it resulted in 24 successfully performed LDKTx in 2012 (Figure 1). We do expect further improvement in the organ donation and transplant activities in all other countries in the following period proving the importance of South Eastern Europe Health Network and other professional societies in yielding significant results in each SEEHN member country.

Conflict of interest statement. None declared.

Acknowledgements: *We would like to thank all national focal points from SEEHNcountries that provided data for this editorial.*

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Viewpoint

Recurrence of Glomerulonephritis after Kidney Transplantation

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The recurrence of glomerulonephritis after kidney transplantation is a cause of graft loss. The true incidence of recurrent disease is difficult to be estimated because not all patients have undergone native kidney biopsy [1]. The risk of graft loss from recurrence is estimated from 0.6% at first post-operative year to 8.4% at the 10th post-operative year. Other potential problems in the diagnosis of recurrence are the difficulty to differentiate between de novo and recurrent disease and the coexistence of histological features of chronic allograft nephropathy or nephrotoxicity due to calcineurin inhibitors [2]. The recurrence rate, clinical course and impact on graft survival vary between different types of glomerulonephritis [1,3].

Reccurence of IgA nephropathy is observed after transplantation in about 30% of patients. However, the recurrence rate fluctuates between 10% and 60% in various studies because of the differences in the follow-up of patients and biopsy policy of different transplant centers. Most centers perform biopsies only in patients with proteinuria, hematuria or decline in renal function. Clinical manifestations are similar to primary IgA nephropathy and typically occur 5-10 years after transplantation. The estimated 10-year incidence of graft loss due to recurrence is about 10% and there is no significant difference in the survival of grafts from living or deceased donors [2,4]. However, potential donors should be carefully evaluated to exclude familial IgA nephropathy, which has a higher risk for progression to end-stage renal failure. In patients with loss of first graft due to reccurent disease the risk of recurrence is signify-cantly higher in the second graft and potential living donors should be discouraged in such cases. No effective therapy exists for prevention or treatment of recurrent IgA nephropathy. New immunosuppressive drugs, steroid free regimen or rapid steroid withdrawal show no influence on the risk of recurrence. Converting enzyme inhibitors and angiotensin receptor blockers are commonly used for reduction of proteinuria and preservation of renal function [2].

Primary focal and segmental glomerulosclerosis (FSGS) has a recurrence rate of 20-50% leading to graft failure in 13-20% of patients, 10 years after transplantation. In patients with graft loss due to reccurent disease the recurrence rate reaches 80%-100% in a subsequent transplant. Secondary FSGS due to an underlying condition causing progressive nephron loss and hereditary FSGS due to mutations of podocyte and slit diaphragm protein genes do not recur after transplantation [2]. Clinical presentation of recurrent FSGS includes early onset of massive proteinuria, usually sometimes hours to days post-transplantation, hypertension and graft dysfunction. A circulating permeability factor is considered responsible for proteinuria. Serum soluble urokinase receptor (suPAR), which activates podocyte β3 integrin, represents a possible permeability factor. Risk factors for recurrence include younger age, rapid progression of the original disease with development of end-stage renal failure within 3 years, mesangial hypercellularity of native kidney and a history of previous graft failure due to reccurence. Living donation for patients with idiopathic FSGS is better to be restricted to patients who are not at high risk for progression [5]. Institution of a short course of plasma exchange pre-emptively should also be considered in this setting. The management of recurrent FSGS is difficult and controversial [5,6]. High doses of either intravenous or oral cyclosporine have achieved satisfacory results. High doses of steroids and cyclophosphamide have not been proved to be beneficial in recurrent FSGS. Plasma exchange or immunoadsorption using protein A columns applied early showed promising results. Rituximab might be a rescue therapy for patients resistant or unable to be treated with cyclosporine and/or plasma exchange but it needs further investigation.

Membranoproliferative glomerulonephritis (MPGN) type I and II have high rates of recurrence after transplantation. Recurrent MPGN should be differentiated from de novo MPGN, which occurs as part of the histological changes in patients with chronic transplant nephropathy. MPGN type I due to viral hepatitis C and systemic diseases has reduced risk of recurrence after treatment of the original disease. MPGN type I recurs in 20-50% of patients whereas MPGN type II in 80-100% of patients. No effective treatment is available for prevention or treatment of recurrent MPGN.

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Idiopathic membranous nephropathy recurs in 10-30% of patients after kidney transplantation [1]. The clinical presentation of recurrent disease is characterized by nephrotic range proteinuria. M-type phospholipase A2 receptor (PLA2R) has been identified as a target antigen for idiopathic membranous nephropathy. Recurrence usually occurs 2-3 years after transplantation [2-7]. No risk factor for recurrence has been identified. Graft failure from recurrence occurs in 10-15% of patients after 10 years. In contrast to idiopathic membranous nephropathy in native kidneys spontaneous remission is rare among post-transplantation cases. Cyclosporine and mycophenolate mofetil, which are used in the management of the primary disease, do not prevent or change the course of recurrent disease. Tacrolimus and cyclophosphamide are not superior to cyclosporine. Rituximab seems promising as in small number of patients with recurrent disease it was followed by partial or complete remission [2].

Recurrence of renal involvement in patients with ANCA-associated vasculitis occurs in about 17% of kidney transplant recipients after 2-3 years post-transplantation [8,9]. Among patients with ANCA-associated vasculitis treated with dialysis, a relapse rate of 0.09 episodes per patient/year has been reported vs. 0.02 episodes per patient/year in transplant recipients. A lower relapse rate of 0.005 per patient per year has been reported with the new drugs tacrolimus and MMF. Pre-transplantation disease course, ANCA titer and donor type do not predict recurrence. However, kidney transplantation is better to be considered in patients with inactive disease. Patients with renal relapses show good response to cyclophosphamide [8,9].

In patients with antiglomerular basement membrane disease (anti-GBM) recurrence has been reported in up to 50% of patients when kidney transplantation is performed while circulating antiglomerular basement membrane antibodies are still present. With the current practice of deferring transplantation until the disease become quiescent and circulating anti-GBM antibody levels become undetectable for at least 6-12 months, clinical recurrence is rare and consists of isolated case reports only. Recurrent anti-GBM disease in the renal allograft should be treated with steroids, cyclophosphamide and plasma exchange. A form of anti-GBM glomerulonephritis in the renal allograft is rarely seen in patients with Alport's syndrome, who develop auto-antibodies in response to the new epitope of type IV collagen a-chain displayed on the graft.

Recurrence of lupus nephritis has been reported in up to 30%. Clinically significant recurrent disease occurs in 2-9% [2,10]. However, the frequency and clinical impact of recurrent lupus nephritis after transplantation vary among centers. Mean time to relapse is 3 years. Although lupus nephritis recurrence in kidney recipients is relatively uncommon, it may increase the risk of graft failure in the long run. Transplantation in patients with end-stage renal failure due to lupus nephritis should be postponed until achieving complete and persistent remission for at least 6-9 months. There are anecdotal reports on the efficacy of mycophenolate mofetil in the recurrent lupus nephritis. Long-term patient and graft survival are similar to kidney allograft recipients with other underlying diseases.

In conclusion, with improving long-term renal allograft survival, recurrent glomerular disease shows an increased incidence as a cause of late graft loss. Apart from plasmapheresis for patients with recurrent FSGS, there is no consensus on strategies to prevent or treat recurrent glomerular disease in the kidney allograft. It is important to emphasize that the majority of patients with primary glomerulonephritis as the underlying cause of renal failure show good graft and patient survival. Thus, living related kidney donation can still be encouraged in carefully selected patients. Caution should be exercised in patients with recurrent disease in the first graft in view of the markedly increased risk for recurrence in subsequent transplants.

Conflict of interest statement. None declared.

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Original Article

Nephroprotection Prevents Incidence of AKI in Patients Undergoing Elective Percutaneous Coronary Interventions

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Abstract

Introduction. It has been described that acute kidney injury due to contrast administration is a common complication after coronary angiography, particularly for high risk patients. The aim of this study was to confirm if current radiocontrast preventive strategy is protective in patients who undergo elective coronary angiography.
Methods. The study included 43 consecutive patients who underwent elective coronary angiography. Patients were divided into subgroups regarding diabetes, age and presence of chronic renal failure. All patients received standard nephroprotective prevention: pre and post-interventional hydration (0.9% saline), N acetyl cystein (600 mg bid, PO), statins (10-20 mg bid, PO), vitamin C (500 mg IV) and iso-osmolar contrast media (Iodixanol- Visipaque), at a dose of 70-100 ml per procedure. Renal function was determined by Cockroft-Gault equation for estimation of Creatinine clearance (CCl) and early marker of acute kidney injury; neutrophil gelatinase-associated lipocalin (NGAL) was measured in urine by using automated platform ARCHITECT (Abbott Diagnostics).
Results. After coronary angiography, CCl and urinary NGAL levels did not change significantly as compared with baseline values in all groups of patients. Also, renal function remained stable after coronary angiography in the subgroup of patients with diabetes, pre-existent chronic renal failure and in the subgroup of elderly patients ($\geq$65 years).
Conclusions. Nephroprotective measures including isotonic contrasts prevent acute kidney injury even in high-risk groups of patients. We need more investigations comprising a larger number of patients to confirm if current preventive measures are sufficient.

Key words: acute kidney injury, contrast media, neutrophil gelatinase-associated lipocalin

Introduction

Acute kidney injury (AKI) is defined by the Acute Kidney Injury Network (AKIN) as functional and structural disorder or signs of renal damage including any defect from blood and urine test, or tissue imaging that is less than 3 months. AKI biomarkers can be components of serum or urine. Traditional biomarkers are far away from satisfying the requirements of the perfect predictors of AKI [1]. Serum creatinine remains the cornerstone of AKI diagnosis, but it has several serious limitations. Its value varies with age, gender, diet, muscle mass, drugs, and exercise. Creatinine is secreted by the urinary tubules and this stands for 10-40% of its clearance. Its values become abnormal when more than 50% of GFR is lost, and it takes up to 24 hours before creatinine increases in blood. Novel markers that rise earlier than creatinine in AKI could allow early detection and intervention to prevent further progression and better outcome of the disease. Among these biomarkers, neutrophil gelatinase-associated lipocalin (NGAL) is a promising predictor for AKI, which shows up in urine or serum 48 h earlier than serum creatinine [1].
NGAL is a small molecule of 178 amino acids that belongs to the lipocalin superfamily of 20 structurally related secreted proteins. Human NGAL was originally identified as a 25-kD protein covalently bound to gelatinase from human neutrophils. NGAL is expressed at very low levels in human tissues, including kidney, trachea, lungs, stomach and colon, and its expression increases in the presence of inflammation and injured epithelia [2]. NGAL is freely filtered by the glomerulus, and it is largely reabsorbed in the proximal tubules.
NGAL as a renal biomarker was discovered in 2003. Both plasma and urine NGALs are increased after a renal insult. Decrease in GFR resulting from AKI would decrease the renal clearance of NGAL, with its accumulation in plasma [3]. Elevated urine NGAL is caused

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by both proximal and distal nephron injury after a nephrotoxic insult [4].

Until now, NGAL was proved to be a reliable and early marker of AKI in different clinical settings, among which radiocontrast nephropathy is one of the leading causes. Recently, there has been major growth in contrast enhanced imaging. It has been described that AKI due to contrast administration is a common complication after coronary angiography, particularly in high risk patients [5]. The causes of radiocontrast induced AKI are multifactorial, such as combination of ischemia due to vasoconstriction and direct cytotoxicity to the renal tubules mediated by reactive oxygen species [6].

Pre-existing risk factors for radiocontrast induced nephropathy (RCIN) include diabetes, advanced age, chronic kidney failure, higher dose of contrast agent, route of contrast administration, congestive heart failure, hypertension, anemia, use of nephrotoxins, and non-steroidal anti-inflammatory medications, volume depletion and some other factors that have been associated with increased risk of radiocontast acute kidney injury [7,8].

The aim of this study was to confirm if current radiocontrast preventive strategy is protective in patients who undergo elective coronary angiography.

Materials and Methods

The study included 43 consecutive patients who underwent elective coronary angiography. Urine and plasma samples were taken at admission, 4h and 24h after the angiography. Patients were divided into subgroups regarding diabetes, age and presence of chronic renal failure. All patients received standard nephroprotective prevention before and after the procedure. The prevention included pre and post-interventional hydration (0.9% saline), use of N acetyl cystein (NAC, 600 mg bid, PO), statins (mostly Atorvastatin or Simvastatin at a dose of 10-20 mg bid, PO), vitamin C (500 mg IV) and iso-osmolar contrast media (Iodixanol- Visipaque), at a dose of 70-100 ml per procedure.

Serum creatinine level was measured in blood samples and clearance was calculated by the Cockroft-Gault equation. For the measurement of NGAL in urine samples we used the automated platform ARCHITECT (Abbott Diagnostics). Values of these two biomarkers were correlated and their trend was followed during the time and within the three subgroups.

Since NGAL is stable in urine if stored at 4^{o}C for up to 7 days and plasma or urine samples are stable if stored at -80^{o}C for a long time, urine was centrifugated to remove neutrophils that may produce NGAL *in vitro* conditions.

Table 1. Characteristics of patient groups

	Diabetic status		Renal function		Age	
	DM	Non DM	CRF	Non CRF	< 65 yrs	≥65 yrs
Male	8(80%)	23(70%)	10(71%)	21(71%)	19(66%)	12(86%)
Female	2(20%)	10(30%)	4(29%)	8(29%)	10(30%)	2(14%)
Age, years	64.3±7.3	58.1±10.0	67.4±9.3	56.3±7.9	54.9±6.4	71.6±5.2
HTA	8(80%)	26(79%)	8(80%)	22(79%)	23(79%)	12(86%)
DM	8(80%)	-	2(20%)	8(80%)	5(50%)	5(50%)
CCl, ml/min	87.6	80.7	52.25	97.2	93.1	60.1

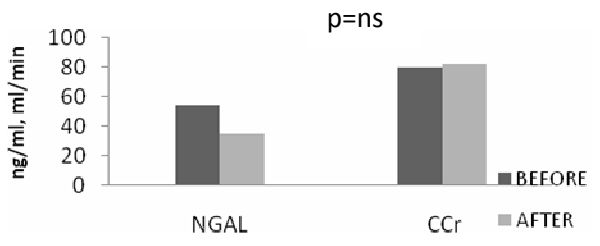

Fig. 1. Creatinine clearance and urine NGAL levels before and after coronary angiogram in all patients

Results

Table 1 shows baseline characteristics of examined patients. There was no statistical difference in gender, CCl, and prevalence of hypertension between patients' groups. From the total number of patients, 10 had diabetes mellitus and this group of patients was older than those without diabetes. At the same time, there was no diffe-

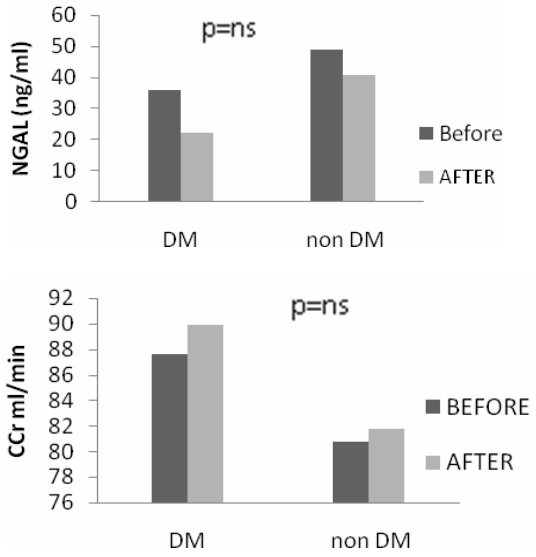

Fig. 2. Creatinine clearance and urine NGAL levels before and after coronary angiogram in patients with and without diabetes mellitus

rence between groups in regard to baseline CCl and prevalence of hypertension. Levels of CCl and urinary NGAL did not change significantly after the procedure as compared with baseline values neither in patients with diabetes nor in patients without diabetes (Figure 2).

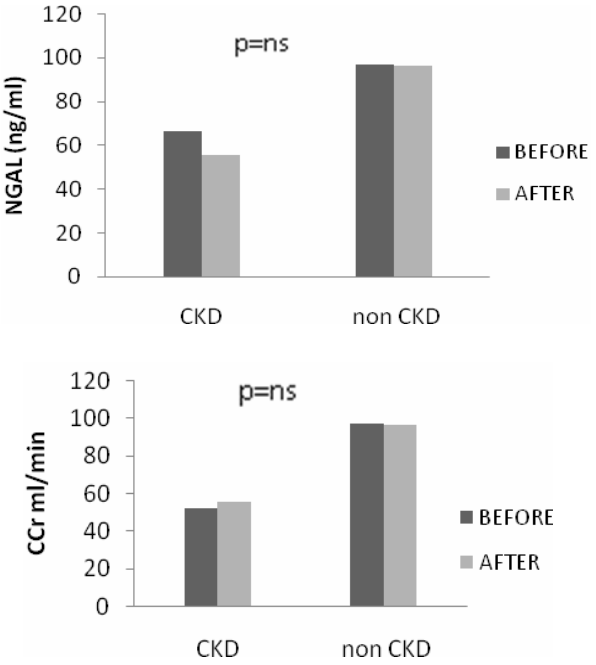

Fig. 3. Creatinine clearance and urine NGAL levels in patients with chronic kidney disease and in patients with normal kidney function before and after coronary angiography

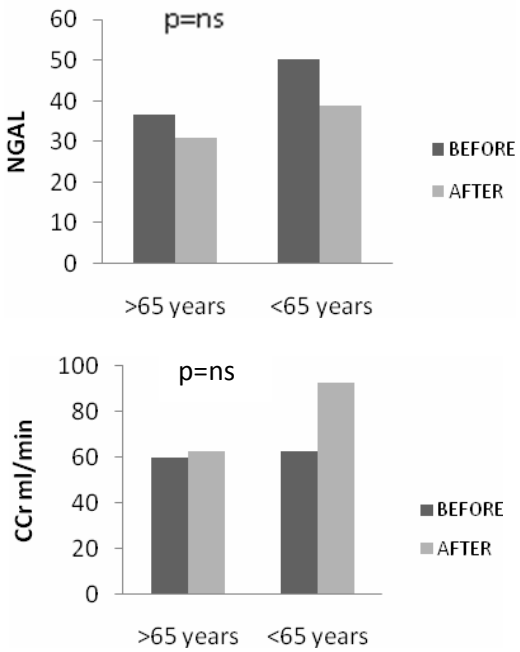

Fig. 4. Creatinine clearance and urine NGAL levels before and after coronary angiogram in patients older than 65 years and younger than 65 years

Similar results were observed in the subgroup of patients with previous chronic renal failure (No=14) and in patients with normal renal function (N=29).Patients with previous CRF were significantly older, but the two subgroups of patients were similar in the prevalence of diabetes and hypertension. In both subgroups, renal function remained stable after the procedure with no significant change in the values of CCl and urinary NGAL (Figure 3).

Finally, renal function remained stable in both elderly (No= 14) and younger (N=29) subgroup of patients with no change in CCl and urinary NGAL after the procedure (Figure 4).

Discussion

Radiocontrast induced nephropathy has been defined as the acute deterioration of renal function after parenteral administration of radiocontrast media in the absence of other causes [9]. There is no effective therapy once injury has occurred; therefore, prevention is the cornerstone in all patients at risk for AKI. There is some evidence showing that prevention of AKI is associated with a reduction in adverse outcomes. The optimal strategy for preventing RCIN has not yet been established [10]. In the present study, preventive strategy included adequate hydration, N acetyl cystein, statins, vitamin C and iso-osmolar contrast media at a dose of 70-100 ml per procedure. This strategy was officious in preventing deterioration of kidney function and AKI even in high-risk patients (diabetic and elderly).

Several studies have been investigating the correlation between the amount of administrated contrast media and risk for developing AKI. Some authors, such as Mc Cullough, *et al*. and Kane, *et al.* [11] have shown that minimizing of contrast amount is one of the most important factors for prevention of AKI especially in the high-risk population.

Also, the type of the contrast media is a key element for nephroprotection in contrast mediated imaging. Contrast media is classified by its osmolality, and it can be high, low or isoosmolar [12]. There have been a lot of randomized trials and several meta-analyses that compared different types of contrast media regarding the development of radiocontrast induced nephropathy [13-17]. Although there was no definite conclusion, we could certainly claim that there is a significant benefit of usage of low and isoosmolar contrast as compared with high osmolarity contrast media. More recent studies have focused on the comparative nephrotoxicity of iso-osmolar and low osmolar contrast media [18-19]. Some of them have showed that use of iso-osmolar contrast is associated with lower risk for AKI, especially in patients with risk factors such as diabetes mellitus, preexisting chronic kidney disease and in geriatric population [20-22]. However, there are also studies that showed no difference or even showed benefit of using low osmolar contrast media [23]. Therefore, no consensus has been reached.

In this study we used lower concentrations of iso-osmolar contrast media and it has been shown that there

was no deterioration in kidney function in patients with diabetes, chronic kidney failure and elderly.

The pharmacological prophylaxis for radiocontrast induced nephropathy includes antioxidant strategy and inhibition of renal vasoconstriction. There have been a lot of investigations regarding the benefit of NAC, vitamin C and statins in prevention of radiocontrast AKI.

NAC is a potent antioxidant that removes oxygen-derived free radicals, and it may be capable of preventing radiocontrast nephropathy by improving renal hemodynamic and by diminishing direct oxidative tissue damage. The first study that proved a decrease of the incidence of radiocontrast AKI by NAC administration at a dose of 600 mg twice a day was performed in 1993 by Tepel, *et al*. [24]. After the initial trial there have been many studies that confirmed this finding. Briguori et al have shown that double dose of NAC can be even more protective [25]. In contrast, the protective effect of NAC was not proved by some other studies [26,27]. Therefore, clinical data regarding the efficacy of NAC in preventing of radiocontrast AKI remains controversial. Considering the very low toxicity and cost of this drug the use of oral NAC at a dose of 1.2 g twice daily on the day before and on the day of the procedure is recommended in patients at risk for contrast nephropathy.

The use of vitamin C in radiocontrast nephropathy prevention is based on the antioxidative effect of the ascorbic acid. Studies that included vitamin C as a prophylaxis for the AKI after the contrast imaging concluded that its role is unclear and its use often is not justified [28-30].

The rationale for the use of statins in prevention of contrast induced AKI is based on its antioxidative and anti-inflammatory properties. The first study by Attallah, *et al*. showed that patient who used statins 24-72h before the coronary catheterization had a significantly lower risk for developing AKI [28]. Even though, many other studies [31,32] confirmed these findings, the first prospective, randomized, double blind, controlled study did not show benefits of taking these medications in AKI prophylaxis [33]. In the meta-analysis that included six cohort studies, four of them showed preventive effects against radiocontrast induced nephropathy [34].

In our study all patients were already using statins before angiography and we can not differentiate the extent of the protective role of statins as compared to other preventive measures that have been used.

The most common and frequent way of preventing radiocontrast nephropathy is periprocedural hydration. There are three types of this method: oral fluids, intravenous 0.45% saline and intravenous 0,9% saline. On the basis of the Mueller's study it has been generally accepted that isotonic saline is superior to hypotonic saline for the prevention of radiocontrast induced nephropathy [35].

The explanation for the prophylactic application of sodium bicarbonate is that the alkalinization of tubular fluid diminishes the production of free radicals and protects renal tissue from the oxidative stress [36,37]. Since the study of Merten, *et al*. [38], which showed significantly lower risk for developing AKI in the group of patients receiving sodium bicarbonate, there have been many trials with controversial conclusions [39-41]. As a result, the use of sodium bicarbonate in a single bolus in addition to pre-interventional hydration could be helpful.

The present study has its limitations. Since we used multiple preventive measures, we cannot determine the contribution of each particular measure. During the study, we used NGAL as a very early marker of AKI. However, apart from NGAL, there are other biomarkers recommended by different authors and combination of these biomarkers could be more conclusive. Finally, we need more patients in every subgroup for better understanding the role of the current strategy during elective coronary angiography.

Conclusions

Administration of the radiocontrast did not cause acute kidney injury in patients who underwent elective coronary angiography including subpopulations of patients with diabetes mellitus, elderly patients and those with preexisting chronic kidney failure. These results were obtained by using nephroprotective strategy including isotonic contrasts. However, further research including a larger number of patients is necessary in order to confirm whether current preventive measures are sufficient in different clinical settings.

Conflict of interest statement. None declared.

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Original Article

Vascular Access for Hemodialysis in Albania: the Past, the Present and Challenges for the Future

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Abstract

Introduction. The hemodialysis treatment in Albania was firstly applied in 1985. Over the 20 years the number of patients was very limited and only 45 were treated in Tirana. Since 2005 due to the positive health policies, the number of patients in HD has been increasing rapidly. Unfortunately, these patients are mostly late referral, resulting in other subsequent serious problems with the vascular access complications: catheter infections, arteriovenous fistula failures due to little experience of surgeons, and then inadequate hemodialysis, repeated interventions, increasing hospitalizations, morbidity and mortality, and in the end, higher costs. The aim of this study was to evaluate the present situation of vascular access and to plan the future with a multidisciplinary team.

Methods. We conducted a cross-sectional study in 6 centers which offer hemodialysis in Albania. There were 484 patients enrolled in the study: 300(63%) males, 18(37%) females that were asked for the actual and past history of vascular access since the beginning of HD treatment. The age ranged from 19-78 years (mean 49.9). Diabetes was encountered in 13.8% of them. Duration of HD treatment ranged from 1 month to 22 years (mean 3.8 years).

Results. At the time of the study 83.5% of patients had an A-V native fistula, 2.25% an A-V graft and 14.25% a catheter. The probability of fistula failure seen by chi square test was statistically higher in diabetics ($p<0.05$) and older age (>50 years) ($p<0.05$), starting hemodialysis without a fistula ($p<0.01$), previous subclavian catheter use ($p<0.001$), non use of antiplatelet drugs ($p<0.01$) and if they had hypotension pre or during hemodialysis session ($p<0.02$).

Conclusions. Arteriovenous fistula is the predominant form of vascular access in majority of patients in Albania**.** The main characteristics of our HD population are the low percentage of diabetics and their younger age, which may prolong the use of their vascular access and render HD treatment more effective. Thus, we are satisfied and proud of our results achieved in the majority of HD patients, but we do have a lot of work to do with the incident ones.

Key words: AVF (arteriovenous fistulae), AVG (arteriovenous grafts), catheters, hemodialysis, vascular access, survival

Introduction

Hemodialysis was applied for the first time in Albania in 1985. A couple of nephrologists were sent to Italy to be trained because our political dictator had diabetic nephropathy that was progressing quickly. He died but some patients did not; they were lucky and lived due to hemodialysis. After 1995 some patients that used to be emigrants in Greece and Italy came to Albania following the HD and their permanent vascular access was already performed there. In 2004, having obtained permission from our authorities, most of our patients were sent to Macedonia to be dialysed and they also got there permanent vascular access. During the 20 year-period, the number of patients was very limited, only 45 ones, thus leading to limited vascular access creation, and only one surgeon performed it. Since 2005 due to positive health policies the number of patients in HD has been increasing rapidly reaching 650 and more (Figure 1).

The hemodialysis now is offered both by public and private sector, which work together to improve patients' healthcare and quality of life.

Despite the benefits, this situation also poses great problems, since most of these patients are late referral, mostly presented to us in very serious conditions, and quite often without any permanent

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vascular access. Dialysing ESRD patients in emergency conditions is well related with temporary catheter use, and in the worst case, with subclavian ones.

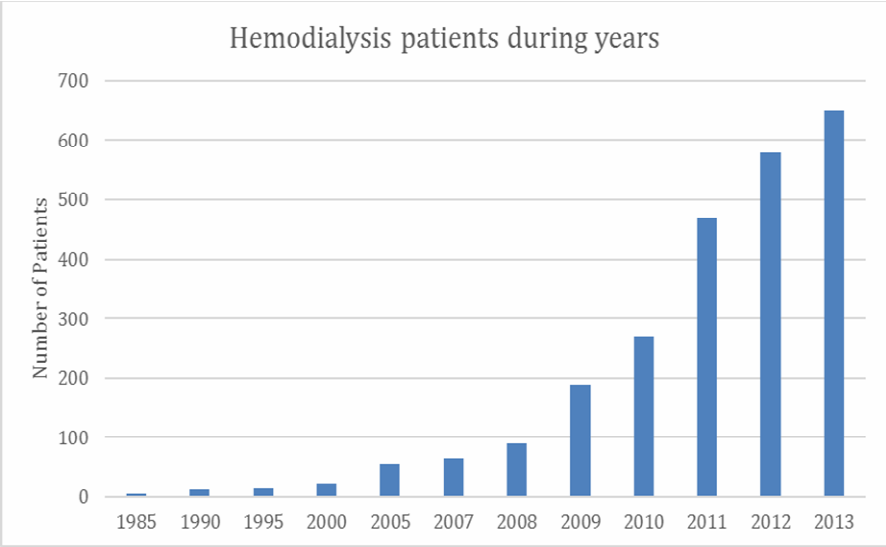

Fig. 1. Growth of hemodialysis patients since the beginning of HD treatment in Albania

Using catheters abundantly in incident patients is hazardous, which leads to many other serious problems of vascular access complications like: catheter infections resulting in increased hospitalizations due to sepsis, lower dialysis adequacy, worse quality of life, higher morbidity, mortality, and, as a result, higher costs [1-6]. Also, during the first steps of this calvary, the initial experience of some surgeons led to arteriovenous fistula failures, and consequently to repeated interventions, longer hospital stays, lowered dialysis adequacy, increased temporary catheter use, all of which created a vicious cycle of problems, which in the end are interpreted into costs and survival. Most of the patients who were known as CKD, and followed-up by nephrologists, were not referred to angiologists for fistula creation, due to many other complex reasons. Patients refuse to accept that dialysis might come soon, and nephrologists are not very alert concerning the proper time of patients' referral to angiologists. However, even if we were more alert, the patients' waiting list is quite long for the simple reason that there is only one surgeon interested and experienced in this field. Thus, here we are with these various and multidisciplinary problems of vascular access, which need to be solved, but the question is then by whom?
The aim of our study was to evaluate the present situation of using vascular access in Albania, factors that contribute to AVF failure and to plan the future with a multidisciplinary team.

Materials and methods

We conducted a cross-sectional study in 6 centers which offer hemodialysis in Albania (three public and three private ones) in May 2013. HD centers of Tirana, Elbasan, Shkodra, American Hospital in Tirana, American Hospital in Durres and Hygeia Hospital were included. The inclusion criteria were: chronic hemodialysis patients over 18 years old, free of any limitations of primary renal disease or time in hemodialysis. The exclusion criteria were metastatic cancer, and severe malnutrition. To accomplish the aims of the study we used hemodialysis registers and a questionnaire. There were 484 patients enrolled in the study: 300 (63%) males, 184 (37%) females, who were asked for the present and past situation of vascular access since the beginning of HD treatment. The primary renal diseases were: chronic pyelonephritis (30.5%), chronic glomerulonephritis (26.5%), nephroangiosclerosis (14%), diabetes mellitus (13.8%), adult polycystic kidney disease (ADPKD) (7.2%) and unknown origin (8%).
The age ranged from 19 to78 years (mean 49.9). Diabetes was encountered in 13.8% of them. Duration of HD treatment ranged from 1 month to 22 years (mean 3.8 years).
Chi square test was used to evaluate the correlation between fistula failure and risk factors. P value below 0.05 was considered significant.

Results

At the time of the study 83.5% of patients had an A-V native fistula, 2.25% an A-V graft and 14.25% a catheter. Data from the questionnaire showed that 84% of patients had begun HD with a catheter although only 37.5% of them were presented as ESRD and 62.5% were known and followed up as having chronic kidney disease (CKD). Only 23.8% of patients with known CKD had a fistula created before initiation of HD. In 65% of patients, a distal native fistula access was firstly applied. The overall failure rate for AVF results was 25.6%.

All factors that contribute to arteriovenous fistula failure are presented in Table 1. The probability of fistula

Table. 1. Factors that contribute to arteriovenous fistula failure

Variable	Total	AVF status		Chi square P-value
		AVF failure	AVF in use	
Sex				
Female	165(36.7)*	47(28.5)†	118(71.5)†	0.129
Male	285(63.3)	63(22.1)	222(77.9)	
Age-group				
≤50 years	210(46.7)	41(19.5)	169(80.5)	0.023
>50 years	240(53.3)	69(28.8)	171(71.3)	
Diabetes status				
Yes	58(12.9)	21(36.2)	37(63.8)	0.026
No	392(87.1)	89(22.7)	303(77.3)	
AVF and HD				
AVF before HD	95(21.1)	13(13.7)	82(86.3)	0.006
AVF after HD	355(78.9)	97(27.3)	258(72.7)	
Referral				
Early	210(56.9)	47(22.4)	163(77.6)	0.370
Late	159(43.1)	42(26.4)	117(73.6)	
Subclavia catheters				
Yes	145(43.5)	56(38.6)	89(61.4)	<0.001
No	188(56.5)	32(17.0)	156(83.0)	
Anticoagulants				
Yes	170(50.3)	56(32.9)	114(67.1)	0.008
No	168(49.7)	34(20.2)	134(79.8)	
Smoking				
Yes	80(25.6)	20(25.0)	60(75.0)	0.601
No	232(74.4)	65(28.0)	167(72.0)	
Hypotension				
Yes	104(38.4)	36(34.6)	68(65.4)	0.018
No	167(61.6)	36(21.6)	131(78.4)	
Ultrafiltration rate				
≤3 l	62(19.8)	14(22.6)	48(77.4)	
>3-4 l	139(44.4)	41(29.5)	98(70.5)	0.533
>4 l	112(35.8)	28(25.0)	84(75.0)	
Eritropoetin use				
No	61(23.8)	14(23.0)	47(77.0)	0.303
Yes	195(76.2)	58(29.7)	137(70.3)	
Hemoglobin level				
<11 g/dl	139(45.7)	39(28.1)	100(71.9)	0.527
≥11 g/dl	165(54.3)	41(24.8)	124(75.2)	

*Absolute number and column percentage (in parenthesis).
†For FAV status, absolute number and row percentages (in parenthesis).

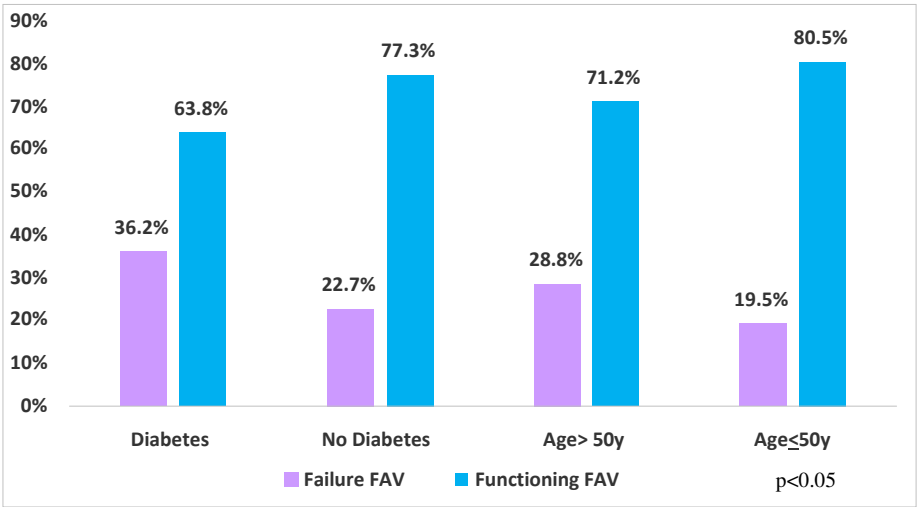

Fig. 2. AVF failure in diabetes and older age patients

failure seen by chi square test was statistically significant in diabetics and patients older than 50 years ($p<0.05$) (Figure 2). Another expected result was the strong correlation between permanent access failure and the time of fistula construction. It was seen that they failed randomly if were made after initiation of hemodialysis ($p<0.01$) (Table 1). Usage of subclavian catheter seems to be very harmful for the fistulas survival and the association between their presence and AVF failure was found in our study ($p<0.001$) (Figure 3). Not receiving antiplatelet drugs ($p<0.01$) and presence of hypotension prior or during hemodialysis session ($p<0.02$) were also correlated with AVF failure (Table 1). A tendency of AVF failure was seen in females, although not significant.

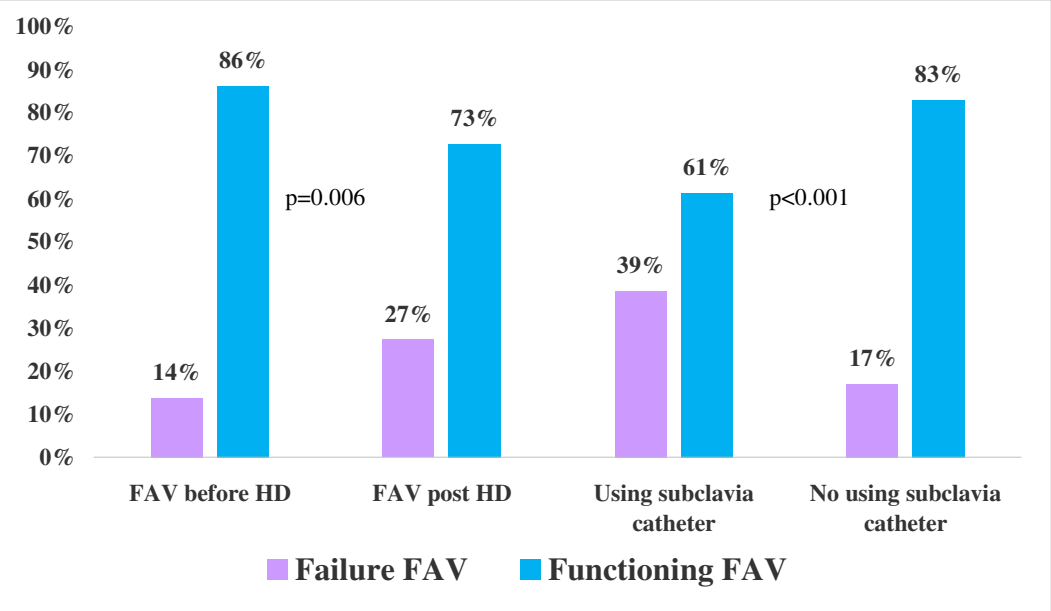

Fig. 3. AVF failure and time of its creation

Discussion

Vascular access morbidity is still high in patients on long-term hemodialysis whose health-related costs are increasing [1-6]. The discussion regarding vascular access in hemodialysis is always open although the guidelines are clearly set and defined [4]. This is most probably due to the wide range of hemodialysis populations all around the world in terms of mean age, primary renal diseases, co-morbidities, percentage of diabetes among them and there is also an evident problem with patients' referral and a different viewpoint of nephrologists in terms of vascular access. Some nephrologists are not convinced when to start with the vascular access and keep treating their patients as much as possible with the "sweet honey" of conservative therapy, until their patients develop uremic states, and receive dialysis only in emergency situations. Ideally, every patient would initiate dialysis with a mature fistula suitable for cannulation. Striving for this goal requires a number of intermediate steps, including pre-ESRD care by a nephrologist, pre-ESRD access surgery, adequate fistula maturation, and successful fistula cannulation by the dialysis staff. This sequence is akin to running a hurdle race, in that all steps have to be performed in sequential order, and failure of any step results in a patient who initiates dialysis with a catheter. Fistula use is much higher among hemodialysis patients in Europe and Japan, as compared with those in the United States [7,8]. Similarly, there are marked differences in fistula prevalence among different dialysis networks within the United States, with the highest frequencies observed in the Northeast and the lowest in the Southeast [9]. These analyses highlight the importance of practice patterns in affecting fistula use and contributed to the 2001 Kidney Disease Outcomes Quality Initiative (K/DOQI) Vascular Access guidelines [4], followed by the "Fistula First" national initiative [10]. Approximately one third of US patients lack nephrology follow-up before initiation of dialysis (8). Among those with pre-ESRD nephrology follow-up, one third do not have access surgery before starting dialysis [11]. Finally, approximately one third (20-50%) of new fistulas fails to mature [12]. The cumulative effect of not overcoming these successive hurdles is that 60 to 65% of patients in the United States initiate hemodialysis with a catheter [7,12]. Even 60 days after initiation of dialysis, 46% of patients are catheter dependent [12].

Our study showed that at present in Albania, 83.5% of prevalent patients make use of an AVF, 2.25% a AVG and 14.25% of a catheter. This is quite good compared with European and US patients as mentioned

above, but majority of incident patients (84%) have started hemodialysis with a catheter, even though they were not that late referral, since 62.5% of CKD patients were followed up by nephrologists. Our patients are younger (mean age 49.9 years) compared to European patients, (60.5 years old) [7], and this is a very important clue.
Another point that needs to be highlighted is the lower percentage of diabetes among our patients, only 13.8%.
Analyzing the rate of failures and the causes of fistula failure, our results are in agreement with the literature reporting that access failure is seen in diabetic patients and in older patients >50 years (Figure 2). As Hayakawa and colleagues [13] showed the age and diabetes mellitus were risk factors for successful maintenance of the initial permanent hemodialysis VA. Also diabetes mellitus was associated with a higher frequency of fistula failure. These findings were in compliance with the results of Garrancho and his colleagues [14].
Hypotension (pre-dialysis and intra-dialysis hypotension) turned to be also a hazardous parameter for fistula surveillance contributing to access thrombosis, as reported by Chang and his colleagues [15]. In our study patients with presence of hypotension prior or during hemodialysis session have significantly more FAV failure ($p<0.02$) (Table 1). Most importantly, lower BP and intradialitik hypotension are two potentially modifiable risk factors for access thrombosis and may account for at least 20% to 40% of access thrombosis in the absence of obvious structural abnormalities. As a result, higher BP targets may be preferred in patients with identified stenosis in their vascular accesses, recent intervention, or other characteristics of increased access thrombosis risk.
Fistula failure was strongly correlated with the fact of having a fistula after initiation of hemodialysis as it is highlighted in the report of Couto A, *et al*. [16]. This reported finding coincides with our results in which patients with FAV creation after starting of dialysis had significantly more FAV failures ($p=0.006$). In our study, it also resulted that if patients used to have subclavian catheters, their access was quite probable to fail in terms of venous stenosis that occurs in 20-50% of them ($p< 0.001$) (Figure 3).
Not using antiplatelet drugs was also related to permanent access failure. In a study of Hasegawa and his colleagues [17], it was consistently demonstrated that the use of aspirin improved the outcome and the survival of permanent vascular access. We found that patients using antiplatelet drugs have significantly low rate of FAV failure ($p<0.005$), (Table 1). Faced with such results, we have some lessons to learn and challenges to bear in the future, and they are:

- To insist on patients' *early referral*, to train the general practitioners to be alert concerning patients with hypertension, inherited kidney disease, diabetics, patients with reflux and calculosis; to assist them. Enhancing pre-ESRD nephrology follow-up requires raising the awareness by primary care physicians of how to diagnose CKD and when to refer patients to a nephrologist [7,18,19].
- *Nephrologists* should be very attentive to follow CKD patients, and prepare them emotionally and surgically for an arteriovenous fistula at the fourth stage of disease because it needs some time to mature [18,19].
- *A close collaboration among* the members of the multidisciplinary team, consisting of nephrologists, surgeons, radiologists, and dialysis staff, which is required to optimize these efforts. It may also be well arranged by assigning a committed access coordinator to streamline the process [20].
- *Preoperative vascular mapping* provides the surgeon with precise information about the diameter of the artery and vein and the presence of vein stenosis or thrombosis and frequently leads to a change in the intended access [21]. A dramatic increase in fistula placement was observed by several centers after implementation of routine preoperative vascular mapping [22-25].
- *To avoid subclavian catheters;*
- *To preselect committed, skilled, and interested surgeons;*
- *To increase efforts in fistula maturation.* Primary fistula failure, as a result of early thrombosis or failure to mature is a major hurdle which leads to fistula prevalence increase [26]. It is more common in women [27], nonwhite patients, older patients, and those with vascular diseases. Relatively, not much has been published on the natural history about new fistulas, about specific reasons of their failure to mature, or best test to use and time to assess their likelihood of success, and the optimal interventions to promote their maturation [28]. The maximal increase in fistula diameter and blood flow occurs within the first few weeks of their placement [28-30]. A post-operative ultrasound may help in assessing fistula maturation. In one study, fistulas with a diameter >4 mm and blood flow >500 ml/min had a 95% likelihood of successful use for dialysis, whereas those that fell below both thresholds had only a 33% chance of success [30].
- *Antiplatelet drug use.* An ongoing multicenter, double-blind, randomized clinical trial sponsored by the National Institutes of Health is evaluating the clopidogel efficiency and safety to prevent early fistula thrombosis occurrence [31].
- *Dialysis staff training* [32]
- *Surveillance of vascular access*: static venous pressure, dynamic venous pressure, access flow recirculation, color flow Doppler, U/S dilution technique.
- *And most important:* "A rule written on the stone": "SAVE THE VEINS" named cephalic and basic, as they are not trombophyllic for the access [4].

Conclusions

Arteriovenous fistula is the predominant form of vascular access in majority of patients in Albania. The main characteristics of our HD population are the low percentage of diabetics and their young age, which make prolong the use of their vascular access and render HD treatment more effective. Thus, we are satisfied and proud of our results achieved in the majority of HD patients, but we do have a lot of work to do with the incident ones.

Conflict of interest statement. None declared.

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Original Article

Iron Status, Iron and Epoetin Therapy in Chronic Hemodialysis Patients-A Single Center Experience

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Abstract

Introduction. Recent KDIGO anemia treatment guidelines encourage the use of iron in treating renal anemia in chronic hemodialysis patients. However, a recent study by Rostoker, *et al*. has demonstrated, by using magnetic resonance imaging, hepatic iron overload in significant proportion of chronic hemodialysis patients. The aim of our retrospective cross-sectional clinical study was to evaluate iron status, levels of iron and epoetin therapy in chronic hemodialysis patients from our center.
Methods. All patients treated by chronic hemodialysis in the Dialysis center for acute and complicated hemodialysis at the University Medical Center in Ljubljana during the month of March 2013 were screened (N=181). Median age was 66 (inter-quartile range 57-80) years, 58.6% were male. The data for the study were obtained from the hospital's laboratory database. Iron and epoetin therapy during that time were recorded from our dialysis charts.
Results. A total of 174 adult chronic hemodialysis patients participated in this study. 150/174 (86.2 %) of the patients have been receiving epoetin therapy, all intravenously, while a lower percentage (100 patients, 57,4%) have received intravenous iron therapy. Out of the patients being treated with epoetins, 36/150 (24%) have received darbabopetin, and the rest of them either epoetin-alfa or epoetin-beta. Average laboratory results were: hemoglobin 11.7±1.1 g/dl, serum iron 10.1±3.9 µmol/l, ferritin 681±440 µg/l, TIBC 40.2±5.8 µmol/l, TSAT 25.7 ±10.4% iPTH 341±314 ng/l, 78.7% of the patients used arteriovenous fistulas as vascular access, 52/174 (29.9%) had elevated CRP levels.
Conclusions. Weekly iron and epoetin dose in our patients were not high. Average serum ferritin level was rather high. Caution on possible iron accumulation should be in focus of renal anemia treatment during the next period.

Keywords: anemia, chronic kidney disease, epoetin, hemodialysis, hemosiderosis, parental iron

Introduction

Anemia is a common complication among patients with chronic kidney disease (CKD). It is present in the vast majority of patients on dialysis, leading to considerable morbidity, mortality and reduced quality of life [1-10]. It reflects a combination of erythropoietin deficiency and iron deficiency, as well as bone marrow resistance to erythropoietin [11]. Diet restrictions, poor absorption of iron, frequent blood tests, or removal of iron and vitamins by hemodialysis also contribute to anemia in a chronic dialysis patient.
As defined by the Kidney Disease: Improving Global Outcome (KDIGO) anemia treatment guidelines, anemia is a hemoglobin (Hb) concentration <12 g/dl for women and <13 g/dl for men [3]. The European Best Practices (ERBP) Guidelines for the Management of Anemia in Patients with Chronic Renal Failure define anemia according to age and sex. Anemia is defined as an Hb concentration of <12 g/dl in women, <13.5 g/dl in men ≤70 years of age, and <13,2 g/dl (in men >70 years of age [1]. Iron deficiency was defined using the Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines criteria: ferritin level <100 ng/ml or the percentage of transferrin saturation (TSAT) <20% when ferritin is <800 ng/ml [2].
Erythropoiesis-stimulating agents (ESAs) are frequently used to treat anemia of chronic kidney disease in the dialysis setting. ERBP guidelines recommend to initiate ESA maintenance therapy when the Hb values are between 9 and 10 g/dl. For patients, who are already receiving ESA therapy, the recommended Hb target value should be kept between 10 and 11.5 g/dl and should not be allowed to routinely fall below 10 g/dl. Individualization of therapy is reasonable as in some low-risk patients (i.e. in younger patients with very few comorbidities), those with ischaemic heart disease with worsening ischaemic symptoms associated with anaemia, or in those in whom a clear benefit on quality of life can be foreseen, ESA therapy may be started at higher Hb values but not exceeding 12.0 g/dl [1].

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Correction of iron deficiency with oral or intravenous iron supplementation can reduce the severity of anemia in patients with CKD and also improve the erythropoietic response to ESA treatment. Due to having a readily available vascular access, intravenous (IV) administration of supplemental iron is preferred among hemodialysis patients. Treatment with IV iron is recommended when transferrin saturation (TSAT) falls below 30% and ferritin is <500 mg/l [1-3,8-10]. According to ERBP guidelines, in adult CKD patients on ESA therapy who are not receiving iron supplementation, a trial of IV iron should be initiated if an increase in Hb concentration or a decrease in ESA dose is desired and TSAT is below 30% and ferritin is <300 ng/ml. In hemodialysis patients with higher serum ferritin levels a course of IV iron therapy can be considered in the presence of hyporesponsiveness to ESA or a risk/benefit ratio going against ESA use [1].

The aim of our retrospective cross-sectional clinical study was to evaluate iron status, levels of iron and epoetin therapy in chronic hemodialysis patients treated in our center.

Materials and methods

For this retrospective cross-sectional clinical study we have used the laboratory database of the University Medical Center Ljubljana, Dialysis center for acute and complicated hemodialysis, for the three month period between January and March 2013. During that time 181 chronic hemodialysis patients were treated at our center, 174 of whom were included in this study. Seven patients were not recruited because they were either absent from our center or have passed away during the observation period. Patients' dialysis charts were retrospectively examined to obtain data regarding the iron and epoetin therapy in the observed time period.

The study population consisted of 102 men and 72 women, with median age of 66 (inter-quartile range 57-80) years. The patients' complete blood count (CBC), serum iron, ferritin, C-reactive protein (CRP) levels, albumin levels (Alb), total iron binding capacity (TIBC) and TSAT were monitored on a monthly basis, prior to the first mid-week hemodialysis session of the second half-month. Intact parathyroid hormone (iPTH) levels were monitored on a 3-month basis.

In accordance with European Best Practice and KDIGO guidelines [1,3], anemia treatment in our hemodialysis center comprised of intravenous administrations of either darbepoetin alfa (Aranesp®, Amgen, Thousand Oaks, USA), epoetin alfa (Eprex®, Janssen-Cilag, Buckinghamshire, UK) or epoetin beta (NeoRecormon®, Roche, Basel, Switzerland) and, if required, iron sucrose (Venofer®, Lek, Ljubljana, Slovenia), with the following targets: hemoglobin 10-11,5 g/dl; TSAT: lower limit 20%, target range 30%-50%; and serum ferritin: lower limit 100 µg/l, target range 200-500 µg/l.

Levels of hemoglobin, serum iron, ferritin, TSAT and TIBC, along with the iron and epoetin therapy doses measured during the month of March 2013 were compared between groups with normal and elevated C-reactive protein (i.e. CRP≥10 mg/l [8]), low serum albumin levels and high iPTH values. We also evaluated the angiotensin-converting enzyme inhibitors (ACEI) use and the choice of dialysis procedure. To evaluate the dose-response effect of erythropoetin therapy, we used the erythropoietin resistance index (ERI), calculated as the weekly weight-adjusted dose of ESA divided by the hemoglobin level. Finally, we also compared the before mentioned laboratory parameters between patients who recevied ESA and iron replacement therapy and those who did not require it.

Results

A total of 174 adult chronic hemodialysis patients participated in this study. 86.2% of the patients have been receiving epoetin therapy, all intravenously, while a lower percentage (57.5%) have received intravenous iron therapy in the last month. Out of the patients receiving epoetins, 27.8% have received darbabopetin alfa, and the rest of them either epoetin-alfa or epoetin-beta.

Table 1. Patients' main treatment and laboratory data

Parameters	All (n=174)	Range
Age (yrs)	66±15	26-92
Male (%)	58.6	
AVF[a] (%)	78.7	
HDF[b] (%)	24.7	
ACEI[c] (%)	25.3	
Hb (g/dl)	11.7±1.1	8.7-16.1
Ht (%)	0.352±0.03	0.271-0.485
ESA[d] (IU/week)[h]	5268±4562	0-24000
Iron Sucrose (mg/month)[h]	136±116	0-533
Ferritin (µg/l)	681±440	13.7-2758
Serum Fe (µmol/l)	10.1±3.9	4.25-23.4
TIBC[e] (µmol/l)	40.2±5.8	22.3-64.3
TSAT[f] (%)	25.7±10.4	8.43-65.5
iPTH (ng/l)	341±314	3-1843
Alb (g/l)	37.6±3.2	26-45.3
ERI[g] (IU/kg/week/g per dl)[i]	7.7±0.5	0.4-24.8

[a]Arterio-venous fistula, [b]Hemodiafiltration, [c]Angiotensin-Converting-Enzyme Inhibitor, [d]Erythropoiesis-stimulating agent, [e]Total Iron-binding Capacity, [f]Transferrin saturation, [g]Erytropoetin Resistance Index, [h]All patients included, [i]n=157

The main patients' treatment and laboratory data are presented in Table 1. Laboratory values, calculated as the mean of the last three values, of serum hemoglobin, iron, ferritin, TIBC and TSAT were 11.7 g/l, 10.2 µmol/l, 662 µg/l, 40.1 µmol/l and 25.7%, respectively. Mean ERI was 7.7 IU/kg/week per 100 ml. 78.7% of the patients had arteriovenous (AV) fistulas as vascular access, postdilutional hemodiafiltration (HDF) was prescribed in 24.7% and standard bicarbonate dialysis (BHD) for in the rest of the patients. 25.4% of patients were treated

with ACE inhibitors. Mean ESA and iron sucrose doses were calculated from the data collected from all patients who participated in the study (N=174). If these doses are calculated by only including patients who were recieving ESA and iron sucroce during the observed period, the results are 6534±4631 IU/week and 239±250 mg/month (Table 2 and Table 3). 29.9% of the patients had elevated CRP levels, 17.8% of the patients had hypoalbuminemia and 41.9% iPTH levels >300 ng/l. Their mean age was higher and a larger proportion of patients used central venous catheters instead of arterio-venous fistulas as a form of vascular access.

Table 2. Comparison of characteristics between patients without and with ESA (erythropoesis-stimulating agent) replacement therapy

Parameter	Without therapy (n=24)	ESA (n=150)	p-value
Age (yrs)	62.7±13.3 (34-88)	66.5±15.6 (26-92)	0,814
Hb (g/dl)	12.9±1.7 (8.4-15.5)	11.5±1.3 (7.9-17.5)	<0.001
ESA (IU/week)[d]	0	6534±4631 (1000-24000)	
Iron Sucrose (mg/month)	121±206 (0-1000)	140±227 (0-1200)	0.7
Ferritin (µg/l)	555±435 (34-1587)	724±500 (15-3334)	0.12
Serum Fe (µmol/l)	9.5±4.3 (3.1-23.2)	10.2±4.6 (3.1-25.7)	0.486
TIBC (µmol/l)[b]	42.4±7.4 (28.2-57.1)	39.9±6.3 (15.1-58.6)	0.08
TSAT (%)[b]	22.9±11.1 (7.2-61.7)	25.4±11.4 (6.7-64.3)	0,612

Data are presented as mean ± standard deviation
[a]Erythropoiesis-stimulating agent, [b]Total Iron-binding Capacity, [c]Transferrin saturation

Table 3. Comparison of characteristics between patients without intravenous iron replacement therapy

Parameter	Without therapy (n=74)	Iron Sucrose (n=100)	p-value
Age (yrs)	64.5±16.7 (28-88)	67±14.8 (26-92)	0.505
Hb (g/dl)	11.7±1.4 (7.9-14.4)	11.6±1.4 (8.4-17.5)	0.642
ESA (IU/week)[d]	4990±4194 (0-20000)	6109±5262 (0-24000)	0.133
Iron Sucrose (mg/month)	0	239±250 (100-1200)	
Ferritin (µg/l)	675±375 (42-2338)	719±568 (15-3334)	0.563
Serum Fe (µmol/l)	10.6±4.3 (3.6-23.2)	9.8±4.7 (3.1-25.7)	0.252
TIBC (µmol/l)[e]	39.5±4.6 (28.2-49.9)	40.8±7.5 (15.1-58.6)	0,189
TSAT (%)[f]	27±10.9 (9.7-61.7)	23.6±11.5 (6.7-64.3)	0,05

Data are presented as mean ± standard deviation
[a]Erythropoiesis-stimulating agent, [b]Total Iron-binding Capacity, [c]Transferrin saturation

Patients with elevated CRP (>10 mg/l) had significantly lower hemoglobin level (11.3±1.3 versus 11.8±1.4, p=0.03) and serum iron (8.3±4.2 versus 10.9±4,4, p<0.001), higher ferritin (764±527 versus 673±475, p=NS), ERI (8.9±7 versus 6.9±5.9, p=0.05) and epoetin doses (6154±4884 versus 5411±4846, p=NS) and a lower iron sucrose dose (113.5±201 versus 142±240, p=NS).

Hypoalbuminemic patients (compared to patients with albumin level >40 g/l) had significantly lower Hb levels (10.7±1.3 versus 11.9±1.3, p<0.001) and serum iron (8.2±4.2 versus 10.6±4.5, p=0.007), similar ferritin levels (714±451 versus 697±505, p=NS), a higher ERI (10.1±6.8 versus 6.7±6, p=0.006) and epoetin dose (6774±4911 versus 5385±4825, p=NS), with lower dose of iron sucrose (105±119 versus 145±240, p=NS).

Patients with iPTH >300 ng/l (compared to patients with iPTH <300 ng/l) had similar Hb levels (11.7±1.4 versus 11.6±1.4, p=NS), similar serum iron (11.6±4.4 versus 10.1±4.6, p=0.03) and ferritin levels (692±499 versus 706±494, p=NS), however ERI was higher (8.2±7.3 versus 7±5.5, p=NS) in parallel with ESA dose (6310±5868 versus 5144±3297, p=NS), The dose of iron sucrose was lower in this group (116±162 versus 154±259, p=NS).

No significant difference was found between the groups with or without ACE inhibitor therapy as concerns hemoglobin level, serum iron, ferritin, ERI, ESA and iron sucrose dose.

Patients treated by postdilutional HDF had lower ERI (5.6±4.9 versus 8.15±6.6, p=0.02) and lower ESA dose (4360±4233 versus 6051±4987, p=0.03). No significant difference was found in the level of hemoglobin, serum iron, ferritin and iron sucrose dose compared to patients treated with bicarbonate hemodialysis.

Discussion

The major finding of our study was that average hemoglobin level was within the guidelines [1] and that average ferritin levels were rather high, including the subgroup in the group with non-elevated CRP, normal albumin and iPTH levels. Interestingly, ferritin levels were elevated even among patients who did not receive

intravenous iron replacement therapy during the month the screening took place. This can be explained by the fact that a vast majority of patients have received intravenous iron in previous months, with only 26.7% of patients not receiving any iron supplements during the observed period. Also, some of the patients were taking iron supplements by oral route and some received intravenous iron outside our center (for e.g. patients treated for hematological conditions in other clinics). There was a noticeable increase of ESA dosage among patients with hypoalbuminemia and high iPTH levels, while patients treated with HDF procedures needed smaller doses of epoetins compared to those treated with BHD procedures

Renal anemia is a common complication among patients with chronic kidney disease, especially among those requiring hemodialysis and can be corrected by erythropoiesis-stimulating agents (ESA) [12]. Current guidelines recommend initiation of ESA therapy when serum hemoglobin drops below 10 g/dl, but should not be used to intentionally increase Hb concentration above 13 g/dl as higher Hb concentrations raise the risk for stroke, hypertension, vascular access thrombosis and may perhaps also increase risk for death or serious cardiovascular events [1-3.8]. Among our study population, a significant proportion was receiving ESA (86.2%). The mean Hb concentration was 11.7±1.1 g/dl, with 5.2% of the patients having target Hb concentration below 10 g/dl, and 10.3% above 13 g/dl.

Failure to achieve adequate iron stores and availability is the main cause of hyporesponsiveness to ESA therapy. For the optimal management of anemia, use of iron supplements in combination with ESA is required [12]. Iron however, is considered to be somewhat of a double edged sword as it is critical for health, yet highly toxic because of its oxidative properties. As a result, the hepcidine system carefully regulates iron absorption from the diet and the availability of iron from storage tissues. Intravenous injection of iron directly into the circulation bypasses these protective controls, and this raises concerns regarding the safety of intravenous iron [13]. In a MRI-based study from Rostoker, *et al.* of hemodialysis patients receiving both erythropoeisis-stimulating agents and intravenous iron, hepatic iron overload was observed in the majority of cases [14]. Iron overload can also increase the risk of infection as it facilitates bacterial growth and impairs host defense against microbial pathogens [15].

KDIGO anemia treatment guidelines recommend that serum ferritin as a measure of iron storage in the body should be quantified every 3 months in patients who are receiving ESA treatment and intravenous iron supplementation. This is required to establish whether an iron deficiency exists or too much iron supplementation is being administered. It must be noted, that high serum ferritin levels in patients with end-stage renal disease (ESRD) may be a result of inflammation, infection, malnutrition, or malignancy and not necessarily the result of iron overload [3]. In our center, we monitor serum ferritin levels of our patients on a monthly basis. The mean ferritin level in our patients was rather high (681±440 µg/l), with only 23.6% of the patients achieving target ferritin values, which raises concerns about possible iron accumulation.

Fluctuation of Hb levels or "Hb variability" during treatment with ESAs is a well-documented phenomenon. Evidence suggests that inflammation is an important factor associated with Hb variability and that high C-reactive protein levels (a widely used surrogate marker of inflammatory activity) are a predictor for less stable Hb control in CKD patients [16]. 29.9% of our patients had elevated CRP levels. Among this group, a higher ESA and a lower dose of supplemental iron was used to maintain target Hb concentration. As expected, despite using lower doses of iron sucrose, ferritin concentration remained higher than in patients with normal CRP levels.

Several possible strategies exist to enhance the response to ESAs and iron in dialysis patients with persistent low-grade inflammation. Occult infections, when found, should be treated with antibiotics. Also, occult infection of old, non-functioning, arteriovenous grafts may be a cause of ESA resistance and a chronic inflammatory state in HD patients. Resection of old non-functioning arteriovenous grafts with occult infection was associated with the resolution of markers of a chronic inflammatory state and improvement in responsiveness to ESA treatment [16]. Chronic heart failure with fluid overload, a common feature in dialysis patients, may be an important cause of inflammation. Thus, rigorous measures should be taken to avoid or treat fluid overload in these patients [16].

Angiotensin-converting enzyme inhibitors are widely used in renal failure patients in the treatment of hypertension, left ventricular dysfunction, and diabetic nephropathy [17]. Much controversy has been generated over whether these drugs can suppress erythropoiesis, and thereby exacerbate anemia, with several studies suggesting that they do [18,19], while others finding no such effect [20]. Among our study population, 25.4% were treated with ACEI. In our experience, ACEI treatment does not seem to have negative effects on hemoglobin levels and erythropoetin-resistance index. When compared to those not treated with these drugs, the epoetin dose was slightly higher in this group, however, the difference was negligible. While ACEI may evoke a degree of epoetin resistance particularly at high doses, this should be able to be counteracted by a corresponding increase in the dose of epoetin [21].

Hemodiafiltration is associated with a lower incidence of neuropathy, carpal tunnel syndrome, joint pain, and partial correction of anemia [21]. Our results show that patients treated with HDF received a lower dose of ESA and had lower ERI, however, selection bias has to be taken into account.

Hyperparathyroidism is usually listed among the possible reasons for impaired response to recombinant human erythropoietin in patients with renal disease. Possible pathogenic links between anemia and parathyroid hormone (PTH) include reduced erythropoiesis due to calcitrol deficiency, and direct or indirect effects of PTH on erythropoietin release, red blood cell production, survival, and loss [22]. Our study shows that increased iPTH levels are present in 41.9% of our patients and are associated with a decreased erythropoietic response in this group.
A large proportion of CKD patients have also protein energy malnutrition and wasting, low serum levels of albumin and other more specific nutritional markers which are predictors of the response to EPO. It is therefore possible that a diminished nutritional status could be a feature of patients who are resistant to ESA treatment [23-25]. In our study, hypoalbuminemic patients had lower Hb levels despite using significantly higher doses of epoetins.

Limitations of the study

The main limitation of this study is that it is from a single center and it is retrospective and cross sectional. Even so we feel that some findings may be important for further focus on renal anemia treatment, since the number of patients included in the study was rather high for a single center study.

Conclusions

In conclusion, ferritin levels in our maintenance hemodialysis patients were relatively high, even after excluding those with elevated CRP levels. Hemoglobin level was in the target range for the majority of patients, with moderate ESA dose. Possible iron accumulation should be a focus of further studies on renal anemia treatment in the next period.

Conflict of interest statement. None declared.

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Original Article

Apoptosis and Gingival Tissue

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Abstract

Introduction. Several factors can influence the gingival tissues such as disrupting tissue homeostasis and the occurrence of pathological conditions. The aim of our study was to investigate and compare the presence of apoptosis in patients undergoing immunosuppressive therapy, patients with periodontitis and healthy patients, as well as to achieve better understanding of the role of apotosis in the same processes.
Methods. The first examined group consisted of 21 patients (10 males and 11 females; mean age 37.4±10.2 years) with neither kidney diseases nor cyclosporine A (CsA) therapy, who had a verified periodontal disease. The second group consisted of 21 kidney-transplant patients (9 males and 12 females), with diagnosed gingival overgrowth (GO) undergoing continuous immunosuppressive therapy. The control group consisted of the same number of patients, clinically healthy subjects (15 males and 6 females; mean age 29±14.0 years) with plaque-induced gingivitis. The following indexes were analyzed: plaque index (PI), index of gingival inflammation (GI) according to Loe-Silnes, and gingival overgrowth index (GOI) according to MacGaw, *et al*. The determination of CsA in blood was performed by a fluorescence polarised immunoassay (FPIA). The tissue samples were estimated by semi-quantitative analysis in order to determine the presence of apoptotic cells and imunohistochemical expression of the bcl-2 and p53 proteins.
Results. In our study we found statistical differences in bcl-2 and apoptotic index, among the groups: the greatest expression of bcl-2 and apoptotic index was registered in the group treated with CsA, and the lowest expression was noted in the gingivitis group ($p<0.01$). There was a statistical significant positive correlation between bcl-2 and apoptotic index, PI, and GI index ($p<0.05$). There was no significant correlation between the blood concentration of CsA and apoptosis ($p>0.05$; $r=0.187$).
Conclusions. Our findings suggest that increased apoptosis may have a role in the pathogenesis of CsA-induced gingival overgrowth in the cases of patients on high dose of CsA.

Key words: gingival overgrowth, cyclosporine A, gingival inflammation, apoptosis, bcl-2, p53

Introduction

Tissue homeostasis is maintained by keeping a balance between cell proliferation and cell death. Inhibition of apoptosis has been implicated in the pathogenesis of malignancy and autoimmune disorders and increased apoptosis is thought to be involved in acquired immunodeficiency syndrome and ischemic injury [1]. Several studies suggest that apoptosis plays an important role in the control of tissue overgrowth. Granulation tissues are going to remodelation of the tissue by apoptosis of fibroblasts. Wounds in which there is inadequate apoptosis may result with the formation of fibrotic tissues [2-4]. This modulation of apoptosis may contribute to the etiology of the occurrence of fibrosis in gingival tissues.
Several studies indicate that bacterium-modulated apoptosis appears to be an important phenomenon in the pathogenesis of infectious diseases [5,6]. Specific pathogens or their exocellular products may directly induce apoptosis of host cell [7,8] and bacterial components such as lipopolysaccharide may delay programmed cell death of terminally differentiated polymorphonuclear leukocytes (PMN) [9,10].
The aim of our study was to investigate and compare the presence of apoptosis in patients treated with immunosuppressive therapy, patients with periodontitis and healthy patients, as well as to achieve a better understanding of the role of apoptosis in these processes.

Materials and methods

The first examined group consisted of 21 patients (10 males and 11 females; mean age 37.4±10.2 years) with neither kidney diseases nor CsA therapy, who had a verified periodontal disease where the average loss of attachment was 1.98. The medical history of all patients excluded use of any medicament causing gingival overgrowth (GO).

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The second group consisted of 21 kidney-transplant patients (9 males and 12 females), with diagnosed GO and subjected on continuous immunosuppressive therapy (175 mg Cs/day). Patients' mean age at time of renal transplantation was 36.2±9.5 years. The mean duration of therapy was 42.4±36.2 months. The post-transplant immunosuppressive therapy consisted of cyclosporine (Neoral®) reaching a satisfactory C2 level (concentration in serum 2 hours after administration of the medicament), prednizolon (0.1 mg/kg/den, Merck), and mycophenolate mofetil (Cellcept 1.5-2g/day, Roche).
The control group consisted of the same number of patients, clinically healthy subjects (15 males and 6 females; mean age 29±14.0 years) with plaque-induced gingivitis, but with no signs of periodontitis. In all patients included in the three groups in our study the use of antibiotics, anti-inflammatory agents and the history of treatment with medicaments known to cause drug-induced GO were excluded.
The patients included in our study underwent the same clinical and para-clinical examinations.

Clinical examinations

The clinical examinations were made by applying the following indexes:

1. Plaque Index (PI) according to Silness-Loe [11];
2. Each of the four surfaces of the teeth (buccal, lingual, mesial and distal) was scored from 0-3. The scores from the four areas of the tooth are added and divided by four in order to show the plaque index for the tooth with the following scores and criteria: 0-No plaque; 1-A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen in situ only after application of disclosing solution or by using the probe on the tooth surface; 2-Moderate accumulation of soft deposit within the gingival pocket, or the tooth and gingival margin which can be seen with the naked eye; 3-Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin;
3. Gingival Inflammation Index (GI) was estimated according to Loe-Silnes scale [12] as: grade 0- normal; grade 1-mild inflammation, slight color change and edema, no bleeding; grade 2-moderate inflammation, redness, edema, bleeding on probing and grade 3-severe inflammation, marked redness and edema, ulceration and spontaneous bleeding.

Tissue Processing and Histochemistry

In the first phase of the periodontal treatment, bioptical material from the overgrown interdental papilla (patients treated with Cs) was taken during gingivectomy procedure under infiltrated anesthesia and was fixed in 10% neutral formalin, while a standard patohistologic processing was made at the Institute for Pathology at the Medical Faculty in Skopje.
Tissue biopsies from the second group (periodontal disease) and the third group (control group) were obtained during the routine dental treatment (gingivoplasty and tooth extraction from orthodontic reasons or any other indication). All patients enrolled in the study gave their informed consent for participation, according to Helsinki Declaration in 1975, revised in 2000.
Furthermore, the tissue samples were placed into the paraffin moulds, out of which tissue cross-sections with 4-6 μm thickness were obtained. These tissue cross-sections were placed on glasses by a standardized manner and colored with hematoxylin eosin (HE), while the cross-sections for the immunohistochemical coloring were placed on silane glasses and colored with ABC-Avidin Biotin Complex method, LSAB + variant.

Immunohistochemistry (ABC-Avidin Biotin Complex method)

Primary antibody, with a determined antigenic determinant, was added to the tissue sample, and thereafter incubated at room temperature (30 min). Later on the samples were rinsed with phosphate buffer, then deluded into 10% normal serum and rinsed again with phosphate buffer. The secondary antibody, which is coupled with biotin was added further on. The Avidin-Biotin Complex contains HRP enzyme (Horse radish peroxidase), which bonds with the biotin molecule of the secondary antibody, and which bonds with determinants of the primary antibody. The samples were incubated at room temperature for 30 minutes and then rinsed with phosphate buffer. The next step involves adding AVS reagent and rinsing with phosphate buffer. The final result as a positive antigenic antibody reaction was followed by forming of a brown precipitate from the polymerized substrate.
Following the immunohistochemical coloring by applying a light microscope on the tissue cross-sections, detection and counting of the apoptotic cells was done, bcl-2 and p53, expressed as average number of cells on ten visual fields (X 400). The level of expression of p53 and bcl-2 as well as the apoptotic cells of each slide was graded on a semi-quantitative manner using a graduation of 0-3+; (0)=no staining; (1+)=stained cells comprising >10% of the inflammatory infiltrate; (2+)=stained cells comprising up to 30% of the inflammatory infiltrate; (3+)= stained cells comprising >30% of the inflammatory infiltrate. The obtained results were photo-documented.

Statistical analysis

Differences between the parodontitis group, CsA-treated group and the control group with respect to the clinical parameters and the histopathological findings were analyzed by using the Student's t-test, Kruskal-Wallis test and Mann-Whitney U test of inversion. Statistical

significance was defined as p<0,05. Correlations between histopathological findings and clinical parameters were tested using Spearman's rank correlation coefficient.
The immunohistochemical findings of the examined groups are shown in Table 1. There was no statistical difference between the groups for p53. In our study statistical differences were found in the levels of bcl-2 among the groups, between the first (group with parodontitis) and the second (group with CsA therapy), the first and the third group (group with gingivitis), and between the second and the third group (p<0,01), respectively. The greatest expression of bcl-2 was registered in the second group, treated with CsA, a bit lower expression in the group with periodontal disease, and thelowest expression was noted in the gingivitis group. Identical results were found when compared the apoptotic index, which was highest in the CsA group (p<0.01), when compared to the other groups.
Patients in the CsA group had significantly higher PI and GI values than did patients in the other groups. There was statistical differences for PI and GI among the groups, between the first (group with parodontitis) and the second (CsA group), the first and the third group (group with gingivitis), and between the second and the third group (p<0.01), respectively (Table 2).

Table1. Distribution of immunohistohemical findings in Parodontitis group, Cs group, and Gingivitis group

Groups	Index	N	Percent %
Parodontitis	p53-1	16	76
	p53-2	5	24
Cs group	p53-1	16	76
	p53-2	5	2
Gingivitis	p53-1	19	91
	p53-2	2	9
Parodontitis	bcl2-1	16	76
	bcl2-2	5	24
Cs group	bcl2-1	5	24
	bcl2-2*	16	76
Gingivitis	bcl2-0	8	38
	bcl2-1	13	62
Parodontitis	apoptosis-0	9	2
	apoptosis -1	16	76
	apoptosis -2	3	15
Cs group	apoptosis -2*	9	43
	apoptosis -3*	12	67
Gingivitis	apoptosis -0	2	9
	apoptosis -1	11	52

*Significantly higher than the gingivitis and parodontitis grops (p<0.01)

Table 2. Distribution of clinical findings in various study groups

Group	Index	N	%
Paraodontitis Group	PI-1	15	71
	PI-2	6	29
CsA Group	PI-2	5	24
	PI -3	16	76*
Gingivitis Group	PI -0	16	76
	PI -1	5	24
Paraodontitis Group	GI-1	16	76
	GI-2	5	24
CsA Group	GI-2	11	52*
	GI-3	10	48
Gingivitis Group	GI-0	20	95
	GI-1	1	5

*Significantly higher PI and GI than in the the other 2 grops (p<0.05)

However, there was no correlation between p53 and the other parameters, between the groups. The findings of our research showed a positive significant correlation between bcl-2 and apoptotic index, PI, and GI index (p<0.01). There was no significant correlation between the blood concentration of CsA and apoptosis (p>0.05; r=0.187) (Table 3).

Table 3. Correlation between different parameters

Spearman Rank Correlations p<0,01	p53	bcl-2	apoptosis	PI
bcl-2	0.041	1.000	0.448**	0.692**
apoptosis	0.045	0.448**	1.000	0.560**
PI	0.108	0.692**	0.560**	1.000
GI	0.082	0.693**	0.553**	0.900**
blood concentr. of CyA			0.187	

**Correlation is significant at the p<0.01 level

Discussion

Although the presence of bacterial pathogens is necessary for the initiation of periodontal diseases, inflammatory and immune responses also play a critical role in the progression of gingival tissue disease [13,14]. The presence of DNA damage-positive cells associated with the expression of the wild type p53 apoptosis-inducing protein in the subepithelial inflammatory infiltrate suggests that apoptotic cell death may be an important phenolmenon in the regulation of the inflammatory response to a chronic bacterial challenge. About 4% of the cells present in the subepithelial mononuclear inflammatory infiltrate displayed apoptosis-associated changes.
Gamonal and coworkers [15] detected presence of p53, Fas, FasL and active caspase-3 in the inflammatory infiltrates only in biopsies performed in the cases with chronic periodontitis, whereas Bcl-2 positive cells were reported to be present in the tissues from the healthy controls and gingival tissues from patients with chronic periodontitis. They also reported presence of apoptotic cells

in the deep area of biopsies taken from sites with probing deep of ≥5 mm and attachment of 3 mm.

Furthermore, it has been well-documented that a variety of bacterial pathogens are able to induce apoptosis in the infected cells. Leukotoxin of a periodontal pathogen and Actinobacillus actinomycetemcomitans have been shown to induce apoptosis in human T cell. Another study reported that bacterial products isolated from different strains Porphyromonas gingivalis may delay neutrophil apoptosis in a dose-dependent fashion [16].

In our study, the value of bcl-2 was highest in the group treated with cyclosporine and in the group with periodontal disease, due to the presence of dental plaque and the consequent inflammatory changes, thereby leading to prevention of dead cell apoptosis. Our results also correspond with the results of Bulut, *et al*. [17], where the frequency of grade3+ expression of bcl-2 was found to be significantly higher in the group with generalized aggressive periodontitis (GAP) than that in the control group. According to the results of Pandilova [18], further progressions of loss of attachment result in increased inflammation and consequently decrease of expression of bcl-2. Identical results were reported by Ellis, *et al.* [14]. Namely, they confirmed the association of decrease of bcl-2 expression in parallel with the greater loss of attachment. On the other hand, Gamonall, *et al.* [19] found no statistical significance between the different amount of bcl-2 in healthy gingiva and in gingiva of patients with periodontal disease.

The presence of functional p53 protein is necessary for certain activators of apoptosis, and therefore, when analysing apoptosis in our study we took into account the possibility that the antioncogen protein p53 takes part in the apoptotic processes of the gingiva.

P53 is a tumor suppressive protein, which in the active phase participates in the regulation of the cell cycle, promotes the reparatory mechanisms of the DNA and in case no reparation takes place, apoptosis occurs.

Although p53 is present in normal tissues and cells, its short half-life makes its expression almost undetectable in healthy normal tissues. Upon activation, p53 stabilizes and hence its expression can be detected with anti-p53 antibodies using standard immunohistochemical techniques [16].

There were no significant differences between the groups regarding the rate of p53 protein expression (Table 2). According to immunolocalization of p53 protein in the epithelia of hyperplastic gingival tissues, these may be in part explained by undergoing DNA damage and by the genotoxic stress of the Cs.

The expression of p53 gene has been confirmed as critical processes in tumorogenesis [20]. Expression of p53 protein has been noticed in the histologically normal epithelia adjacent to oral carcinomas and other carcinomas. It has been reported that normal epidermis, when exposed to UV radiation, results in DNA damage and shows sporadic patterns of p53 protein expression and mutations of the p53 gene [21].

Bulut, *et al*. [22] found no significant differences between CsA and gingivitis group with respect to immunolocalization of p53 and bcl-2. Furthermore, Saito, *et al.* [23] evaluated the expression of p53 immunohistochemically in overgrowth tissues induced by nifedipine and phenitoin. They observed positive expression of p53 protein in the nuclei of epithelial cells in overgrowth tissues, while no expression was found to be evident in non-overgrowth control tissues. It was suggested that bcl-2 may lead to cell accumulation, leading to acanthosis and that p53 may be implicated in the pathogenesis of nifedipine- and phenition-induced gingival overgrowth through impaired DNA.

The number of apoptotic cells was significantly higher in the CsA-treated group compared to the group with periodontitis and the inflamed gingiva of healthy individuals. Tonetti, *et al*. [24] demonstrated that the apoptotic process is involved in chronic, bacterially induced gingival inflammation. Analysis of data showed that inflammation led to an increase in apoptosis in "no overgrowth" control gingiva, and inflammation similarly appeared to stimulate apoptosis within the context of gingival overgrowth, but to a lower degree. We believe that the obtained results are mainly due to the fact that inflammation of the gingival tissue is greatest in the CsA tissues, and hence the activation of apoptosis with a consequently proliferative activite as a compensatory mechanism occurs. At the same time, the effect of cyclosporine on the gingival tissue was also present, which cannot be neglected, despite the fact that its influence was not a subject of interest in our study.

Alaaddinoglu, *et al.* [25] demonstrated that keratinocyte apoptosis in the gingiva of kidney recipients with CsA-induced GO is similar to that observed in inflamed gingiva of healthy individuals.

The patients in the CsA group had significantly higher PI and GI values than those in the other groups, as a result of the pseudopockets, which maintain more plaque and at the same time create difficulties in maintaining good oral hygiene.

The absence of any correlation of p53 with all of the analyzed parameters, as well as the participation in the cell-cycle control, is a limitation to connect it with apoptosis during peridontitis.

Our findings showed a positive significant correlation between bcl-2 and apoptotic index, PI, and GI index ($p<0.05$) (Table 3). These results support the idea that inflammation causes apoptosis, and that relatively overgrowth gingiva is a result of the compensational epithetlium proliferation. Similar results were reported by Minovska, *et al.* [26], who proved the existence of a strong positive correlation between inflammation and apoptosis, which participates in the loss of attachment, but does not participate in the recession occurrence and progression. Taking into account the obtained results for the increased mononuclear cell infiltrate in tissues treated with CsA, compared to the non-treated ones, Bulut, *et*

al. [27] supported the idea about the participation of inflammation and local immune stimuli in the development of GO, which nonetheless remains a multifactorial process. Cell composition and the existence of inflammatory cells reflect its chronic nature, which may result in a long-term local stimulation process leading to GO. The largest degree of expression of apoptosis was registered in the group treated with the highest dose of cyclosporine, most probably due to the cyclosporine action which increases the apoptosis, and at the same time this effect is strengthened by the level of inflammatory infiltrate in the fibrous tissue. The level of inflammation in the gingival tissues and the action of cyclosporine are indisputable and important factors in triggering apoptosis. In the group with gingivitis, a greater presence of apoptosis was registered, compared to the group with periodontitis, which we believe is due to the inflammatory changes (acute stage) of the gingival tissue, accompanied with a present enlargement which influences the interrupted homeostasis.

In conclusion, our findings suggest that increased apoptosis may have a role in the pathogenesis of CsA-induced gingival overgrowth in the case of high dose of CsA**.**

Conflict of interest statement. None declared.

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Original Article

Long-Term Outcomes of Renal Transplantation: A Single Center Experience

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Abstract

Introduction. Successful outcome of renal transplantation depends on various factors, of which immunologic is one of the most important. Accumulated experience of a single center, with the same surgical and immunological team contributes significantly to safe conclusions. Purpose of this study was the evaluation of potential factors, in particular immunologic, that influence renal allograft survival.
Methods. During the period 1991-2013, 20784 surgical operations have been performed in our Department of Surgery-Transplant Unit, of which 575 were renal transplantations. We examined donor and recipient demographic factors, immunologic characteristics along with patient and graft survival. Results: Renal allograft was retrieved from living-related donor in 103 cases and in 472 from cadaveric donor. Donor age was 46.7±18.5 years old and 49.9% (287) were male. Recipient age was 48±12.3 years old and 402 were male. HLA histocompatibility was carefully matched resulting in 85.5% renal transplants with 2-4 HLA mismatches and 93.8% renal transplants with at least one HLA-DR. Renal graft survival the first, fifth and tenth year was 89%, 76% and 67% and patient survival was 95%, 89% and 83%, respectively. Statistical analysis revealed that only donor age influenced renal graft survival ($p<0.05$). HLA mismatches were not correlated with graft survival (log rank $p=0.495$) but identification of panel reactive antibodies (PRA) class I and class II post-transplantation had a statistically significant impact on long-term renal graft survival (log rank $p<0.001$ and $p=0.021$ accordingly).
Conclusions. Analysis of potential prognostic factor showed that only donor age was correlated with allograft survival. Development of PRA following renal transplantation influenced long term graft survival. Good HLA matching with at least one HLA DR resulted in excellent graft and patient survival.

Key words: renal transplantation, donor age, immunologic compatibility, HLA matching, panel reactive antibodies

Introduction

Successful outcome of renal transplantation relies on many factors, one of the most important being the immunological compatibility donor-recipient. Key factor to survival analysis is the accumulated experience of a single transplant center that has the advantage that the procedures followed and operative techniques are the same as well as the immunologic handling (selection of the recipients with immunological criteria, administration of immunosuppression to prevent acute rejection and chronic renal allograft dysfunction, dealing with rejection episodes). The effect of immunologic histocompatibility on the graft and patient survival has several conflicting opinions, from minimum to mandatory.
We present the analysis of 575 renal transplantations from a single center and the identification of potential factors that influence long-term allograft survival.

Materials and Methods

During the period of 23 years (1990-2013), 20784 surgical operations have been performed at the First Department of Surgery-Transplant Unit of Evangelismos General Hospital of Athens and 575 renal transplantations. Renal transplant recipients' records were reviewed and data were recorded regarding donor characteristics, recipient characteristics, warm and cold ischemia times, immediate or delayed graft function, immunosuppressive regimens. Immunologic data were collected regarding typing of Human Leukocyte Antigens (HLA), histocompatibility donor-recipient, cross-match procedures with complement depended cytotoxicity (CDC) and flow

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cytometry (FC). Renal allograft and patient survival was recorded along with major complications.
Values of continuous parameters with normal distribution are expressed as mean±standard deviation. Comparison analysis of continuous and categorical data was perfomed with the Student's t-test and chi-square, respectively. Kaplan-Meier curves show patient and renal graft survival and log-rank was used to compare survival based on examined parameters.

Results

Demographic Characteristics

In a period of 23 years 575 renal transplantations were performed in our Transplant Unit. Renal allografts were retrieved from 103 living-related donors and 472 cadaveric donors. Mean donor age was 46.7±18.5 years old and 49.9% (287) were males. Mean recipient age was 48±12.3 years old and 69.9% (402) were males. In more detail demographic characteristics according to donor type, mean living-related donor age was 62.4±9.4 and the vast majority were females. Demographic data are presented in Table 1.
Comparison of renal transplantations from living-related and cadaveric donors showed that mean cadaveric donor age was 43.5±18.3 years vs living-related donor age 62.4±9.4, and in 56.5% the donor was male. In donation from living-related donors, older age is justified because usually parents donate renal graft to their children. Characteristically, 76.6% of living-related donors were women and more precisely 70% were mothers, 4.6% sisters and 2% wives while 19.4% were fathers, 2% brothers and there was one case of donation form grand-father and one case from cousin (Figure 1). As expected, the mean recipient age was lower in living-related donor group. It is interesting that the majority of recipients either of cadaveric or living-related renal grafts were men, 68.9% and 74.8%, respectively. This difference does not depict the incidence of chronic kidney disease, but could be attributed to the fact that women are more frequently sensitized resulting in difficulties to find a suitable renal graft.

Table 1. Demographic characteristics

	Renal transplantations	Renal grafts from cadaveric donors	Renal grafts from living-related donors
Total No	575	472	103
Donor Age	46.7±18.5	43.5±18.3	62.4±9.4
Donor Gender Men (No)	49.9% (287)	56.5% (266)	21.4% (21)
Recipient Age	48±12.3	50.1±11.5	38.7±11.5
Recipient Gender Men (No)	69.9% (402)	68.9% (325)	74.8% (77)

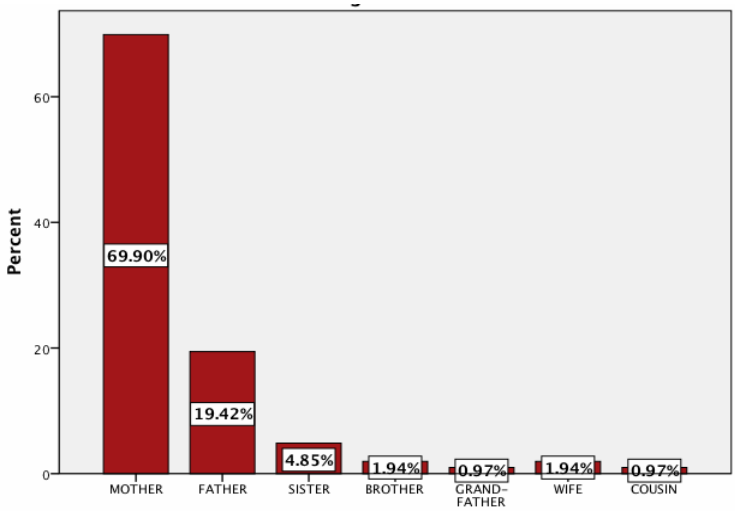

Fig. 1. The origin of living-related donors

Immunologic Characteristics

All renal transplantations were performed with compatible ABO blood group. The histocompatibility cross-matching test for T-cells with CDC and FC was negative in all cases.

HLA mismatches between donor and recipient were identified in 98% of cases, of which 85.5% had 2-4 HLA mismatches (Table 2). In particular, 93.8% of renal transplantations had at least one compatible HLA-DR, resulting in just a few cases with none HLA-DR matches. As far as HLA class I compatibility is con-

cerned, HLA-A mismatches 0, 1 and 2 were identified in 17.9%, 60.7% and 21.4%, respectively and HLA-B mismatches 0, 1 and 2 were found in 10.1%, 59.8% and 30.2%, correspondingly.

Panel reactive antibodies class I and class II were monitored regularly prior to transplantation, at 15 days post-transplantation and thereafter according to indication of renal graft dysfunction. Before renal transplantation, PRA class I and class II were negative in 75.5% and 73.95% of potential recipients. Positive PRA class II and class II were identified in 24.5% and 26.05% while sensitized candidates with positive PRA above 70% were 3.8% and 9.7%, respectively. Donor specific antibodies prior to renal transplantation were recognized in 0.57%. Presence of PRA class I and class II before renal transplantation was found in 20.8% and 27.2% of female candidates and in 16.8% and 18.2% of male candidates. This difference between men and women for PRA class II was statistically significant (p=0.02) and could be a possible explanation of the higher percentage of male renal transplant recipients.

Table 2. HLA mismatching donor-renal transplant recipient

Mismatch (MM)	Percentage (%)
6 MM	0.35
5 MM	6.86
4 MM	25.88
3 MM	39.78
2 MM	19.89
1 MM	5.28
0 MM	1.93

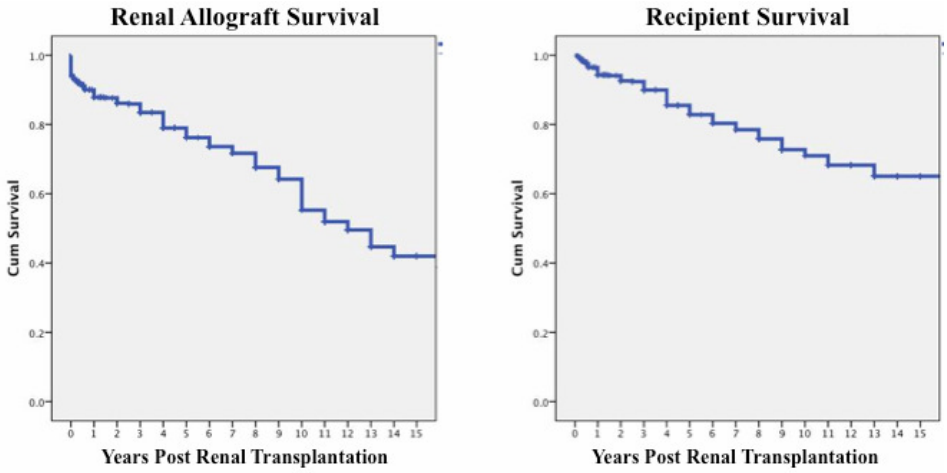

Fig. 2: Kaplan Meier survival curves of renal allograft and renal transplant recipient

Monitoring of PRA after renal transplantation revealed that 32.4% and 34.32% of recipients had positive class I and class II, respectively. Positive PRA class I and class II above 70% were detected in 14.8% and 15.3% of renal transplant recipients. More specifically donor-specific antibodies after transplantation were identified in 11.85%. The number of recipients with positive PRA increased following renal transplantation but the gender was not correlated with the presence of PRA.

Further analysis revealed that 18% of recipients with 0-1 HLA mismatches had positive PRA class II while positive PRA class II was tested positive in 36% of recipients with 5-6 HLA mismatches and 30% of recipients with 2-4 HLA mismatches. Even though differences are evident, they do not reach statistical significance.

In addition, positive PRA class II were identified in 21% of recipients with zero HLA-DR mismatches, 33% of recipients with 1 HLA-DR mismatch and in 30% of recipients with 2 HLA-DR mismatches. Even though recipients with zero HLA-DR mismatches had the lowest percentage of PRA, the difference was not statistically significant (p=0.103).

Renal Allograft and Recipient Survival

Renal graft survival the 1st, 3rd, 5th and 10th year was 89%, 82%, 76% and 67%, while respective recipient survival was 95%, 93%, 87% and 75% (Figure 2).

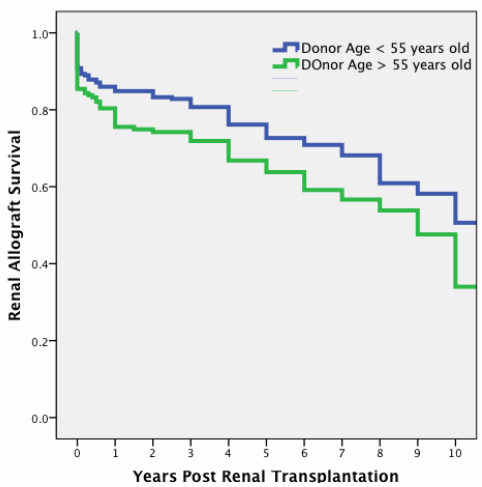

Fig. 3. Kaplan-Meier survival curves of renal allograft based on donor age

Renal allograft for living related or cadaveric donors had equivalent survival (log rank 0.799). Similarly, donor's gender, recipient's age and gender did not have an impact on graft survival. However, donor's age was a significant parameter, resulting in better short-and long-term graft survival if the graft was procured from donors younger than 55 years old (log rank: 0.01), which is depicted in Figure 3. Characteristically, 30.6% and 39.6% of grafts from older donors were lost by the third and fifth year post-transplantation, respectively. HLA mismatching was not correlated with long-term allograft survival (log rank 0.495).

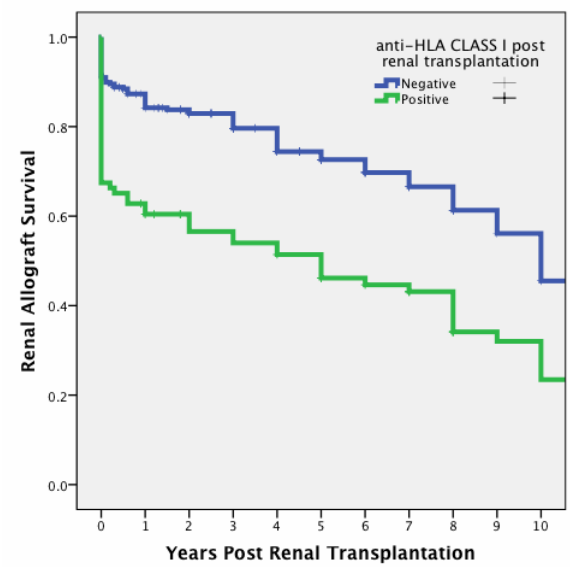

Fig. 4. Renal allograft Kaplan Meier survival curves based on presence of anti-HLA class I

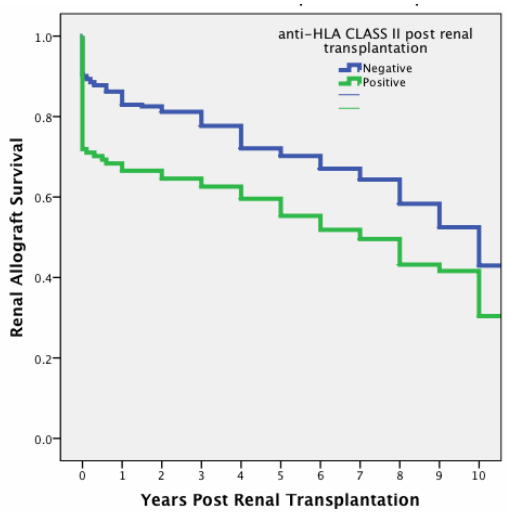

Fig. 5. Renal allograft Kaplan Meier survival curves based on presence of anti-HLA class II

The detection of either anti-HLA class I or II after renal transplantation influenced significantly on renal graft survival (log rank <0.0001 and 0.003 respectively), which is presented with Kaplan-Meier curves in Figure 4 and 5.

Discussion

In the early 1980s, Paul Terasaki described the significant impact of donor-recipient HLA DR matching on renal allograft survival [1]. Large multicenter studies have shown the inverse relation between renal graft survival and HLA mismatch [2]. The introduction of cyclosporine in the treatment of transplantation at the end of the 1970s was revolutionary with an increase in prolonged allograft survival, while since 1995 with newer immunosuppressive agents such as tacrolimus, mycophenolic acid and induction antibodies against Interleukin-2 receptor, minimization of rejection episodes the immediate post-transplant period was observed. It was suggested that due to more potent immunosuppressants, the donor-recipient histocompatibility may not be a priority. In the UNOS study that included 135000 renal transplant recipients, comparing the impact of HLA matching on renal graft survival during a period of 20 years prior and after change of immunosuppressive regimens it was shown that even though survival percentages improved, HLA compatibility had statistically significant correlation with 5-year graft survival [3]. Analysis of 575 renal transplantation at our Transplant Unit has shown very good renal graft and patient survival results that were attributed to our policy of seeking for the best donor-recipient HLA matching. Given that 93.8% of renal transplantation were performed with at least one HLA-DR and just 7% had zero or one common HLA confirmed this policy and as a result the restricted number of renal transplantations with low compatibility has not affected the overall good survival rates. Furthermore, optimal HLA matching protects against sensitization and development of panel reactive antibodies especially in patients who receive their first transplant who may require retransplantation in the future [4,5]. The recognition of PRA post renal transplanttation affected significantly renal graft survival [6]. Our results reinforce the policy of most favorable HLA matching and additionally sequential monitoring may be required for early detection of PRA and prompt change of immunosuppression regimen to avoid allograft dysfunction and ultimately loss of renal graft [7].

Except for immunologic parameters, donor age was another variable that correlated with renal graft survival, especially after the third post-transplant year. Several studies have shown that advanced donor age increases not only the incidence of delayed graft function, but also chronic allograft dysfunction and finally leads to graft failure [8]. Even though renal graft survival from older donors is inferior of that of younger donors, the benefit of transplantation over dialysis should be reviewed.

Analysis of demographic data showed that living related donors are older than cadaveric donors, which is explained because they are usually parents who donate their kidney to their children. Furthermore, the vast majority of living donors are mothers, showing their undoubted

and altruistic devotion. Another interesting point is that approximately 70% of recipients are men, percentage that does not reflect the incidence of chronic kidney disease in the general population but probably is explained by the fact that women have more frequently positive PRA and as a sensitized group it is more difficult to find compatible renal allograft.

In conclusion, development of PRA class I and II post-renal transplantation had significant impact on renal graft survival. Analysis of potential factors that may affect survival showed that donor age was the only one that reached statistical significance. We believe that optimal HLA matching (at least two HLA matched and one HLA DR) is the key to good results and long-term allograft survival.

Conflict of interest statement. None declared.

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Short communication

Comparative Analysis of Hypertension and Target Heart Damage in Two Ethnic Groups in Macedonia

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Abstract

Introduction. Hypertension (HTA) is one of the most common, non-communicable diseases. HTA is strongly associated with higher cardiovascular diseases incidentce, and target organ damage. Left ventricular hypertrophy (LVH) is one of the most frequent target responses to untreated HTA. HTA is number one risk factor for the mortality rate in the world, and significantly increases the risk of myocardial infarction, congestive heart failure and sudden cardiac death. The aim of our study was to compare the prevalence of the hypertensive target heart damage (LVH) in two ethnic groups in Macedonia.
Methods. We performed a cross-sectional study including hypertensive Roma patients (examined group), and equal number of patients with HTA from Macedonian nationality (control group). In both groups we analyzed left ventricular hypertrophy according to ECG signs and recommendations of the American College of Cardiology/American Heart Association. The examined group (EG) was consisted of 431 Roma patients with HTA. All EG subjects from Suto Orizari community were included in the study by the inclusion criteria for access into the study. In all subjects we performed ECG which was the basis to have a representative sample. The controls (CG) comprised an equivalent number of Macedonian patients with HTA. Both groups were matched by sex and age.
Results. ECG findings confirmed significantly higher prevalence of LVH among examined group of patients, compared to control group of subjects; e.g. there was statistically significant association of the cardiac hypertensive target damage (LVH) with nationality.
Conclusions. Uncontrolled and untreated HTA is a possible cause for more intensive target heart damage and higher incidence of LVH in Roma population.

Key words: hypertension, LVH, Roma population

Introduction

Hypertension (HTA) is one of the most common chronic, non-communicable diseases, in the world. The estimation is that more than one billion of people worldwide have HTA, and around 7.1 millions of fatal events are due to untreated HTA. WHO reports that suboptimal systolic blood pressure (>115-120 mm/Hg) is responsible for 62% of the cerebrovascular diseases and for 49% of coronary heart diseases. On the other hand, HTA is one of the most important risk factors for fatal events worldwide [1,2]. HTA is not only the most important cardiovascular risk that decreases the quality of life, but it is also a factor that significantly increases the health expences, too. Recent evidence shows that 33.5% from the US adult population, or 76.4 million people have HTA. Further data report that 80% of them know that they have HTA, and that 71% take antihipertensives, but only 48% have adequate antihypertensive response [3]. Another source says that from the total population in the US over 18, 6.6% don't know they have HTA (>140/90 mm/Hg), and only 64% from the patients who take antihypertensive drugs achieve the preferred blood pressure values [4]. Up to 75% Americans with coronary arterial disease, diabetes, or stroke are hypertensive, but despite they take antihypertensive drugs, only 40% touch optimal blood pressure [5]. The data from the Institute for Public Health in R. Macedonia show that from total the primary health care morbidity in Macedonia, HTA takes very important part because the number of visits due to HTA was 120.942 for male, and 176.991 for female [6]. These indicators show that the "rule of halves" is not suitable any more, and that the patients became more aware of the HTA, and about the importance of the quality of antihypertensive response. Nevertheless, the HTA remains unsatisfactory controlled worldwide.
Left ventricular hypertrophy (LVH) defined as morphologic, adaptive, abnormal increase of the ventricular mass, as a response of the chronic overload of the left ventricle, is detected in 16-19% of the general population. According to other data cardiac hypertrophy is a morphological adaptive increase in myocardial mass in response to chronic work overload and is a common clinical finding affecting 23% of men and 33% of wo-

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men over the age of 59. Pressure or volume overload on the myocardium results in an increase in myocardial wall stress and hypertrophy may be seen as an attempt to normalise wall stress and oxygen demand. Although initially protective, the increased myocardial mass requires an increase in coronary blood flow to keep function [7,8]. HTA and/or chronic myocardial overwork lead to consecutive hypertrophy reaction, in order to normalize the situation with oxygen supply [8]. LVH significantly increases the risk for myocardial infarcttion, congestive heart failure, and sudden death [8-11]. It is also close related to increased prevalence of arrhythmias, as well as to cardiovascular morbidity and mortality [8,12-14]. In addition, there are many factors that can influence the prevalence of LVH such as: age, sex, values and duration of the HTA, drugs used, comorbidity etc.

The Roma population typically lives in groups and closed communities, which are separated from the majority populations in a number of countries. Results from many articles and reports studying Roma population health and social position notify higher prevalence of infectious and non-communicable diseases, as well as shorter life expectancy that is closely related to the living conditions [15]. The literature review shows that there are only few articles studying the health status in the Roma population, especially target organ damage caused by HTA. We did not find any study on hypertensive target heart damage in the Roma population at all. Thomas, *et al*. suggests that the prevalence of HTA in the US is 73%. Life expectancy for Roma population in the USA is 48-55 years, due to the high prevalence of the sub-diagnosed and sub-treated cardiovascular diseases [16]. Ruprecht, *et al.* concluded that the life surroundings and inadequate lifestyles are main causes for significantly worse health status in Roma population in comparison with majority populations in Europe [17]. Further, higher prevalence of undiagnosed HTA, and other cardiovascular diseases, non-adequate therapy, complications, and lifestyle are the crucial causes for target heart damage and premature mortality in Roma population [18-21].

The aim of our study was to compare the prevalence of the hypertensive target heart damage in two ethnic groups in R. Macedonia.

Material and methods

We performed a cross-sectional study in PZU Intergin and Health Center Skopje in 18-month-period. The Roma group included 431 randomly selected subjects with HTA from Suto Orizari community. The inclusive criteria were Roma ethnicity and confirmed HTA. The same number of Macedonian subjects with HTA, matched to Roma group by sex, age, type of medications and duration of treatment, was studied as a control.

The HTA diagnosis was confirmed by blood pressure measurement with auscultatory method with a mercury sphygmomanometer (sfigmomanometro a mercurio-Artsana) as follows:

1. Cuff with standard dimensions was used (14x35 cm);
2. The measurements were taken from either the right or the left arm with the patient in the sitting position. It was performed at least 2 times, with an interval of 2 minutes. The arm with the higher reading was accepted as valid value;
3. Cuff deflations was done with 2-3 mm/Hg per second;
4. Measure in the siiting position, after 10 minutes rest.

We confirmed HTA if the blood pressure value was >139/89 mm/Hg in at least 2 measures per day in at least 4 consecutive days, according to the recommendations in the Cochrane evidence-based medicine guidelines, and/or if patients were treated with antihypertensive therapy no less than 6 months.

All examined subjects underwent ECG with 12 channel ECG machine.

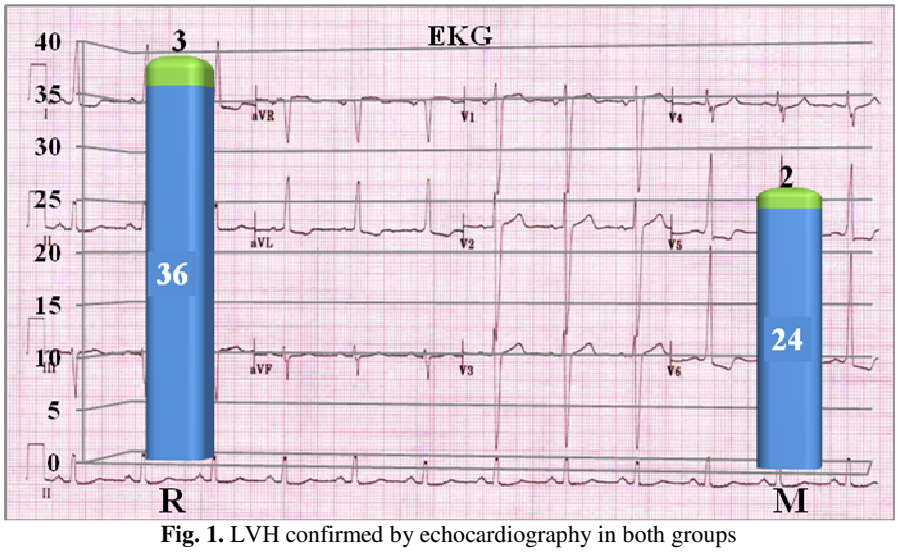

Fig. 1. LVH confirmed by echocardiography in both groups

According to the American College of Cardiology/American Heart Association (ACC/AHA) criteria, LVH was considered if the summary of the R (V5/V6)+S (V1/V2) was bigger than 35 mm (Sokolov-Lion index) and/or R wave (V5/V6) was higher than 27 mm.
In order to confirm LVH diagnosed by ECG findings, we performed echocardiography as a gold standard for verification of the existence of LVH. It was done randomly by selection of the every fourth ECG with LVH.
From the many comorbidities we took into account anemia (level of hemoglobin), and level of glycemia.
The statistical analysis was done by the software package SPSS 18.

Results

The ECG findings of LVH were registered in 156 examinees of the Roma group (36.2%) and in 101 controls (23.4%).
The difference was statistically significant (P=0.0026), i.e. the relation between LVH and nationality was significant.
From the group of examinees that performed echocardiography the LVH was not confirmed in 3 subjects from the Roma group, and in 2 controls (Figure 1).

Discussion

Our findings suggest higher prevalence of the LVH among the Roma subjects with HTA in comparison with controls.
The prevalence of LVH increases with the severity of hypertensive disease. Approximately one third to one half of hypertensive patients have LVH. The presence of LVH is a very strong independent risk factor for future cardiac events and all cause mortality [22]. An increase in left ventricle wall stress is the principal mechanical factor in the development of LVH, and blood pressure the most powerful determinant of LV mass. However, some additional hemodynamic factors play important roles in the development and maintenance of LVH [23]. Thus, volume overload also contributes importantly to the development of LVH.
A pattern of LVH evident on the ECG is a precursor of morbidity and mortality from cardiovascular (CV) disease. Echocardiography permits the non-invasive determination of left ventricular mass and the examination of its role as a forerunner of morbidity and mortality [24]. Hypertensive heart disease can also be defined as the response of the heart to the afterload imposed on the left ventricle by the progressively increasing arterial pressure and total peripheral resistance produced by HTA. Although sometimes the response appears to be out of proportion to the level of the arterial pressure, it is mainly the result of the hemodynamic overload. HTA can cause or can be related to various cardiac manifestations, among which the LVH is one of the most important [25].
Ethnic/race background plays a main, but still not completely defined, role in the development of HTA. Ethnicity/race is incorporated in almost all lists of HTA risk factors, and there are probable some underlying genetic mechanisms that have yet to be clearly recognized. Still, the precise risk contributed by ethnicity is not absolutely agreed upon by major researchers, with some claiming a high stage of risk and some claiming that ethnicity is a factor because it is correlated to other variables that can affect outcome. African-Americans, for example, consistently lead incidence profiles in HTA studies, with about 36% of the population developing HTA at some point. This is compared to about 20% in the Caucasian, Native Americans, and Hispanic populations. African-American populations also tend to develop target organ damage at a higher rate than their Caucasian counterparts, and tend to have poorer outcomes overall. The answer to the question why African-American populations have higher prevalence of HTA the majority of researchers find in a clear genetic rationale for the higher incidence of HTA among them. Some other data suggest that lower place on the socioeconomic steps, poorer health care and less healthy lifestyles lead to HTA at increased rates. At the end, it is clear that some ethnic groups are at increased risk for developing HTA. It is not obvious, however, whether this increased risk is a function of real genetic influences, or whether some social aspects and socioeconomic factors contribute more strongly than genetics [26,27]. The conclusion is that there is a clear connection between HTA and genetics. HTA runs in families, and is more common in some ethnic groups than in others. Both facts point to a genetic cause of some cases of high blood pressure. Identifying the exact genetics of HTA, however, is likely to take time. Many articles, but not all, have confirmed that renin activity is lower than in white people in both hypertensive and normotensive black population [28]. Some other sources suggest different reasons. For example, there is evidence that diastolic heart function is significantly worse in hypertensive subjects of African-Caribbean origin than in white Europeans. This difference in diastolic performance is not due to known confounding variables (genetics or lifestyle differences) [29]. These ethnical/racial differences in health measures are seen clearly in CV risk factors and outcomes for Americans of African descent, or African-Americans, compared with those of European descent, or European-Americans. Compared with European-Americans, African-Americans have higher mortality rates for most CV diseases, including coronary heart disease and stroke [30].
ECG left ventricular hypertrophy contributes more to the risk of CV mortality in African-Americans than it does in whites. Using regression of ECG left ventricular hypertrophy, as a goal of therapy, might be a means to reduce racial differences in CV mortality; however, prospective validation is required [31]. LVH is more prevalent in black than in white subjects with HTA [32].
Roma population is amongst the most depressed social minorities worldwide, suffering profound discrimination

for centuries, often living in extreme poverty, almost always isolated due to deep-seated prejudices and therefore excluded from the regular life that other people take for granted, such as going to school, seeing the medical providers, job applying etc. [33]. The Roma community has all the time been, both excluded and blamed for not wanting to engage with the rest of the society, a paradox, which is shaping the contemporary situation of Roma as it has done throughout their history. This paradox, so often overlooked, has contributed to reinforcing the wheels of Roma history, moving them approximately the vicious circle of discrimination, disadvantage, and exclusion [34]. Lifestyle, genetics, and a lack of routine medical care contribute to a high incidence of chronic conditions such as heart disease, HTA, and diabetes [35]. Medical data on 58 Gypsies in the area of Boston, Massachusetts suggested that HTA was found in 73% [36]. According to Sutherland, *et al.* life expectancy in Roma population is 48-55 yrs, and main reasons are under-diagnosed HTA and diabetes. Ruprecht's results show that similar situation is with Roma ethnicity in Europe.

The results of this study indicate that there is statistically significant difference in the prevalence of the ECG findings suggesting LVH between the Roma group and controls, i.e. there was a significant correlation of the cardiac target damage (LVH) with ethnicity. We could not find studies with identical or analogous content in order to compare and evaluate our findings. Facts that HTA in Roma population become visible earlier, is more severe, and give more target organ damage are reason why LVH is higher in Roma ethnicity. Perhaps uncontrolled and untreated HTA is possible reason for more intensive target heart damage and higher incidence of LVH in Roma population. Ethnicity/race is incorporated in almost all risk factors, and there are probable some underlying genetic mechanisms that have yet to be clearly recognized. Why the Roma population has more HTA and more expressed LVH, perhaps will be the trigger to start a new research, and a topic for some future investigations.

Conclusions

The target heart damage (LVH) is more frequent in the Roma group than in controls. Uncontrolled and undertreated HTA may be a reason for higher LVH prevalence among the Roma subjects with HTA that should be a subject for further investigations.

Conflict of interest statement. None declared.

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Teaching point

Implementation of Immunoadsorption in a Nephrology Unit: The Toulouse Experience

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Abstract

Introduction. Plasmapheresis is widely used to remove potential deleterious antibodies from the blood. Because the volume of treated plasma is limited, plasmapheresis can be replaced by immunoadsorption (IA), a more tedious but sophisticated technique that enables treatment of larger volumes of plasma, i.e., >4 L vs. 1.5-2 L. We have implemented in our Department IA technique to replace plasmapheresis when we launched our ABO-incompatible (ABOi) and HLA-incompatible (HLAi) kidney-transplant programs with living kidney donors. In this setting, isoagglutinin titers (ABOi) or donor-specific alloantibodies (HLAi) have to be decreased drastically at pretransplant by apheresis and immunosuppression.
Methods. We designed a desensitization program based on IA, which was started in the first trimester of 2010 within the Acute Polyvalent Hemodialysis and Apheresis Unit (Toulouse University Hospital, France). We describe all the steps for implementing this IA technique. So far, we have performed >225 IA sessions.
Results and Conclusions. The IA sessions were associated with a net body-weight gain of ~1 kg. Normally, IA is performed first and then hemodialysis on the same or the following day; however, we were able to simultaneously perform IA with hemodialysis (tandem procedure). We are now able to conduct this procedure 24 h/7 days a week. This tandem procedure has reduced costs. Implementation of IA has enabled the successful transplantation of 32 kidney patients.

Key words: ABO-incompatible kidney transplantation; desensitization; hemodialysis; HLA-incompatible kidney transplantation, immunoadsorption; living kidney transplantation

Introduction

In France, ~36,000 patients have end-stage kidney disease treated by dialysis, chiefly hemodialysis (>92%) [1]. Of these, ~13,000 are on the national kidney-transplant waiting list; however, only ~3,000 patients per year receive a kidney transplant, and this number is reaching a plateau (Agence de la Medicine, 2010) because the annual number of brain-dead donors is stable or even slightly decreasing. Meanwhile, the number of living-related or unrelated kidney donors increased from <10% to 12% in 2012, now accounting for >30% of the donors in our center (i.e., for ~180 kidney transplantations annually).
When implementing a living transplant-kidney program, to avoid denying a potential living donor, we also need to accept those with ABO-incompatibility. Moreover, many kidney-transplant candidates, such as those with a previous failed transplant and many women, are sensitized, i.e., they have anti-HLA alloantibodies (HLA incompatibility), which makes it difficult to find a suitable deceased and HLA-compatible donor. In this setting, it may be easier to find a potential suitable living-related donor. Of the ~500 patients with end-stage kidney disease and listed for a kidney transplant in our center, HLA-sensitized patients represent ~20% of cases. Thus, in order to develop a living-kidney program, ABO-incompatible (ABOi) and/or HLA incompatible (HLAi) kidney pairs will be encountered. For HLAi, the only way to succeed is to implement a desensitization protocol. Several desensitization protocols have been published: they report good kidney-allograft outcomes, even though treatment costs are increased within the first year posttransplantation [2-4].
ABOi kidney transplantation was developed initially in Japan (in the 1980s) because the concept of a brain-dead donor was not recognized. It was achieved using preparatory plasmapheresis, intraoperative splenectomy, and maintenance immunosuppression based on calcineurin inhibitors, azathioprine or mycophenolate mofetil, and steroids. However, many postoperative infectious

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complications have occurred and many grafts have failed due to acute or chronic humoral rejection [5,6]. In the early 2000s, many Japanese teams implemented ABO-incompatible transplantation by administering intravenous rituximab prior to transplantation instead of conducting a splenectomy. This enabled chemical elimination of B-lymphocytes, which are implicated in the humoral rejection that takes place after ABO-incompatible renal transplantation. Thereafter, ABOi kidney transplantation was disseminated worldwide with very good results in terms of graft survival.

Desensitization of kidney-transplant candidates

In the setting of ABOi, pre-transplant desensitization currently relies upon: i) removing antibodies, i.e., isoagglutinins, by means of several plasmapheresis sessions, with the aim of lowering the titer of isoagglutinins to <1/10; ii) preventing their subsequent synthesis by rituximab infusion; and iii) initiating conventional immunosuppression, i.e., tacrolimus, mycophenolic acid, and steroids, at 7-10 days pre-transplantation. The long-term posttransplant results for such patients are very good, particularly since pre-transplant splenectomy has been replaced by rituximab, i.e., results are as good as those observed in patients that have received an ABO-compatible living-kidney transplant [7,8]. However, in candidates that have very high titers of isoagglutinins, pre-transplant plasmapheresis may be insufficient. This is why Tyden, *et al*. in Sweden in the 2000s, replaced plasmapheresis with specific immunoadsorption (IA) sessions, using columns coated with either A or B blood-group antigens, which deplete isoagglutinins quickly and in a sustainable way [9].
In the setting of HLAi kidney transplantation, the recipient may have donor-specific alloantibodies (DSA) at pre-transplant. DSA can then act against HLA class I (A, B, or Cw) and/or HLA class II (DR, DQ, or DP) antigens. If DSAs are left, they quickly cause acute antibody-mediated rejection at posttransplant, despite immunosuppression [10]. Therefore, for pairs in whom the recipient has DSA(s) against the donor, we need to implement a desensitization protocol at pre-transplantation. This relies on i) removing DSAs by plasmapheresis, ii) preventing their subsequent synthesis using rituximab infusion, and iii) starting conventional immunosuppression, i.e., tacrolimus, mycophenolic acid, and steroids at 7-10 days pre-transplantation [11]. Desensitization can also include intravenous IV-Ig infusions (for its immunomodulatory properties); however, this treatment is costly and there is no conclusive evidence that it improves this protocol [3,12].

Semi-specific immunoadsorption vs. plasmapheresis

Immunoadsorption can replace plasmapheresis as a desensitization protocol for HLAi pairs. Because IA can treat greater volumes of plasma in one session (compared to plasmapheresis), it may be more efficient in reducing anti-HLA antibody titers. When addressing semi-specific IA, we use columns that are covered by *Staphylococcus* protein A and that can be reused up to 20 times provided they are carefully rinsed (Immunosorba & Globaffin, Fresenius Medical Care) [13], thus saving significant costs. The long-term post-transplant results for HLAi patients who undergo pre-treatment desensitization with IA are very good. In addition, this strategy is cost-effective when compared to matched kidney-transplant candidates who remain on a waiting list for a deceased kidney transplant [11,14].
The kidney-transplant program at Toulouse University Hospital is one of the top three French kidney-transplant centers and performs the greatest number of living-related or unrelated kidney transplantations. Thus, the Nephrology/Transplantation Department decided to implement desensitization strategies in the setting of ABOi and/or HLAi kidney transplantation using pre-transplant IA instead of plasmapheresis.

Presentation of the APHA structure

The University Hospital of Toulouse (Rangueil, France) made the strategic choice to create one dialysis Unit for Acute Polyvalent Hemodialysis and Apheresis (APHA), within the Department of Nephrology and Organ Transplantation (DNTO), concentrating expertise into a single location and creating high-quality collaboration between medical and paramedical personnel. The APHA team is composed of one attending physician, one senor nurse, twelve nurses, six nurse aides, and two biomedical assistants. In 2012, the APHA unit conducted 3,400 hemodialysis sessions, 520 plasmapheresis sessions, 130 IA sessions, 60 liver-dialysis sessions, and 580 continuous veno-venous hemodiafiltration sessions. The Unit is open from 8:00 a.m. to 7:00 p.m., Monday through Saturday, and a nurse is on call 24-h/7-days for emergencies.

Immunoadsorption technique

Table 1 lists the prerequisites needed to implement IA in an apheresis unit, and our outcomes. Phase 1 took place in the first quarter of 2010. During this period, the medical team, led by Pr. L. Rostaing and Dr. A. Allal, implemented a desensitization program using IA to treat highly sensitized kidney-transplant candidates who could not receive a deceased renal allograft because they had high levels of anti-HLA antibodies (HLAi patients).
IA removes the antibodies of interest from the plasma, in this instance anti-HLA antibodies, by passing plasma through a column covered with Staphylococcus protein A (Immunosorba®system, Fresenius). Non-specific IA was initially performed in partnership with an experienced manufacturer (Fresinius Medical Care). In addition,

Table 1. Immunoadsorption (IA): implications for practice

Prerequisites
- Mastering hemodialysis basic procedures, mastering fistula and/or catheter procedures
- Mastering plasmapheresis
Implementation of immunoadsorption
- Training to use an Adasorb® monitor
- Training on how to handle, save, and store IA columns
- Use of non-specific usable columns, e.g., Immunosorba®, and specific non-reusable ABO columns
- Set-up and manage hemodialysis and IA circuits simultaneously
Outcomes
Three goals:
- increase patients' safety by developing a multi-skilled caregiver team
- time saving: only 6 h of treatment cf. 11 h with hemodialysis given *after* IA. Less stress and less tired patients during the desensitization sequence
- cost effectiveness: total procedure time of <6 h, only one nurse, and reduction in total consumables
Time saved means a nurse can look after two or three patients treated by apheresis or hemodialysis. Coupling IA and hemodialysis can be routinely performed by a single caregiver
vailable
The care unit can treat patients 24 h/7 days a week: patients must be effectively desensitized whenever a transplant is a

a nurse from the company trained two nurses from our team. Training was carried out using two different monitors: Art Universal® and ADAsorb®. The Art Universal separates the plasma by filtration: heparin and sodium citrate are used to prevent coagulation. TheADAsorb® treats the plasma using two non-specific (protein A) columns. These columns can be reused up to 20 times after thorough rinsing with distilled water. Plasma flow within the columns is 40-50 mL/min for a blood flow of 70 mL/min. The procedure takes 4-5 h to treat the plasma plus ~1.5 h of total nursing time, making a total of 6.5 h. An IA session is associated with a weight gain of up to 1 kg.

Table 2: The four phases used to implement immunoadsorption (IA) in our apheresis unit

	First phase: non-specific IA	**Second phase: specific/specific + non-specific IA**		**Third phase: coupling IA + HD**	**Fourth phase: generalization of coupling IA + HD**
Generators / columns	Art Universal®;Adasorb®/ Immunosorba® system	Com.Tec® Glycosorb ABO®	Com.Tec®+Adasorb®/Immunosorba®+Glycosorb ABO®	Life 18™, **Therasorb**™/ Therasorb® Ig flex + hemodialysis monitor	Any type of monitor: Com.Tec® Abasorb® -Life™, Therasorb™
Type of patients	Recurrent glomerulonephritis on the allograft (n = 4), or highly sensitized kidney transplant candidates(n=4); Autoimmune peripheral neuropathy (n=2)	ABO-incompatible kidney-transplant candidates	ABO-incompatible + HLA-incompatible kidney-transplant candidates	ABO-incompatible + HLA-incompatible patient	ABO incompatible/ HLA incompatible/ ABO+HLA incompatible patients
Number of transplant patients	Four: one with a living kidney; Donor: no suitable cadaveric donors for the other three patients	8 patients with a living kidney donor	2 patients with a living kidney donor	1 patient	9 patients
Outcome	Excellent renal function	1 vascular rejection treated successfully; excellent renal function	Excellent renal function	Excellent renal function	3 acute rejections (1 cellular, 1 vascular, 1 humoral); or excellent renal function

The four phases in the technique

During phase 1, i.e., implementing IA, the medical team maintains close surveillance for signs of blood contamination at the entry point of the column using a dipstick as an additional safety measure as well as the monitor'salarm. It is essential to prevent the columns becoming clotted, which would render them unusable.
The outcomes from phase 1 confirmed the usefulness of this technique. Hence, four highly sensitized patients on chronic hemodialysis, with high levels of anti-HLA alloantibodies and high mean fluorescence intensities were treated by IA (average of eight sessions per patient). There was a dramatic decrease in mean fluorescence intensity for some anti-HLA alloantibodies.
However, because these patients were waiting for a deceased donor-kidney transplant and were not prioritized on the national French transplant waiting list, they could not receive a kidney transplant within the 4 months following the IA sessions. Thus, at this point, we changed our strategy by offering IA only to highly sensitized kidney-transplant candidates who had a suitable potential living-kidney donor. We were then able to perform two kidney transplantations with living donors in two highly sensitized patients who had been desensitized by IA sessions with a negative complement-dependent cytotoxic crossmatch at transplantation, even though they still had DSAs at that time. At the last follow-up, these two patients had good renal function.
Phase 2 took place in the first trimester of 2011 with the goal of implementing IA in the setting of ABOi kidney transplantation (Table 2). In the context of ABO-incompatible renal grafts, IA can be either non-specific (cf. supra) or use specific columns that contain blood type A or B antigens on a sepharose matrix (GlycoSorb ABO®; Glycorex Transplantation AB, Lund, Sweden); the latter allows targeted elimination of isoagglutinins.
The following factors were needed: i) a central line or arteriovenous fistula access, ii) plasma separation by centrifugation (no longer by filtration, i.e., we replaced the Art Universal monitor with the Com.Tec© monitor [Fresinius Kabi AG©]) (see Figure 1), iii) adoption of a new circuit that allowed adaptation of specific IA columns on the Com.Tec® monitor, and iv) anticoagulation with citrate (citric-acid monohydrate) in the arterial line.

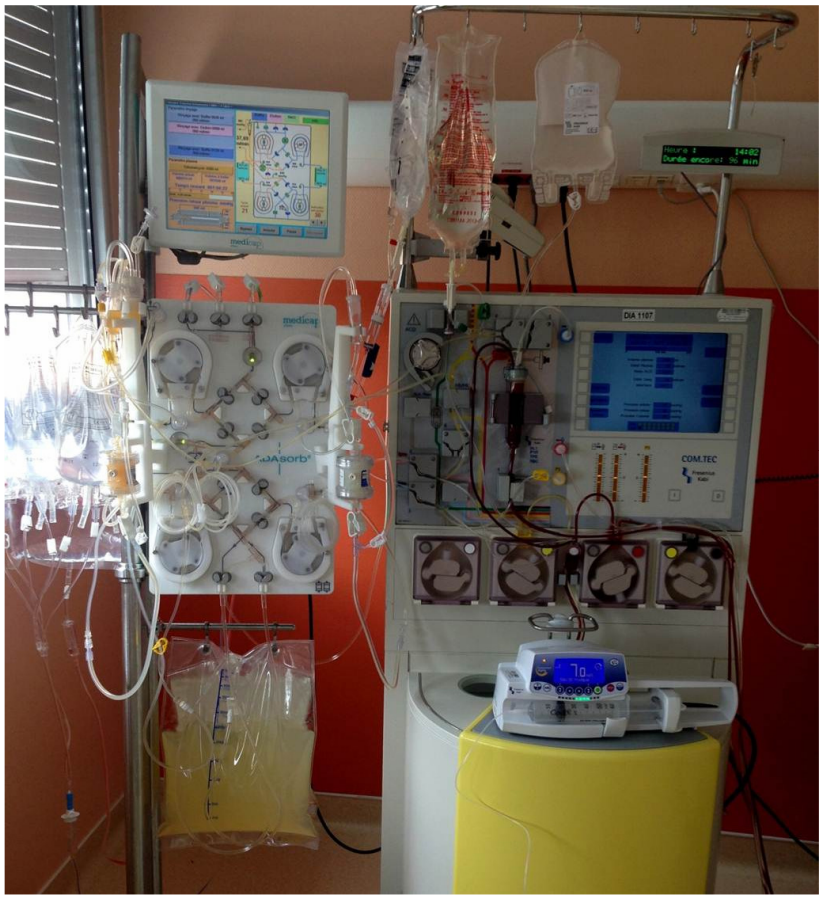
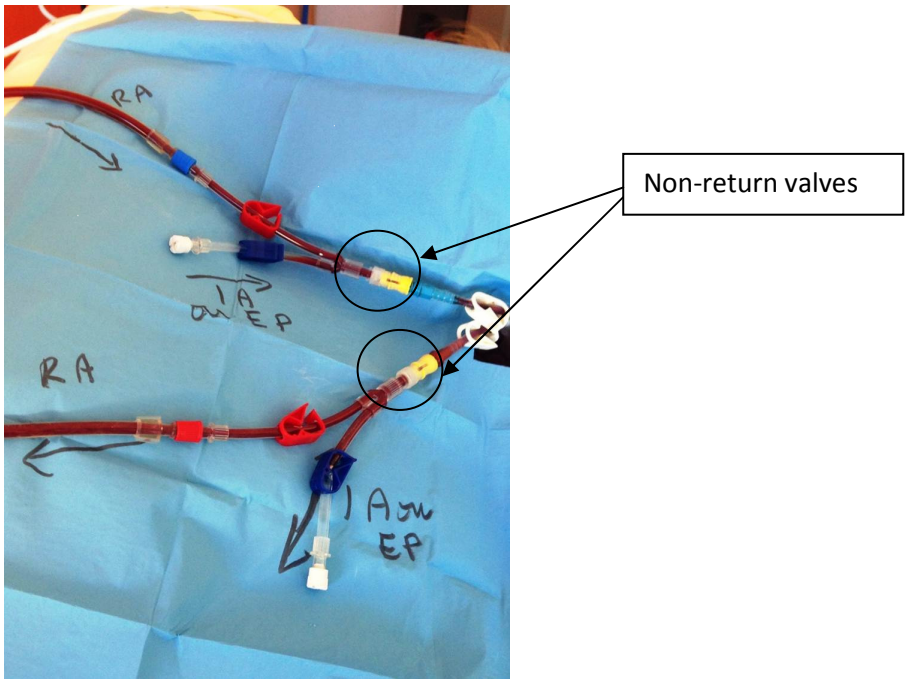


Fig. 1. The generators for performing immunoadsorption. On the left of the image is the Adasorb® generator, and on the right hand side, the Com.Tec® generator

To respond to medical needs, the nursing team proposed the following recommendations: a 'Y'-connector on the return line to compensate, when appropriate, for blood-calcium depletion (citrate is a calcium chelator), and a three-way valve positioned at a point before plasma arrives at the column, dedicated to detecting blood contami-

nation using a dipstick. Biological surveillance before and after the procedure included assessing calcium and magnesium levels, and anti-A or anti-B isoagglutinin levels. Samples were transported to the lab and blood-testing results were available within 30 min for calcium and magnesium, and in <2 h for isoagglutinins.
Compared to phase 1, the nursing time in phase 2 was reduced to 2-3.5 hours for the IA procedure and to ≤15 min for setting-up the monitors and rinsing the columns. The team used the Com.Tec® plasmapheresis monitor, which is mandatory for ABO-incompatible IA, in tandem with the ADAsorb® plasma-treatment monitor (used during phase 1), which uses non-specific sepharose + protein A reusable columns (Immunosorba® Fresenius Medical Care). This combination made it possible to eliminate anti-HLA antibodies from sensitized patients as well as anti-A or anti-B isoagglutinins at the same time, and particularly benefited those patients with concomitant ABOi and HLAi living-kidney transplantation. This new procedure provided more security and comfort for the patients, and use of the non-specific columns cut costs. Thus far, seven patients have been successfully treated and grafted using this procedure.
Phase 3 was implemented concomitantly with phase 2. During phase 2, in October 2012, we encountered a difficult situation with a living-kidney transplant candidate on hemodialysis. This patient was highly sensitized with two DSAs that had high mean fluorescence intensities in the setting of ABO-incompatibility and elevated anti-A isoagglutinin titers (>1/128), which were remarkably resistant to both non-specific and specific IA sessions (>10), performed as described above. This resistance to IA led the team to implement a new strategy (phase 3) that coupled IA with hemodialysis. This patient was treated using a new LIFE18™ IA monitor (TheraSorb™). This monitor has two specialized functions: i) plasma separation through centrifugation/filtration and ii) it uses non-specific columns (TheraSorb® Ig Flex, Miltenyi Biotec GmbH: sepharose + sheep immunoglobulins directed against human anti-Ig). Because of its small-volume circuit (80 mL), IA and dialysis can take place simultaneously via the 'Y' assembly (Figure 2).



Abbreviations: IA: immunoadsorption; EP: plasmapheresis; RA: hemodialyis.
Fig. 2. The Y-system, which allows concomitant hemodialysis plus immunoadsorption [bottom: (red): arterial line; top (blue): venous line]

This configuration has multiple benefits for the patient: the procedure is quicker; it is better tolerated because hemodialysis corrects for electrolytic problems caused by citrate anticoagulation with IA (variations in calcium and/or magnesium levels, *de novo* alkalosis); and body-weight increases can be avoided. In contrast, each IA session, when performed alone, leaves the patient with 0.5-1 L of hyperosmotic fluid. In addition, using our new method, less time is required by caregivers per patient and, thus, other patients can be treated simultaneously.
However, this procedure requires i) vigilance by the caretaker, i.e., proficiency in using the IA circuit assembly at the same time as the hemodialysis circuit, and ii) a small number of trained paramedics because of the complexity of the two new techniques.
Achieving a low isoagglutinin titer (<1/10) in the patient described above at pre-transplantation was a long and difficult process because she required 20 non-specific immunoadsorptions, four specific IAs, and three plasmapheresis sessions at pre-transplant. Nonetheless,

she successfully received a transplant and her current serum creatinine at 9 months posttransplant was 90 µmol/L. Phase 4 was implemented in January 2013 (Table 2).
This consisted of systematically performing hemodialysis in tandem with an IA session; the latter could be either specific or non-specific. Combined hemodialysis-IA has now become a common procedure: it achieves the best possible tolerance for the patient, regardless of the IA monitor used.

Conclusions

Today, thanks to excellent doctor/nurse/biomedical technician interactions, the Toulouse DNTO has performed 16 ABO-incompatible, 7 ABOi/HLAi, and 7 HLAi renal transplantations, which have resulted in very good outcomes with regards to kidney-allograft function.
Throughout the process of designing and implementing this new IA technique, the team has been creative, flexible, committed, and available (e.g., some posttransplantation IA sessions took place at night). In addition, it requires technical competence and know-how.
The implementation of this new IA technique by the CHU Toulouse team did not exclusively involve the transplantation program. During this time, we also treated patients with other conditions, e.g., focal segmental glomerular sclerosis recurring after kidney transplantation, myasthenia gravis, and Guillain-Barre syndrome.

Conflict of interest statement. None declared.

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Case report

Benign Transient Hyperphosphatasemia in a Renal Transplant Recipient - A Case Report

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Abstract

Transient hyperphosphatasemia (TH) is characterized by isolated elevation of serum alkaline phosphatase (ALP). This condition was first recognized in children but only few cases reported this problem in adult population. There is no evidence of liver or bone disease and no signs of infection and usually levels of ALP return to normal levels within few months.
Here we report a case of benign TH in a 60-year-old male renal transplant recipient.

Key words: renal transplantation, alkaline phosphatase, elevated, hyperphosphatasemia, tacrolimus

Introduction

Most common conditions in which elevated serum alkaline phosphatase may be found are liver disease, some infections and bone disease as the most frequent condition in patients with renal dysfunction. Idiopathic elevation of serum alkaline phosphatase was first described in 1954. It was considered as benign condition which occurred mainly in children under 5 years old [1]. Few years later, the term of transient hyperphosphatasemia (TH) in infancy was established [2].
This condition is characterized by elevation of serum alkaline phosphatase several fold the reference upper limits, with no evidence of liver or bone disease and spontaneous return to normal levels after approximately 4 months [3,4]. Later on, there have been some publications of transient hyperphosphatasemia in pediatric renal transplant recipients [5-7]. Only few cases reported this condition in adult population and it was associated with liver disease, antiviral therapy, acute infection and lymphoma [8]. Just few years ago the first three cases of TH were described in renal transplant recipients without any other condition responsible for these elevated levels of serum ALP [9,10]. After the first cases that have been reported of these conditions in adult population it has been suggested that the term should be changed from transient hyperphosphatasemia in infancy to benign transient hyperphophatasemia [11].

Case report

A 60-year-old male was diagnosed with polycystic renal disease 12 years ago. He started with hemodialysis in January 2012. After no contraindications have been found he received renal transplant from deceased donor in June 2012. Immunosuppressive protocol included basiliximab induction, tacrolimus, corticosteroids and mycophenolate mofetil. The patient was discharged from the hospital 10 days after the transplantation with serum creatinine within the reference range. Shortly afterward serum creatinine started to rise and obstructive uropathy caused by lymph collection was found. After a surgical intervention the problem was solved and the patient was regularly followed up at the transplant outpatient clinic with a stable graft function. In January 2013 routine laboratory analyses demonstrated isolated elevation of ALP to 333 IU/I. As all the other results were in the reference range and the patient was asymptomatic, control on outpatient basis was planned. One month later ALP was 1040 IU/I, so we started diagnostic evaluation to exclude all the possible conditions which might explain ALP elevation. There was no evidence of acute infection, no acute gastrointestinal disease,

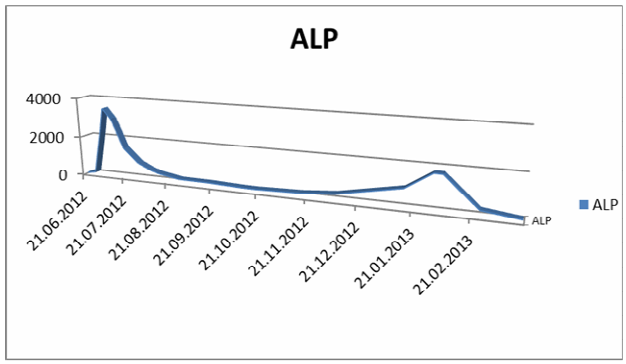

Fig. 1. Timeline of ALP (alkaline phosphatase) levels

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no ultrasonographic and CT evidence of lymph node enlargement or malignancy, and the bone scan showed no pathology. The tumour markers (CEA, AFP, CA 19-9, PSA) and other serum parameters were normal. One month later ALP was 1444 IU/I (Figure 1), but then for the first time serum calcium was slightly elevated to 2,74

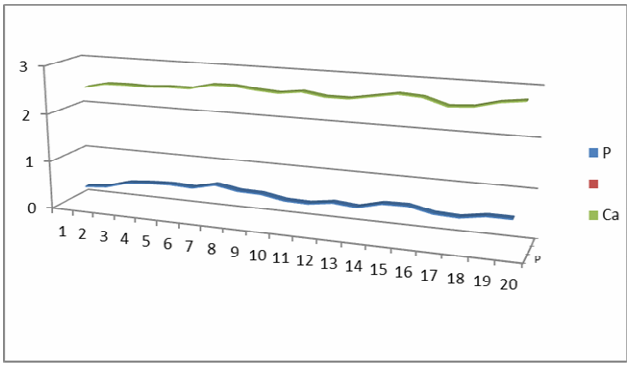

Fig. 2. Timeline of phosphorus and calcium levels

mmol/l and calciuria was detected (Figure 2). Intact parathyroid hormone was 18 pmol/L (range 1.0-6.0 pmol/L). Parathyroid glands scan revealed hyperfunctional parathyroid tissue near the lower pole of the left thyroid lobe. ALP isoenzymes showed most of ALP was bone related. So far mystery was solved. In the presence of hypercalcemia and high rise in serum ALP we considered to introduce calcimimetics in the therapy, but in March 2013 the regular control revealed serum ALP within the normal range.

There was no correlation of tacrolimus trough level on elevation of the serum ALP (Figure 3).

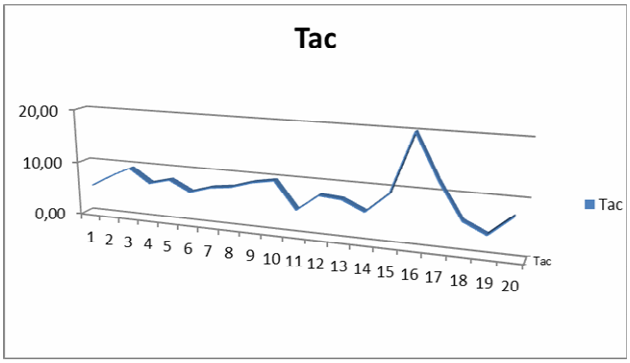

Fig. 3. Tacrolimus trough concentrations were not connected to alkaline phosphatase elevation

As the ALP levels returned spontaneously to 103 IU/l 3 months after diagnosis we suspected that benign transient hyperphosphatasemia was responsible for this condition.

Discussion

Little is known about transient elevations of alkaline phosphatase in adults. All our knowledge is based on several case reports, which differ significantly. Our patient is a good example. Many tests, which were done, might explain ALP elevation in the context of bone disease but still there is an open question why ALP return to normal level spontaneously. And considering this, the possibility of occurrence of benign TH in adults in association with renal transplantation must be observed [9,10]. However, while isolated hyperphosphatasemia in renal recipients may be a benign, self-limited condition, such cases must be differentially diagnosed from other conditions that can trigger hyperphosphatasemia, which include malignancy, infections, bone and liver disease [12,13].

Interestingly, recent studies have suggested that tacrolimus can induce in vitro bone formation. In such study investigators cultured rat bone marrow cells with tacrolimus hydrate (FK506) and observed that numerous cell clusters became positive for alkaline phosphatase activity. Later on by electron microscopy they revealed mineralized bone matrix in the cell clusters [14]. Having in mind these new research results, when we approach our patients and analyze laboratory test results we should think also about the effect of therapy that our patients receive,which is still unknown or is still the object of investigation. All the immunosuppressive drugs beside their main activity probably influence all the other organs which we still do not know, and therefore new studies and research are necessary.

Conflict of interest statement. None declared.

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Case report

A Rare Etiology among the Huge Burden of Herbal Nephrotoxicıty

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Abstract

Kidney is the target of many natural toxicants because of its rich vascularization and the major role in their metabolism and elimination. Herbs are believed to be "natural" and safe but they may have serious side effects such as nephrotoxicity. Eucalyptus is used as an antiseptic, expectorant, antibacterial remedy especially for asthma, bronchitis, sore throat and rhinosinusitis in varying forms. The main clinical side effects of eucalyptus preparations are epigastric pain, vomiting, symptoms from central nervous system (CNS), a burning sensation in the mouth/ throat and respiratory distress. Nephrotoxicity due to eucalyptus exposure is very rarely reported in the literature.

Key words: eucalyptus; acute kidney injury; renal dialysis

Introductıon

The kidney is very susceptible to toxicity. The use of herbs and herbal supplements has been increased among people as an alternative treatment in chronic diseases. The best known example of these remedies resulting in renal failure is Aristolochic acid-Chinese herbs nephropathy which may cause uroepithelial malignancies, renal interstitial fibrosis and tubular atrophy as well as Fanconi syndrome [1]. Traditionally, herbs have been considered to be non-toxic and harmless, because of their natural origin. However, they may contain contaminants, such as heavy metals and pesticides. Herein we report a rare case of herbal nephrotoxicity presenting with acute renal failure (ARF) requiring temporary hemodialysis that was probably due to eucalyptus tea. In cases of unexplained ARF, alternative/herbal medicine use should always be considered.

Case

A 61-year-old man with a 2-year history of hypertension treated by amlodipin 10 mg tb 1*1, valsartan/hydrochlorothiazide 160/12.5 mg tb 1*1 was admitted to the Department of Emergency Service. He suffered from fatigue and dizziness for five days. He had no history of renal failure, contrast media exposure or surgical intervention. The family history of the patient was not contributory. Blood pressure was 160/100 mm/Hg, heart rate 80/min, temperature 36.5^{o} C. The rest of the physical examination was normal apart from bibasiler rhonci. Chest X-ray was normal without any pulmonary infiltration, nor enlarged cardiothoracic index. Blood tests showed the following: ESR: 99 mm/first hour, random blood sugar: 148 mg/dl, serum urea: 231 mg/dl, creatinine: 6.12 mg/dl, uric acid: 14.1 mg/dl, calcium: 10.2 mg/dl, potassium: 5.8 mmol/l, sodium: 124 mmol/l, phosphorrus: 6.8 mg/dl, PTH: 129.8 pg/ml, albumin: 3.8 gr/dl, WBC 13400, hemoglobin: 11.3 g/dl, hematocrit: 32.7%, platelets: 250000 and CRP: 127.58 mg/dl. Urinalysis revealed specific gravity of urine sample of 1010 and pH: 5.

Fig. 1. Leaves of Eucalyptus spp.

No protein and leukocytes were found in the urine but some erythrocytes (3/high power field) were present whereas spot urine sodium was 20 mmol/l. A consultation from nephrologists was requested because of in-

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creased urea and creatinine leves. Two months ago the patient had normal renal function (serum creatinine 0,8 mg/dl). As we deepened the medical history to better understand the cause of renal failure, we found out that he had been drinking eucalyptus tea (approximately 20-30 ml per day) for natural treatment of his hemorrhoids for the last 7 days (Figure 1).

Venous blood gas analysis showed metabolic acidosis; Ph 7.29, pCO_2 48.1, HCO_3 22.7. Glomerular filtration rate was calculated as 10.21 ml/min/1.73 m2 according to MDRD formula. Renal ultrasonography of the right kidney showed that right kidney was 102*56 mm with parenchymal thickness of 16 mm and normal echogenicity, with a focal ectatic area of 1.5 cm in the upper anterior pole. The left kidney was 105*57 mm with parenchymal thickness of 17 mm and normal echogenicity. There was no sign of stone on ultrasonography. A central catheter was placed and he was subjected to intermittent hemodialysis. Amlodipine 5 mg tb 1*1, sodium hydrogen carbonate 500 mg tb 3*1 and calcium polystyrene sulfonate 1*1, bronchodilatator therapy were added to the medication. Daily urinary protein excretion was 196 mg/day and microalbuminuria 72 mg/day was detected in the twenty-four hour urine sample. Serum immunoglobulins and complement components were within normal limits. Antinuclear antibodies, AMA, ANCA, ASMA, Anti HCV Ab and hepatitis B surface antigen were also negative. The rest of the biochemical tests revealed transferrin saturation of Fe%: %27, ferritin 572.35 ng/ml, vitamin B12: 132 pg/ml, folate: 3.7 ng/ml, TSH: 1.19 mIU/ml, free T4: 1.42 ng/dl. The ECG was normal with sinus rhythm, without any pathological findings. After undergoing hemodialysis for 4 sessions, his urinary output remained satisfactory throughout. During the follow-up period without hemodialysis his urea and creatinine levels regressed to 107 mg/dl and 1,84 mg/dl, respectively. He was discharged from hospital. A renal biopsy was not planned at first step due to the rapid regression of urea and creatinine levels. During the close follow-up his laboratory findings stayed stable; after 2 months he was re-addmitted to the hospital. Serum urea was 48 mg/dl and creatinine value 1.41 mg/dl. Arterial blood gas analysis was normal. Finally, after 5 months he had urea 36 mg/dl and serum creatinine 1.04 mg/dl. Since there was no evidence of potential etiology for acute renal failure, this might be possibly attributed to the exposure of eucalyptus.

Discussion

The market for the herbal remedies and natural products is growing rapidly worldwide. It has been documentated that about 40% of Americans use dietary supplements [2]. In general, herbs are believed to be "natural" and safe but many dangerous and sometimes lethal side effects have been reported. This risk may be attributed to the contamination with pesticides and/or heavy metals in the botanical product, and by minerals and/or prescription drugs in patent medicines. The high percentage of people who use herbal remedies do not mention this use to their physician or pharmacist. This paradigm has been well-shown [3]. Because of this, many herbal poisonings are not diagnosed or treated correctly in every day clinical practice. The interactions between herbal components and concurrent pharmacotherapy represent a cause of increasing risk of side-effects from the use of these remedies [4].

Kidney is particularly susceptible to the action of many natural toxicants because of its anatomic and physiologic features, rich vascularization and its major role in metabolism and elimination of toxicants. The clinical manifestations of toxic nephropathy may vary from a mild reduction of renal function, hematuria, proteinuria, and urolithiasis to a severe progressive toxicity even to end-stage renal disease.

The prototype nephrotoxic supplement that is used so frequently is Chinese herbs containing the nephrotoxin-aristolochic acid [5-7]. Chronic exposure to Aristolochia acid (AA) can be involved in the renal fibrosis as well as in urothelial cancer [8]. Nephrotoxicity is an important issue when selecting Chinese herbal formula for the treatment of chronic diseases. Some of the other well-known herbal supplements are Chaparral, Ephedra, Yellow oleander, Thunder god vine, Willow bark [9]. Also, some natural medicines may be associated with kidney stones or increased urine excretion of kidney stone precursors as Calcium, Sorrel, Wood sorrel [10]. Such agents may cause urothelial cancers, acute renal failure and chronic renal failure. Possible interactions between herbal and conventional medications must be considered [11]. Ginkgo, St. John's wort, Ginseng, Echinacea, Sawpalmetto, Kava are some of the examples for these dangerous interactions between herbs and drugs [12].

There are about 600 species of Eucalyptus worldwide. Of all of these species, Eucalyptus Globulus is the most widely cultivated in subtropical and Mediterranean regions. Eucalyptus is used as an antiseptic, expectorant, antibacterial remedy especially for asthma, bronchitis, sore throat, rhinosinusitis in varying forms such as oil, tea, mouthwash. The main clinical side-effects of eucalyptus preparations are reported as epigastric pain, vomiting and CNS symptoms. In addition, a burning sensation in the mouth/throat and respiratory distress represent other adverse reactions. Respiratory symptoms may include bronchospasm and tachypnoea, with dangerous respiratory depression following severe intoxication. Central nervous system involvement includes seizures, diminution or loss of reflexes, and depression of consciousness which may progress to coma. Convulsions are rare in the adult but may be prominent in the child. Death is usually the result of respiratory failure or convulsions. Nephrotoxicity due to eucalyptus exposure is rarely reported in the literature [13,14]. Associated with ingestion of large amounts of mouthwash; in-

cluding eucalyptol; anion-gap acidosis and renal failure has been shown at a fatal case [14]. Direct nephrotoxicity may follow the ingestion of large quantities [15]. Mechanisms of kidney injury may include direct nephrotoxicity, which may be augmented by underlying predisposing conditions such as dehydration, contamination, inappropriate use or preparation of a remedy, or interactions with other medications. Eucalyptus preparations are capable of hepatic microsomal induction hence it may affect the metabolism of other drugs and chemicals using the common pathway. Studies with human liver microsomes have characterized the hydroxylation of eucalyptol by CYP3A enzymes [16]. In severe poisoning, peritoneal dialysis or haemodialysis may be necessary [13]. In some of the case reports hemoperfusion has been recommended, but these reports are poorly substantiated [17]. Leaves of eucalyptus are reported to contain a high content of eucalyptol (cineol) together with terpineol, sesquiterpene, alcohols, aliphatic aldehydes, isoamyl alcohol, ethanol, terpenes and tannins [18]. Worth, *et al*. reported no renal side effects whereas cineole was used in a multicenter study [19]. However, it was shown in animal studies that the presence of tannic acid, present in high concentrations in eucalyptus, potentiated the nephrotoxic effect of cisplatin on rats [20]. The nephrotoxic content in eucalyptus has not been clearly defined yet. Although in the related references the lethal human dose for menthol, thymol, and eucalyptol is estimated at 50-500 mg/kg, there is still no exact information on adequate estimates of toxic dose levels for eucalyptol [21].

Conclusıon

Eucalyptus poisoning is not so frequently seen in clinical practice, especially among adults. Thus, we have found interesting enough to present this case report. Healthcare professionals should ask their patients about the use of herbal products and consider the possibility of herb-drug interactions. We should keep in our mind that the possibility of interactions between herbal and synthetic drugs can be fatal.

Conflict of interest statement. None declared.

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Case report

A Case of Venous Thrombosis of the Upper Extremity in Patient with Minimal Change Disease

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Abstract

Introduction. Thromboembolism is unexpected in nephrotic syndrome in remission unless there are other factors causing thrombosis. Herein, we present a case of deep venous thrombosis in the upper extremity in a patient with nephrotic syndrome in remission during an excessive exercise program.
Case. A 33-year-old male was diagnosed with minimal change disease. He had complete remission with steroid treatment. He was admitted to our Clinic with swelling of the left arm; and was found to have acute thrombus lying from left brachial vein to left subclavian vein. He had no additional diseases causing hypercoagulability, but he had been swimming for about 30-60 minutes every day. The symptoms resolved with low molecular weight heparin treatment followed by warfarin therapy within one month.
Conclusions. Thromboembolic events may rarely occur in patients with nephrotic syndrome in remission although there are no other inducing factors. Paget-Schroetter syndrome should be kept in mind in cases with UEDVT together with excessive physical activity of the specific muscles of the upper arm.

Key words: nephrotic syndrome; Paget-Schroetter syndrome; remission; thrombosis

Introduction

The hypercoagulabilty state with changes in the proteins of coagulation cascade and increased thrombocyte aggregation may lead to deep venous thrombosis (DVT), especially of the renal veins in patients with nephrotic syndrome (NS). Immobility, infections and hemoconcentration are other facilitating factors. DVT has been reported in 10% of adult patients with NS [1]. The most commonly used parameter for determining thrombosis risk is serum albumin level, which values under 2g/dl increase the risk substantially [1-4]. Thromboembolism is unexpected in NS in remission unless there are other concomitant factors such as immobility, malignant disorders, inherited causes of procoagulant states etc.
Upper extremity deep vein thrombosis (UEDVT), the term used for thrombosis of the brachial and subclavian veins, is fairly rare composing 4-6% of cases with DVT [5-7]. Most of the cases (80%) are secondary to central vein catheterization and malignancy [5-7]. Primary UEDVT may be either idiopathic or due to thoracic outlet syndrome or effort related thrombosis (Paget-Schroetter syndrome). Hereditary or acquired thrombophilia may underlie in idiopathic UEDVT (8-11). But, it is speculated that the frequency of thrombophilic disorders is lower in UEDVT compared with thrombosis of the lower extremity deep veins [12,13].
Herein, we present a case of acute UEDVT in a patient with NS in remission during an excessive exercise program.

Case history

A 33-year-old male was diagnosed with minimal change disease (MCD) by renal biopsy three years ago when he had been admitted to our Clinic with clinical findings of NS. He responded well to corticosteroid treatment; followed by two attacks of relapses that also responded well to the same treatment. He was accepted as steroid resistant and was treated with low dose corticosteroid treatment under which no relapse occurred again. The patient, who was in remission for the last 12 months, was admitted again to our Clinic with pain and swelling of the left arm for the last three days.
His past medical history revealed no history of concomitant disease, immobilization, surgical procedure, catheterization or trauma; and no history of smoking and alcohol use. He was right-handed and had been swimming for about 30-60 minutes every day. He was using ramipril, asetylsalycilic acid, lansaprazol and methylprednisolone (4mg methylprednisolone every other day) at the time of admission. Pathological findings on physical

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examination were edema of the left arm and hand without change in the color and temperature of the overlying skin. Basic laboratory results were within normal limits as follows: Leukocyte: 11600/mm³, hemoglobin: 14.5g/dl, thrombocyte: 359000/mm³, creatinine: 1.0mg/dl, albumin: 4.8 g/dl, proteinuria: 180 mg/day. Doppler ultrasonographic examination of the left arm revealed an acute thrombus extending from left brachial vein to left subclavian vein. He was given low molecular weight heparin followed by warfarin; and screened for diseases associated with thrombophilia. PT G20210A allele, FV Leiden mutation, antiphospholipid antibodies, anti-nuclear antigen and anti ds-DNA were negative. Levels of homocysteine, protein C and S were within normal levels. He was thought to have idiopathic UEDVT and discharged with warfarin therapy. His symptoms resolved completely within one month. Warfarin was stopped after six months at which time no thrombus was detected with Doppler ultrasonography. He has been followed up in our outpatient clinic without relapses for 12 months since the time of the thrombotic attack.

Discussion

Thromboembolism is a frequent extrarenal complication of NS. Decreased levels of anti-thrombin III and plasminogen, thrombocyte activation, hyperfibrinogenemia, inhibition of plasminogen activation, systemic effects of procoagulant activity due to immune complex injury within the glomeruli are among the defined hemostatic abnormalities [14-17].

Risk of thromboembolism is directly correlated with the severity of the disease and hypoalbuminemia with the highest risk encountered in patients with serum albumin levels below 2 g/L [14-18]. Thrombotic risk is not the same in NS of various origin; with membranous nephropathy having the highest risk followed by membranoproliferative glomerulonephritis and MCD [19,20]. MCD is the most common cause of NS in childhood; whereas it represents 10-15% of cases in adults [21,22]. Thromboembolic events usually occur in case of hypoalbuminemia in MCD as in other etiologies of NS. In a review by Waldman, *et al.* [5], severe hypoalbuminemia (0.6-1.5g/L) was detected in all four cases with thromboembolic events (three cases of DVT and one with arterial thrombosis) among 95 patients with NS. Kerlin, *et al*. [23] detected older age and proteinuria as predictive of thromboembolic events in 326 children with MCD. With these data and the current knowledge, it can be said that thromboembolic events are unexpected in patients with NS in remission. It has been shown that thrombin activation products (fibrinopeptide A and thrombin-antithrombin III complex) and prothrombin activation products (prothrombin fragment 1 and 2) are increased in patients with proteinuria less than 1gr/day [17]. This may support the existence of a subclinical state of coagulation in those patients; although there is a need for further studies to correlate these findings with clinical events.

Our case has a peculiar presentation due to atypical localization of the thrombus, atypical timing ie. one year after complete remission with serum albumin level of 4.8 g/L, and lack of congenital or acquired risk factor for thrombosis. The only risk factor delineated for this patient was history of forceful activity of upper extremities associated with regular swimming exercise.

UEDVT is fairly rare composing 5% of all cases with DVT. In more than 80% of cases, there are secondary factors like central venous catheterization, malignancies, pregnancy and use of oral contraceptives [5-7,11]. The presented case had none of these in his medical history.

Primary UEDVT may be either idiopathic, or related to thoracic outlet syndrome or Paget-Schroetter syndrome. Paget-Schroetter syndrome (effort related thrombosis) was described in the end of the 19th century in young (mostly aged 24-37 years) and otherwise healthy subjects presenting with UEDVT after repeating and forceful activity of the upper extremities like painting, swimming and heavy lifting [24-26]. Costoclavicular joint abnormalities may accompany this syndrome [11]. The presented case has similarities with the mentioned syndrome with his age and history of regular swimming exercise. It was thought that the procoagulant state of NS and prior corticosteroid treatment together with regular swimming facilitating Paget-Schroetter syndrome has led to UEDVT in the presented case.

Conclusions

Thromboembolic events may occur rarely in patients with NS in remission, unless there is a predisposing factor. Thoracic outlet syndrome and Paget-Schroetter syndrome should be kept in mind in cases with UEDVT together with excessive physical activity of the specific muscles of the upper arm.

Conflict of interest statement. None declared.

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Letter to the Editors

Renal Transplantation in Macedonia - the Endless Story!

Dear Editors,

With a substantial interest we have read the editorial paper "Improvement in Kidney Transplant Program of Macedonia-What Might be the Clue", written by G. Spasovski, I Rambabova-Busletic, J. Masin- Spasovska *et al.* and published in BJ 2012; 10(2): 47-48 [1]. As pioneers in Renal Transplantation in our country we are obliged to put some comments.

1. Facing the lack of organized deceased donor transplantation in the entire region during the past 20 years we introduced a very ambitious living donor transplant program from the very beginning accepting the so-called expanded criteria living donors including marginal, elderly, non-related and ABO incompatible donors [2-7]. Thus, we could continue our transplant activity in the country during the past very turbulent time of 25 years. The usual number of transplants in the last decade was among 16-20 per year. Bearing in mind that in 2003 we reached 20 transplants per year, 24 living renal transplantation in 2012 mentioned in the editorial is not a big difference! On the other hand it is known that the expanded criteria living donor program in Macedonia were very well-established in the last decade with a substantial success and safety for the donors and recipients. The surgical technique, the post-transplant immunological and clinical monitorring was already very well-established many years ago. We are continuing with the use of expanded criteria living donors as our original experience. Most of our original works were published and/or presented at the most prestigious international transplant meetings all over the world [7-10].

2. The reduced number of only 6 transplants in 2011, as Spasovski, Rambabova-Busletic and Masin-Spasovska mentioned, was due to the financial difficulties and reorganizations in the system of health in the country. The above-mentioned authors, as members of our transplant team, are very well familiar with this issue. Our team was not responsible for such dramatically reduced transplant activity in the country in the year 2011. So, 6 transplants in 2011 could not be accepted as a reference for our continued activity over the past years.

3. The real challenge for all of us is the introduction of deceased donor donation program using the Croatian experience with a help of SEEHN, TTS and ESOT. As we all know, the initiative started in 2010 and, unfortunately, until now regarding our country has not had any success. There is still not any deceased donor transplantation in the country. Many productive and less productive meetings with known and unknown authorities were held and nothing happened. After the recent change of our NFP (National Focal Point) the things have improved. We have now ambitious young people, very well-trained in European centers (not one week, but several months and years) and we will start with our Deceased Donor Transplant Program (DDTP) very soon. In the meantime we will nominate National Transplant Coordinator (the final decision is already done) and network of local coordinators. We can conclude that now there is a real team ready for a real work.

4. We strongly believe that DDTP is a question of local organization and interest. If there is a support from the local authorities it is quite possible to have Deceased Donor Transplants. In other words, DDTP has always belonged to the local organization at first. Any help could not be efficacious without any support of the transplant team by local authorities. Otherwise, everything is waste of time and waste of money.

5. Anyway, we encourage our successors to continue our work in the field of renal transplantation bearing in mind that the safety of the living donors and recipients remains the most important duty of all members of the transplant team. At the same time, we suggest them to take in hands Deceased Donor Transplantation as a real challenge which could finally develop the transplantation as a regular medical procedure in the country.

Conflict of interest statement. None declared.

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Reply

Regarding Renal Transplantation in Macedonia - the Endless Story! - Reply

We have read the letter to the editor on our editorial "Improvement in Kidney Transplant Program of Macedonia-What Might be the Clue" [1], and we are pleased to have a couple of comments from the pioneers and our predecessors of the kidney transplant program in Macedonia, Ivanovski and Popov [2]. In fact, we do not see a lot of different positions or any published data related to the content of our editorial to be confronted with, but we'll try to respond to each point by point:

1. There is no doubt and disagreement that the kidney transplant program in our country was well-established in the previous years and regardless of the type of donor the numbers were pretty low for the reported period 1996-2011 (13.5/year). The problem of multidisciplinary organisational infrastructure as common obstacle for deceased donor program development in majority of emerging countries and possibilities for organ commercialism has been recognized [3]. Hence, the question was what should have been done by the transplant professionals in order to increase the number of transplantations as best option to discourage paid transplantations [4]. In this regard, the number of transplants reported in 2012 (24) was worth mentioning as the best result ever achieved in R. Macedonia, particularly because it was achieved over the period of only 7 months [5].

2. We also agree that the reduced number of only 6 transplants in 2011 may be partially due to the financial difficulties and reorganizations of the health-care system in the country. However, it's a bit intriguing that the authors confirmed 20 kidney transplantations performed in 2003 under the same conditions.

3. The South-Eastern Europe Health Network (SEEHN) initiative and the support from the Regional Health Development Centre (RHDC) on Organ Donation and Transplantation established in Croatia (Zagreb) was initiated in 2011 with the first scientific meeting in Skopje, 27-28 May [6,7], and not in 2010 as stated in the letter of Ivanovski. As we reported the SEEHN initiative had shown to be of great value for improvement in the transplant program in Macedonia with 28 living donor kidney transplants performed in the first 7 months of 2013 [8]. We hope for the same or even better results reported in the forthcoming period under the changes pointed out by Ivanovski.

4. We agree that deceased donor program is a question of local organization and interest with a support from the local authorities. Nevertheless, at present, we have succeeded in our commitment as professionals that the number of living related transplants have been increased as an immediate and prompt action [3,4].

5. We would like to express once again our appreciation and gratitude to our predecessors for their encouragement to continue their work in the field of renal transplantation and hope they might be proud of already reported results [9].

Conflict of interest statement. None declared.

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