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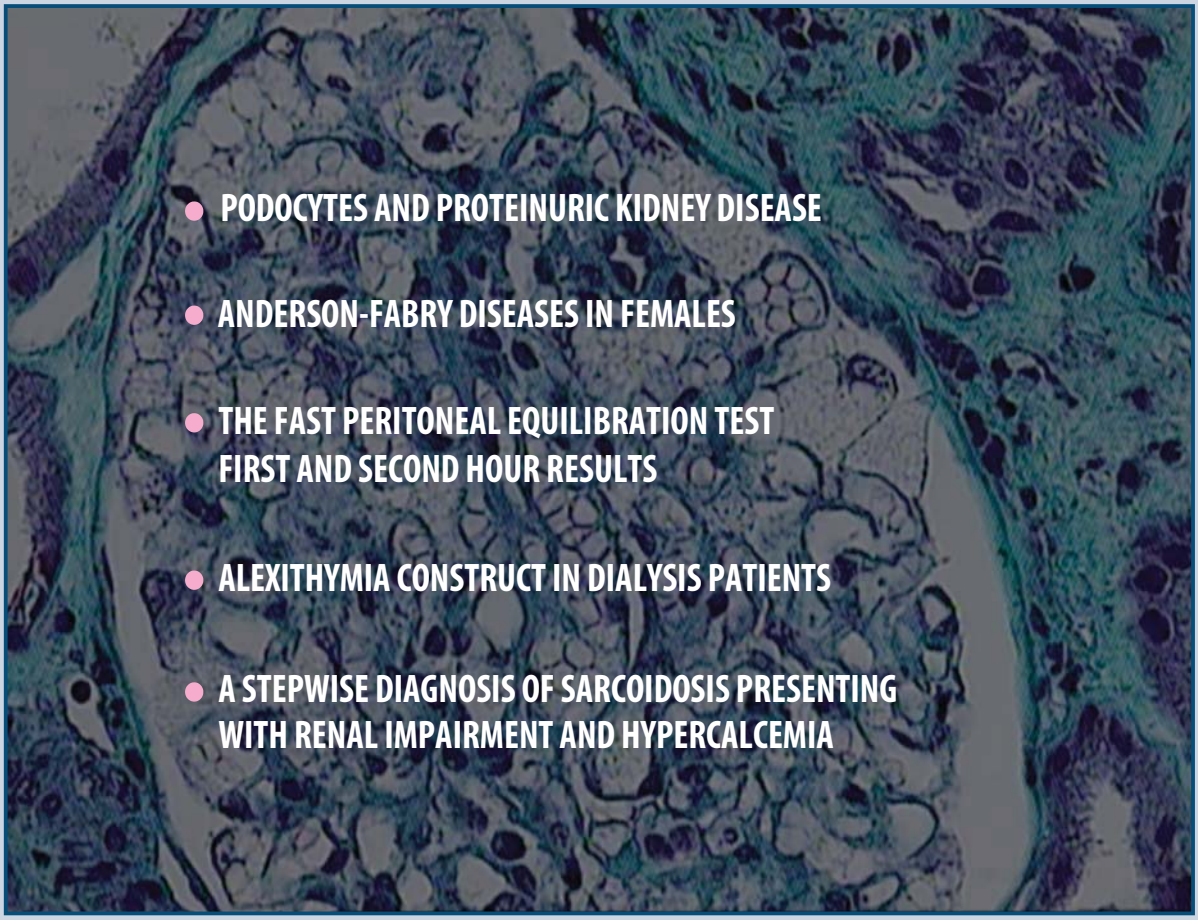

- PODOCYTES AND PROTEINURIC KIDNEY DISEASE
- ANDERSON-FABRY DISEASES IN FEMALES
- THE FAST PERITONEAL EQUILIBRATION TEST FIRST AND SECOND HOUR RESULTS
- ALEXITHYMIA CONSTRUCT IN DIALYSIS PATIENTS
- A STEPWISE DIAGNOSIS OF SARCOIDOSIS PRESENTING WITH RENAL IMPAIRMENT AND HYPERCALCEMIA

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Special feature

European Renal Best Practice Guideline on Kidney Donor and Recipient Evaluation and Perioperative Care

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Abstract

The Clinical Practice Guideline on evaluation of the kidney donor and transplant recipient was developed following a rigorous methodological approach aiming to provide information and aid decision-making to the transplant professionals. Thus, this document should help caregivers to improve the quality of care they deliver to patients with no intention it is defined as a standard of care. In this short version of the guidelines we present 112 statements about the evaluation of the kidney transplant candidate as well as the potential deceased and living donor, the immunological work-up of kidney donors and recipients and the perioperative recipient care. The extended version of the guidelines with methods, rationale and references is published in Nephrol Dial Transplant (2013) 28: i1–i71; doi: 10.1093/ndt/gft218 and can be downloaded freely from http://www.oxfordjournals.-org/ our_journals/ndt/era_edta.html.

Key words: donor evaluation, ERBP, guideline, kidney transplantation, perioperative care, recipient evaluation

Introduction

Caring for kidney transplant recipients (KTRs) requires specialized knowledge in areas as varied as nephrology, immunology, pharmacology, endocrinology, infectious disease, and cardiology. In this context of increasing complexity coupled with an exponential growth in medical literature, clinical practice guidelines (CPGs) aim at helping clinicians and other caregivers to deliver evidence-based medicine and thereby, to improve patient outcomes. Furthermore, guidelines also help to expose gaps in our knowledge, and thereby suggest areas where additional research is needed.

This guideline was developed following a rigorous methodological approach:

1. identification and selection of a representative work group, consisting of experts in transplantation (neph-

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rologists, surgeons, immunologists) and guideline methodologists;
2. identification of clinical questions;
3. prioritisation of questions;
4. systematic literature review and critical appraisal of available evidence;
5. formulation of recommendations and grading according to GRADE;
6. comparison to existing guidelines, when available;
7. suggestions for future research.

The GRADE system allows provision of guidance even if the evidence base is weak, but makes the quality of the available evidence transparent and explicit. The strength of each recommendation is rated 1 or 2, with 1 being a "We recommend" statement implying that most patients should receive the course of action, and 2 being a "We suggest" statement implying that different choices will be appropriate for different patients with the suggested course of action being a reasonable choice. In addition, each statement is assigned an overall grade for the quality of evidence, A (high), B (moderate), C (low), or D (very low). Although there are reasons other than quality of evidence to make a grade 1 or 2 recommendation, in general, there is a correlation between the quality of overall evidence and the strength of the recommendation. Even if the evidence is weak, clinicians still need to make clinical decisions in their daily practice, and they often ask 'what do the experts do in this setting'? Therefore, the ERBP board opted to give guidance, even if evidence was weak or non-existing, which unfortunately is often the case in nephrology.

The draft guidelines were submitted for review to selected European experts, all ERA-EDTA members and reviewers selected by the European Society of Organ Transplantation and The Transplantation Society. Where appropriate, changes based on these comments were made in the final document. We felt this is an important step in the development of guidelines, as it fuelled the base of expertise that enhanced the overall quality of the guideline. We owe a special debt of gratitude to all those who took time out of their busy schedules to share their comments with us. They have been instrumental in improving the final guidelines.

We hope that this document will help caregivers to improve the quality of care they deliver to patients.

Daniel Abramowicz, Transplantation work group Co-chair
Wim Van Biesen, ERBP advisory board Chairman
Pierre Cochat, Transplantation work group Co-chair
Raymond Vanholder, President of ERA-EDTA

RECOMMENDATIONS

Chapter 1. Evaluation of the Kidney Transplant Candidate

1.1. Should we actively screen for presence of malignancy in kidney transplant candidates? Is presence or history of malignancy a contra-indication to kidney transplantation?

We recommend screening kidney transplant candidates for cancer according to the recommendations that apply to the general population (Ungraded Statement).

We suggest screening kidney transplant candidates for presence of kidney cancer by ultrasound (Ungraded Statement).

We suggest screening for the presence of urothelial cancer by urinary cytology and cystoscopy in kidney transplant candidates with an underlying kidney disease associated with an increased risk of this type of cancer (Ungraded Statement).

We recommend screening HCV and HBV-infected kidney transplant candidates for presence of hepatocellular carcinoma according to the EASLEORTC Clinical Practice Guideline on the management of hepatocellular carcinoma (Ungraded Statement).

We suggest that patients with current or previous cancer should be discussed with an oncologist and considered on a case by case basis. The following factors should be considered when determining the appropriate time that wait-listing should be delayed: a) the potential for progression or recurrence of the cancer according to its type, staging and grade; b) the age of the patient; c) the existence of co-morbidities, in order to define the appropriate period of time that wait-listing should be delayed (Ungraded Statement).

1.2. Under which conditions can HIV infected patients be enrolled for the waiting list?

We recommend that HIV per se in not a contra-indication for kidney transplantation (1C).

We recommend wait-listing HIV patients only if 1) they are compliant with treatment, particularly HAART therapy 2) their CD4+T cell counts are >200/μL and have been stable during the previous 3 months 3) HIV RNA was undetectable during the previous 3 months 4) no opportunistic infections occurred during the previous 6 months 5) they show no signs compatible with progressive multifocal leukoencephalopathy, chronic intestinal cryptosporidiosis, or lymphoma (1C).

We suggest that the most appropriate anti-retroviral therapy should be discussed before transplantation with the infectious diseases team in order to anticipate potential drug interactions after transplantation (Ungraded Statement).

1.3. Is there a role for immunisation against herpes varicella-zoster (HVZ) prior to renal transplantation?

We recommend immunisation against varicella zoster virus (VZV) all paediatric and adult patients negative for anti VZ antibodies, preferable still when they are waitlisted (1D).

1.4. Should haemolytic uremic syndrome (HUS) as underlying cause of end-stage renal disease preclude waitlisting for transplantation and does it influence graft and patient survival post-transplantation?

We recommend that typical, proven shiga-toxin E-coli associated Haemolytic Uremic Syndrome (HUS) is not contra-indication to transplantation from either deceased or living donors (1B).
We suggest considering renal transplantation as an acceptable option 1) in renal transplant candidates with aHUS and a proven MCP mutation, and 2) in those displaying anti-CFH auto-antibodies (Ungraded Statement).
We suggest that kidney transplantation in patients with aHUS should only be undertaken in centres with experience in managing this condition and where appropriate therapeutic interventions are available (Ungraded Statement).
We do not recommend living donation from a genetically related donor in patients who are suspected to have aHUS as their underlying kidney disease unless the responsible mutation has been conclusively excluded in the donor (1D).
We recommend evaluating the potential of living donation from a genetically unrelated donor to a recipient with aHUS on a case by case basis. It should only be considered after appropriate counselling of recipient and donor on the risk of disease recurrence in the transplanted graft (Ungraded Statement).

1.5. Should focal segmental glomerulosclerosis (FSGS) as underlying cause of end-stage renal disease preclude waitlisting for transplantation and does it influence graft and patient survival post-transplantation?

We recommend that primary focal segmental glomerulosclerosis per se is not a contraindication to kidney transplantation from either a living or a deceased donor (1D).
We recommend informing the recipient and in living donation, the potential donor, about the risk of recurrence of focal segmental glomerulosclerosis in the graft (Ungraded Statement).
We recommend that when a first graft has been lost from recurrent focal segmental glomerulosclerosis, a second graft from either a deceased or a living donor should only be transplanted after an individual risk/benefit assessment and careful counselling of the recipient and potential donor in the case of living donation (Ungraded Statement).
We suggest using an updated management protocol in cases of recurrent focal segmental glomerulosclerosis (Ungraded Statement).
We suggest that children with steroid-resistant nephrotic syndrome undergo appropriate genotyping before wait listing them for kidney transplantation (Ungraded Statement).

1.6. Does pre-transplant alcohol and drug abuse in patients influence patient or graft survival?

We recommend that women who drink >40g and men who drink >60g of alcohol per day stop or reduce their alcohol consumption to below these levels (1D).
These patients can be waitlisted, but a careful surveillance of reduction of alcohol consumption should be exerted (Ungraded Statement).
We recommend that patients with alcohol "dependence" should not be waitlisted (Ungraded Statement).
Strategies to stop alcohol consumption should be offered, according to the WHO Clinical Practice Guideline (Ungraded Statement).
We recommend that patients with an on-going addiction to "hard drugs" resulting in non-adherence should not be waitlisted for transplantation (1D).

1.7. Does pre-transplant tobacco smoking in patients influence patient or graft survival?

We recommend that patients stop smoking before transplantation (1B).
Smoking cessation programs should be offered (Ungraded Statement).

1.8. Should obesity preclude waitlisting for kidney transplantation and is there a difference in outcomes posttransplantation between those with and without obesity?

We recommend that patients with a BMI>30 kg/m² reduce weight before transplantation (Ungraded Statement).

1.9. Should kidney transplantation be delayed in patients presenting with uncontrolled secondary hyperparathyroidism? Does uncontrolled secondary hyperparathyroidism in the immediate pre-transplant period have an impact on transplant outcomes?

We recommend not to refuse a cadaveric graft only because of uncontrolled hyperparathyroidism (1D).
However, for patients on the waiting list, effort should be made to comply with existing CKD-MBD guidelines, including parathyroidectomy, when indicated (Ungraded Statement).

1.10. How should screening for potential cardiovascular disease in the potential recipient be done in a cost-effective way?

We recommend that basic clinical data, physical examination, resting ECG and chest-X ray are a sufficient standard work-up in asymptomatic low risk kidney transplant candidates (1C).
We recommend performing a standard exercise tolerance test and cardiac ultrasound in asymptomatic high risk patients (older age, diabetes, history of cardiovascular disease). In patients with a true negative test, further cardiac screening is not indicated (1C).

We recommend performing further cardiac investigation for occult coronary artery disease with non-invasive stress imaging (Myocardial perfusion or Dobutamine Stress Echocardiography) in kidney transplant candidates with high risk and a positive or inconclusive exercise tolerance test (1C).
We recommend performing coronary angiography in renal transplant candidates with a positive test for cardiac ischemia. Further management should be according to the current cardiovascular guidelines (1D).

1.11. When and for which indications should native nephrectomy be performed in kidney transplant candidates awaiting kidney transplantation?

We recommend native nephrectomy before transplantation (unilateral or bilateral) in patients with autosomal polycystic kidney disease (ADPKD) when there are severe, recurrent symptomatic complications (bleeding, infection, stones) (1C).
We suggest unilateral nephrectomy of asymptomatic ADPKD kidneys when space for the transplant kidney is insufficient (2C).
We do not recommend routine native nephrectomy, unless in cases of recurrent upper urinary tract infections or when the underlying kidney disease predisposes to enhanced cancer risk in the urogenital tract (Ungraded Statement).

Chapter 2. Immunologic Workup of Kidney Donors and Recipients

2.1. How should HLA typing be performed in renal transplant candidates and donors?

We suggest that at least one typing is performed by molecular HLA typing of patients and donors to avoid mistakes in the classification of the HLA antigens (2D).
We suggest that HLA typing is performed in duplicate, preferentially on separate samples obtained at different occasions to avoid logistical errors (Ungraded Statement).
In case of sensitized patients, we recommend additional serological typing of the donor cells to be used for cross-matches in order to check the proper expression of the HLA antigens on the target cells (1D).
For highly sensitized patients with allele specific antibodies we suggest to consider high resolution molecular typing in both recipients and donors (2D).

2.2. In a renal transplant recipient, how should HLA matching be used to optimize outcome?

We suggest to match for HLA-A, -B and -DR whenever possible (2C).
We recommend to balance the effects of HLA matching with other parameters that affect patient and graft outcomes when deciding the acceptance of a potential graft (1D).
We recommend to give preference to an HLA identical donor and recipient combination (1B).
We suggest to give more weight to HLA-DR matching than to HLA-A and -B matching (2C).
We recommend to give more weight to HLA matching in younger patients, in order to avoid broad HLA sensitization that might impair re-transplantation (Ungraded Statement).

2.3. In renal transplant candidates, what HLA antigens and non-HLA antigens should be defined in addition to HLA-A, -B and -DR?

We recommend to perform HLA-DQ, HLA-DP and HLA-C typing of the donor only when the intended recipient has HLA antibodies against those antigens (1D).
We do not recommend routine typing for Major Histocompatibility Complex class I related chain-A (MICA) and other non-HLA antigens in either recipient or donor (1D).

2.4. In HLA sensitized kidney transplant candidates what measures should be attempted to improve the probability of a successful transplantation?

We recommend establishing programs to select a donor towards whom the recipient does not produce antibodies (1C).
In recipients from cadaveric kidney donors, this aim can be achieved by an acceptable mismatch program (1C).
In living donation this goal can be achieved by paired exchange (Ungraded Statement).
We recommend to transplant patients with donor specific antibodies only if these abovementioned measures cannot be accomplished and after successful intervention (2D).

2.5. Should in renal transplant candidates a failed allograft that still is in place be removed or left in place?

Evidence comparing patients with a failed transplant with versus without nephrectomy is insufficient and conflictive, hampering a meaningful general recommendation on whether or not nephrectomy of failed grafts should be recommended (Ungraded Statement).
We suggest that in following conditions an explantation of the failed kidney graft be considered: clinical rejection, chronic systemic inflammation without other obvious cause, or recurrent (systemic) infections (Ungraded Statement).
We suggest to continue low level immunosuppression and to avoid a nephrectomy of a failed graft when residual graft urinary output is >500ml/day and there are no signs of inflammation (Ungraded Statement).

2.6. In renal transplant candidates, what technique of cross-match should be used to optimize outcomes?

We recommend a complement-dependent cytotoxic (CDC) cross-match be performed in HLA sensitized patients to prevent hyperacute rejection (1B).
We suggest that in HLA antibody negative patients with negative regular quarterly screening samples a cross-match can be omitted, unless a potential HLA sensitizing event has occurred since last screening (2B).
We do not recommend to perform Luminex cross match, or endothelial cell cross match because their additional value needs further evaluation (1D).
We recommend a positive CDC cross-match should only be accepted as truly positive when donor specific antibodies are known to be present (1B).

2.7. In renal transplant candidates planned to undergo living donor transplantation but for whom the available donor is ABO incompatible, what measures can be taken to improve outcome after transplantation?

We recommend both inhibition of antibody production and ABO antibody removal before transplantation applied together in one and the same validated protocols (1C).
We recommend transplantation of an ABO incompatible kidney only if the ABO antibody titre after intervention is lower than 1:8 (1C).
We suggest to consider paired exchange when available (Ungraded Statement).

2.8. In previously transplanted patients, what is the effect of repeated mismatches for HLA antigens on outcome, as compared to avoiding repeated HLA mismatches?

We recommend that repeated HLA mismatches are not considered a contra-indication for transplantation in the absence of antibodies against those repeated mismatches (Ungraded Statement).
We suggest that the presence of antibodies against the repeated mismatch detectable by other techniques than CDC be considered as a risk factor rather than a contra-indication. (Ungraded Statement).

Chapter 3. Evaluation, Selection and Preparation of Deceased and Living Kidney Donors

3.1. When is dual kidney transplantation preferred over a single kidney transplantation?

We recommend that before the kidneys of a cadaveric donor is discarded because they are deemed unsuitable for single transplantation, transplantation of both kidneys into one recipient (dual kidney transplantation) is considered as an option (1C).
We suggest that in cadaveric donors where there is uncertainty on the quality of the kidneys, the decision to either discard the kidneys, or use them as a dual or a single transplant, is based on combination of the clinical evaluation and history of the recipient and donor, and when available, a standardised assessment of a pre-transplant donor biopsy (2D).
We recommend that before a kidney from a paediatric donor is discarded because it is deemed unsuitable for single transplantation in an adult recipient, en bloc transplantation is considered. due to low donor age for single transplantation in adult recipients, en bloc transplantation is considered (1B).
We suggest that the option of using kidneys for en bloc transplantation is always considered for donors weighting less than 10 kg (1D).

3.2. Which perfusion solution is best suited for kidney preservation in recipients of living donation? Which perfusion solution is best suited for kidney preservation in recipients of deceased kidney donation?

There is insufficient evidence to favour a particular preservation solution for kidneys that carry a low risk of delayed graft function (Ungraded Statement).
We recommend not using Eurocollins as a preservation solution for kidneys that carry a high risk of delayed graft function (long projected CIT, extended criteria donors) (1B).

3.3. Is machine perfusion superior to standard perfusion?

There is conflicting data regarding the generalizability of the benefit of machine perfusion over static cold storage. Until further evidence emerges, no firm recommendation for the use of machine perfusion in preference to cold storage can be made (Ungraded Statement).

3.4. Is there a critical cold ischemic time beyond which a donated organ should be discarded?

We suggest that cold ischaemia time is kept as short as possible (2D).
We recommend keeping cold ischaemia time below 24 hours when transplanting kidneys from donors after brain death (1B).
We recommend keeping cold ischaemia time less than 12 hours when using kidneys from donors after cardiac death (1D).
We recommend that the decision to use donor kidneys with a cold ischaemia time of more than 36 hours should be made on a case per case basis (1D).

3.5. On which criteria should we select living kidney donors to optimize the risk/benefit ratio of their donation?

General remarks

We recommend encouraging living kidney donors to exercise on a regular basis and when relevant, to lose

weight and stop smoking (1C).
We recommend that the individual risk of donation should be carefully discussed with the donor, taking into account the situation of both donor and recipient. Ideally, this should be done using standardised check lists to ensure all items are discussed (Ungraded Statement).
We suggest that the donor be evaluated by an independent physician who is not part of the transplant team and is not involved in the daily care of the recipient, and when possible, by a psychologist (Ungraded Statement).
We recommend that the process of donation is stopped should any doubt on donor safety arise, especially in younger donors, or when the benefit for the recipient is limited (Ungraded Statement).
We recommend that the simultaneous presence of more than one risk factor (hypertension, obesity, proteinuria, impaire glucose tolerance, haematuria) precludes donation (Ungraded Statement).

Hypertension

We recommend considering potential donors with a blood pressure <140/90 mmHg on at least three occasions without antihypertensive medication, as normotensive (1C).
We suggest measuring ambulatory blood pressure in potential donors who have office hypertension (blood pressure >140/90 mmHg) or who are taking pharmacological treatment for hypertension (2C).
We suggest well-controlled primary hypertension, as assessed by ambulatory blood pressure <130/85 mmHg, under treatment with maximum 2 anti-hypertensive drugs (diuretics included) is not considered a contra-indication to living kidney donation (2C).
We recommend that in hypertensive donors with evidence of target organ damage such as left ventricular hypertrophy, hypertensive retinopathy, and micro-albuminuria, donation should be discouraged (1C).

Obesity

We suggest a BMI above 35 kg/m2 is a contraindication to donation (2C).
We recommend counselling obese and overweight donors for weight loss before and after donation (Ungraded statement).

Impaired glucose tolerance

We recommend diabetes mellitus is a contraindication to donation, other than in exceptional circumstances (1D).
We suggest impaired glucose tolerance is not an absolute contraindication to donation (2C).

Proteinuria

We recommend to quantify urinary protein excretion in all potential living donors (1C).
We recommend overt proteinuria is a contra-indication for living donation (24-hour total protein >300 mg or spot urinary protein to creatinine (mg/g) ratio >300 (>30 mg/mmol) (1C).
We recommend that potential living donors with persistent (more than 3 measurements with 3 months interval) proteinuria <300mg/24hrs be further evaluated by quantification of micro-albuminuria to assess their risk of living donation (Ungraded statement).
We suggest to consider persistent (more than 3 measurements with 3 months interval) micro-albuminuria (30-300mg/24hrs) a high risk for donation (Ungraded statement).

Haematuria

We recommend considering persistent haematuria of glomerular origin as a contraindication to living donation, because it may indicate renal disease in the donor (1B).
However, we acknowledge thin basement membrane disease might be an exception (Ungraded statement).

Old age

We recommend that old age in itself is not a contra-indication to donation (1B).

3.6. What lower level of kidney function precludes living donation?

We recommend that all potential living kidney donors should have their glomerular filtration rate (GFR) assessed (1C).
We recommend that in cases where more exact knowledge on GFR is needed or where is doubt regarding the accuracy of GFR from estimation methods, a direct measurement of GFR should be undertaken by exogenous clearance methods (Ungraded Statement).
We recommend that all potential donors should have a predicted GFR that is projected to remain above a satisfactory level after donation within the lifetime of the donor as indicated in the graph below (Ungraded Statement).

3.7. What are the risks of pregnancy in a woman with a single kidney after living kidney donation?

We recommend informing women of childbearing age that as they are a selected from a very healthy subpopulation, donation increases their individual risk from below that of the general population, to that of the general population (1B).

3.8. What is the best surgical approach for living donor nephrectomy for the donor? What is the best surgical approach for living donor nephrectomy for the recipient?

For living donor nephrectomy we suggest either a minimally invasive or laparoscopic approach rather than a flank subcostal retroperitoneal one.
The choice between minimal invasive and laparoscopic procedure should be based on the local expertise (2C).

Chapter 4. Perioperative Care of the Kidney Transplant Recipient

4.1. What are the indications for an additional haemodialysis session in the recipient immediately before the transplantation procedure?

We recommend to not routinely perform a haemodialysis session immediately before the actual transplantation procedure unless there are specific clinical indications (1C).
When additional haemodialysis is performed immediately before the transplantation procedure, we recommend that ultrafiltration is not used unless there is evidence of fluid overload (1C).

4.2. Does the use of central venous pressure measurement as a guidance tool for fluid management in kidney transplant recipients improve the outcome after transplantation?

We suggest that central venous pressure is measured and corrected in the early post-operative period to prevent hypovolemia and delayed graft function (2D).

4.3. In kidney transplant recipients during the perioperative period, does the use of intravenous solutions other than 0.9% sodium chloride improve patient and/or graft outcome?

There is no evidence to prefer one type of solution (crystalloids versus colloids, normal saline versus Ringer) for intravenous volume management of the recipient during kidney transplant surgery (Ungraded Statement).
In view of the available data in the general literature, and in line with the ERBP position on prevention of AKI, we suggest to be cautious with the use of starches in the perioperative kidney transplant patient, although specific data on the use of starches in the perioperative period in kidney transplant recipients are lacking (Ungraded Statement).
We recommend to monitor for metabolic acidosis when normal saline is used as the only intravenous fluid in the perioperative and postoperative period (1B).

4.4. Does the use of dopaminergic agents (dopamine and its alternatives) improve early postoperative graft function?

We do not recommend the use of "renal doses" of dopaminergic agents in the early postoperative period, since it does not influence graft function or survival (1B).

4.5. Should we use prophylactic antithrombotic agents during the perioperative period?

We do not recommend routinely using low molecular weight heparin, unfractionated heparin or aspirin before transplantation to prevent graft thrombosis (1B).

4.6. In renal transplant recipients, what are the effects of using a JJ stent at the time of operation on renal outcomes?

We recommend prophylactic JJ stent placement as a routine surgical practice in adult kidney transplantation (1B).
We suggest that when an JJ stent is in place, cotrimoxazole is given as antibiotic prophylaxis (2D).
We suggest removing the JJ stent within 4 to 6 weeks (Ungraded Statement).

4.7. What is the optimal postoperative time for removal of the indwelling bladder catheter in kidney transplant recipients?

We suggest removing the urinary bladder catheter as soon as possible after transplantation, balancing the risk of urinary leak against that of urinary tract infection (2D).
We recommend monitoring adverse event rates (urinary tract infection, urinary leakage) in each centre, to inform the decision over when to remove the indwelling bladder catheter (1D).

Conflict of interest statement. None declared.

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1. European Renal Best Practice Transplantation Guideline Development Group. ERBP Guideline on the Management and Evaluation of the Kidney Donor and Recipient. Nephrol Dial Transplant 2013;28 Suppl 2:ii1-71.

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Editorial Comments

BANTAO Journal: A Story of Ten Years

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On the occasion of the 10th anniversary of the BANTAO Journal, the Editorial team is presenting a brief report on the current status and achievements trying to get an answer whether it has met the expectations since its establishment.

The first issue was published in September 2003 by Kidney Foundation of Bulgaria, ten years after BANTAO was born in Ohrid on October 9, 1993. In fact, the first issue was an abstract book composed of the selected 330 submitted abstracts from the 6th BANTAO Congress held in Varna from 6-9 October. The Editor was Veselin Nenov and Editorial board members were: Ljubica Djukanovic, Dimitar Nenov, Momir Polenakovic, and Charalambos Stathakis, all founders of the BANTAO Association. We decided to nourish the practice of publishing additional supplement from the Congress abstracts. At the same time, the authors with presentations at the BANTAO Congress were invited to submit their valuable papers to be published into the Journal. Over time, there were editorial changes: since 2005 until 2009 the Editor was Ali Basci (Izmir, Turkey) and Deputy Editor Goce Spasovski (Skopje, Macedonia). Since 2009 G. Spasovski has held the Editor-in-Chief Office. Over the last couple of years, as a result of the great effort and engagement the Journal has been included into the EBSCO, DOAJ and SCOPUS/SCIMAGO database. It is a great progress and we are grateful to all of the contributors to the Journal, and especially to Veselin Nenov who was very devoted to the work in the Journal, applying for its inclusion in various databases and maintaining the Journal official web page for many years.

In addition, we appreciate the highly scientific contribution of the world-recognized experts in the field of nephrology. However, we are pleased that there is a growing number of original papers from Balkan authors reflecting the high quality research and scientific potential from the Balkan nephrologists. Reasonably, along with the imposed higher standards for publication, the number of papers decreased over time. Table 1 shows the number of papers published in two periods: 2003-2007 and 2008-2013. Herewith, we could observe an increasing number of papers from Croatia, related maybe to the acquisition and effort of their associate editor. We would like very much to see such an increased enthusiasm from the other countries in the future. Apart from the originality of our authors, we tried to put some challenges asking for their comments on topics we found important for our region such as: improving transplant program, vascular access in developing countries, peritoneal dialysis in AKI, dilemmas about RRT selection, vascular calcifications, Balkan endemic nephropathy, guideline controversies, novel therapy of primary and secondary glomerular diseases, etc.

Table 1. Number of papers published in the BANTAO Journal and the country of origin

	MKD	SRB	GR	TR	BG	AL	RO	BiH	CRO	SLO	Others	Overall
Papers 2003-2007	52	50	46	35	21	12	9	7			35	267
Papers 2008-2014	27	13	18	29	9	10	2	5	16	6	12	147

In addition, in our publications we also tried to shed some more light on the nephrology and nephrologists from the Balkan region. Although working in difficult conditions, they keep pace with the nephrologists from developed countries, and their effort is really worth mentioning. This is even more important, having in mind

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that the number of their papers in journals with a high impact factor is increasing. Of note, the contribution of nephrologists from the region in the field of clinical trials has also increased recognizing our hard work and enthusiasm when selecting the sites for clinical trials and more and more sites from the region are involved in the last couple of years.

Let's remember the goals proposed when BANTAO Association was established: *"The main goal of BANTAO is to promote scientific and technical cooperation in the field of renal diseases and artificial organs between the Balkan cities. This goal will be achieved not only through a periodical congress, but also through lecturers fellowship and scientific research methods exchange, joint meetings and courses, publications and cooperation in the field of renal transplant"*.
This Journal, for sure, has contributed to these goals and we want to express our gratitude to all who incorporated their spirit for the benefit of our association.

Still, there is a lot to be done in the forthcoming period: to increase the submission rate and quality of papers and to think about paper citations from the journal since we have obtained Scientific Impact factor. Finally, our aim in the near future is to include BANTAO Journal in the Medline database which is the way to increase Journal Impact Factor (Thomson Reuters) once it's Medline cited.

On behalf of the Editorial Board of
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Editorial Comments

Update on the management of lupus nephritis

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Abstract

The treatment of lupus nephritis still represents a therapeutic challenge for the clinician. Besides early recognition, appropriate guiding by the histologic classification at presentation as well as at relapsing disease, is essential. The most severe proliferative and mixed forms require aggressive induction therapy. Nevertheless, recent but established by RCTs advances, as low dose iv cyclophosphamide, lower doses of corticosteroids and mychophenolate acid (MPA) allow us to achieve remission induction with lower toxicity without any cost in terms of efficacy. For maintenance, azathioprine and mycophenolate acid with concomitant low dose steroids have shown both good results with a slight superiority of mycophenolate acid. Emerging therapies as B cell targeting-either by depleting agents as the anti-CD 20 mAb Rituximab, or by modulating agents as the anti-Bliss Belimumab, further contribute to the effort to minimize toxicity. This review mainly focuses on the recent efforts to treat the most aggressive form of lupus nephritis effectively with the minimal possible toxicity.

Key words: lupus nephritis, treatment, cyclophosphamide, corticosteroids, mychophenolate acid

Introduction

Systemic lupus erythematosus (SLE) is a complex disease with variable presentations, course and prognosis. Nephritis is a common manifestation of SLE; it occurs in up to 60% of patients at some time in the course of the disease [1]. Nephritis not only leads to ESRD in 10-20% of patients, but it is also a major contributor for morbidity and mortality. Despite signifycant improvement in early mortality, long-term prognosis still remains suboptimal [2]. Severe SLE manifesttations, including nephritis require more aggressive treatment. Therefore, those patients are exposed to higher doses of immunosuppression [3]. So, accurate treatment of SLE nephritis still remains a challenge.

At first, effective treatment of lupus nephritis depends on early recognition of the renal involvement, since the presenting features may be subtle. Secondly, renal injury varies widely from mild to very severe; the spectrum of renal injury can most accurately be assessed only by renal histology. Based on the newest ISN/RPS 2003 classification, SLE nephritis comprises six classes (I to VI) [4]. Lupus nephritis (LN) class I and II are the mildest and generally do not warrant specific immunosuppressive therapy. Based on the recently published EULAR-ERA/EDTA recommendations [5], LN class I in the presence of podocytopathy on electron microscopy and clinical evidence of nephrotic syndrome should be treated as minimal change disease. In the cases of class II nephritis with proteinuria>1g/24hrs despite adequate rennin-angiotensin-aldosterone-system (RAAS) blockade, low-to-moderate doses of corticosteroids (0.25-0.5mg/kg/d) alone or in combination with azathioprine should be used. Class VI describes a kidney with advanced sclerosis (more than 90% of glomeruli) and requires only supportive treatment.
Proliferative (class III and IV) as well as the mixed classes of proliferative with concomitant membranous lupus nephritis (class III+V, IV+V) with the even worst prognosis, represent the most severe forms of lupus nephritis. Those classes have to be treated promptly and aggressively.

Adjunctive treatment

Strict blood pressure control with a target BP of 130/80 mmHg is warranted in all patients with lupus nephritis. When proteinuria is more than 500 mg/24 hrs, renin-angiotensin-aldosterone-system (RAAS) blockade is the antihypertensive treatment of choice or needs to be given without hypertension. Since SLE is associated with accelerated atherosclerosis, cholesterol lowering with statins is indicated for persistent hyperlipidemia with a target low-density-lipoprotein (LDL) of ≤100 mg/dl. Hydroxychloroquine (HCQ) should be administerred to all patients with LN; it seems to reduce flares and to prevent thrombotic events [6].

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Treatment of proliferative lupus nephritis

Induction therapy

The initial induction period consists of combined intensive immunosuppressive therapy.

Corticosteroids

Corticosteroids as intravenous pulses of 500-1000 mg/d for three days and thereafter given orally as 0.5-1mg/kg/day of prednisone for one month tapered to 5 mg/day at six months, still remain the cornerstone of induction therapy for severe, proliferative lupus nephritis. The use of intravenous methylprednisolone (MP) pulses in current induction treatment protocols is based on circumstantial data that support the evidence for its use [7]. One study has evaluated the efficacy of conventional (1 mg/kg/d) versus low-dose (0.5 mg/kg/d) steroids in conjunction with a MPA (enteric-coated mycophenolate sodium, EC-MPS) as induction in severe, proliferative LN. It showed equal efficacy of both steroid regimens in inducing remission at 24 months [8].

Cyclophosphamide

The pioneering studies by investigators at the National Institute of Health (NIH) have demonstrated the importance of intravenous cyclophosphamide in the management of lupus nephritis [9,10].The so-called "NIH-regimen", consisting of monthly, intravenous pulses of 0.5-1 g/m^2 of cyclophosphamide (CYC) and steroids, became the standard of care for three decades, despite its high toxicity. The Eurolupus Nephritis Trial (ELNT) showed that equal efficacy could be achieved with lower doses (a total of 3 g) and shorter duration (3 months) of cyclophosphamide [11]. Nevertheless, responses are often slow [12] and treatment with cyclophosphamide is associated with significant toxicity. In order to completely avoid cyclophosphamide, other immunosuppressive agents have been tested for induction therapy.

Azathioprine

One effort to use azathioprine instead of cyclophosphamide as induction failed; not only cyclophosphamide was superior to azathioprine in means of efficacy and safety, but also repeat biopsies after two years showed progression of chronic lesions with the use of azathioprine [13].

Mycophenolate acid

Recent studies have focused on the use of mycophenolate acid (MPAs), for induction. The recommended dose in non-Asians is 3000 mg of mycophenolate mofetil or its equivalent 2160 mg of the enteric-coated mycophenolate sodium daily. Asian patients seem to respond as good to lower doses of 2000 mg of MMF daily [14].The largest randomized controlled trial in lupus nephritis was the Aspreva Lupus Management Study (ALMS) [15] comprising a mixed population of 370 patients with lupus nephritis class III, IV and V. Although in the induction phase the study did not meet its primary objective of showing that MMF was superior to CYC, it was at least equally effective. After subgroup analysis, MMF showed superiority in African-Americans and Hispanics.
One meta-analysis of trials comparing MMF and CYC as induction in a total of 306 patients, published in 2006 [16], showed that MMF was more effective than CYC, with a remission rate for MMF of 66-80% versus 54 for CYC and lower rates of serious adverse events.
A second meta-analysis [17], 6 months later, in 268 patients, showed again better efficacy of MMF over CYC but most importantly, lower risk of death and end-stage renal disease (ESRD).
In terms of safety concerns, MPA therapy may be also preferable to those young patients with proliferative LN, for whom fertility preservation is of essential importance; it is known that six or more iv courses of high-dose CYC causes sustained infertility in at least 10% [18].
So, both based on efficacy and safety data, there is substantial evidence to conclude that MPA may be considered a first line treatment for induction therapy of class III and IV lupus nephritis.
The only exception seems to be crescentic lupus nephritis i.e. the presence of cellular crescents and necrotic lesions in renal biopsy. In this case, despite the lack of evidence by RCTs, as also stated in the American College of Rheumatology Guidelines, experts still favor the use of high-dose iv pulses of CYC in combination with iv steroid pulses followed by high-dose (1 mg/kg/d) oral steroids as induction [19].

Maintenance therapy

Despite high remission rates, relapses are common; nephritic or nephrotic flares occur in about 30% of patients and severe relapses are a prognostic factor for adverse renal outcome [20]. The goal of maintenance therapy is to sustain remission and to prevent relapses with the minimal possible toxicity long-term. Currently, the most common choices for maintenance therapy are MPA and azathioprine (Aza) in conjunction with low-dose steroids. MPA and Aza act both as purine antimetabolites; they have similar but also distinct mechanisms of action. Currently, the two largest multicenter RCTs directly comparing the two agents as maintenance therapy are the European MAINTAIN Study and the multiethnic US ALMS Study.
In the MAINTAIN trial [21], 105 patients were randomized after 12 weeks of induction therapy with Euro-

lupus regimen, either to azathioprine or to mycophenolate mofetil (MMF). Over three years, there was no difference in terms of efficacy.These results differ from those of the ALMS study. In this study, from a total of 370 patients, those patients who achieved complete remission (n=227) after 6 months either on MMF or CYC, were re-randomised to MMF or Aza again in conjunction with low-dose steroids. There was superiority of MMF over Aza in terms of efficacy: there was a significantly higher percentage of patients reaching the primary end-point i.e. treatment failure at 3 years in the Aza than in the MMF group (32% versus 16%) [22]. If this difference reflects the larger number of patients in the ALMS study or the differences in ethnicity (since MMF is known to be more effective in blacks and Hispanics), or the fact that the ALMS included patients only after remission, remains to be elucidated. The last EULAR/ERA-EDTA recommendations suggest as a reasonable approach, continuation of MPA without switching to Aza, if MPA therapy has proven successful as induction therapy.
Today, based upon current evidence, we can assume that maintenance therapy with either MPA or Aza with low-dose steroids is both safe and effective with possible advantages of MPAs. Those refer to the lower risk of malignancy long-term, its better cardiovascular profile, a trend towards less flares and their superiority in certain ethnic groups and may-at least in part-be counteracted by its higher cost and its contraindication in pregnancy.
The optimum duration of maintenance therapy is still a matter of debate; current evidence suggests that once patients enter remission, maintenance should be continued for at least 3 years [23].

Emerging therapies in lupus nephritis: B-cell depletion

In the past years there has been important success in the development of B-cell targeted therapies in the treatment of lupus nephritis.

Rituximab

The anti-CD20 mAb Rituximab was the first biological widely used agent in the treatment of autoimmune diseases including SLE.
The rationale for its use, especially in lupus nephritis, is that B-cells play a central role in the autoimmune response of this disease. Besides autoantibody production and immune deposit formation, B-cells also interact with autoreactive T-cells. In a study by our group [24], we investigated the therapeutic effect of Rituximab in 10 patients with proliferative lupus nephritis; moreover, we examined the changes in peripheral T-cell subsets after B-cell depletion. One month after B-cell depletion, a 4-fold decrease in the expression of the costimulatory molecule CD40 ligand on CD4+ cells and a significant decrease of the T-cell activation markers CD69 and HLA-DR was observed, in parallel with partial and complete clinical remission which was achieved in 8/10 and 5/10 patients, respectively. Cumulative evidence, mostly from small, open-label trials suggests that Rituximab is effective in SLE and lupus nephritis with minimal toxicity [25,26]. As induction, it is most often used concomitantly with low-dose "conventional" therapy, or in refractory cases [25]. However, a multicenter phase II-III trial in 144 patients with SLE nephritis (LUNAR) [27] that was designed to detect a beneficial effect of Rituximab, given additionally to MMF and high-dose steroids on the induction of renal response, did not attain its primary end-point at 52 weeks.
This study disappointed many investigators worldwide about the true efficacy of Rituximab in lupus nephritis but one of the possible explanations in favor of Rituximab seems reasonable: the trial was intended to detect a large clinical effect in patients with very active disease and this was not possible for a biological therapy given additionally to high-dose conventional therapy.
Additional studies in targeted populations are needed. For the use of Rituximab as maintenance, again data from small, uncontrolled studies show that B-cell depletion with Rituximab is effective and safe. In these trials Rituximab is used with different dosing-regimens, alone or in combination with maintenance therapy, as a single or as repeated doses, either after B-cell reappearance or after clinical indication of relapse. In another study by our group [28], we used Rituximab in 10 young women with a relapse of proliferative lupus nephritis. A single course of four weekly doses of 375 mg/m^2 of Rituximab was given additionally to 2 g/d of mycophenolate mofetil and 0.5 mg/kg/day of prednisolone for one month, with rapid tapering thereafter. Complete remission was achieved in 7/10 and partial remission in the remaining 3 patients, with excellent tolerability. Remission was sustained for 38 months in 6/10 patients [22]. Overall, with all the limitations of the small, uncontrolled trials, Rituximab shows promising efficacy with good tolerability and can be used in order to either minimize therapy-related toxicity for a considerable period or to control resistant disease.

Newest B-cell targeting agents: Belimumab, Atacicept

Besides B-cell depletion, B-cell modulation is another option and a growing field of research. New biological agents that inhibit B-cell activating factor (BAFF/BLyS) are currently under investigation. The most promising agent is Belimumab, a fully human monoclonal antibody against soluble BAFF. In humans, BAFF levels are elevated in SLE and correlate with disease activity. Preliminary results of two phase III trials of Belimumab in patients with moderate to severe lupus (BLISS-52 and BLISS-76) showed promising efficacy.

The primary end-point that was SRI (systemic lupus erythematosus response index) at 52 weeks, showed a significant effect of Belimumab compared to placebo and given additionally to conventional therapy, with very good tolerance [29].
Atacicept, a chimeric molecule formed by a receptor for BAFF and a proliferation-inducing ligand (APRIL) with IgG, which binds both BAFF and APRIL, has induced profound depletion of plasma cells, resulting also in a reduction of immunoglobulin levels. Unfortunately, a phase II study of Atacicept with mycophenolate in patients with lupus nephritis was stopped because of the high rate of infections [29].
Combining BAFF-blockade with B-cell depletion is the next step in B-cell targeted therapies.

Treatment of membranous lupus nephritis

Pure membranous (class V) nephritis is a relatively rare entity, comprising 8-20% of patients with lupus nephritis and its optimal treatment is still not fully answerred. Most of the therapeutic evidence comes from small, open-label trials. There are only a few small RCTs. Non-immunosuppressive strategies including angiotensin-converting-enzyme inhibition, should be instituted early in all cases. Immunosuppressive treatment should be initiated when proteinuria exceeds 3 g/24 hrs.

Alkylating agents

The first RCT in membranous lupus nephritis comes from the NIH [30]. In this study, 42 patients received either alternate-day oral prednisone (control group) or alkylating agents i.e. alternate-month intravenous pulse cyclophosphamide for 6 months or cyclosporine for 11 months. Both iv CYC and cyclosporine were more effective in inducing remission (60% and 83% remission rates, respectively) than prednisone alone (27% remission rate). However, a significantly higher relapse rate upon withdrawal of cyclosporine was noted.

Mycophenolate acid (MPA)

Given its milder toxicity profile than alkyating agents and CNIs, MPA therapy has emerged in this class of LN, too. A pooled analysis of two RCTs demonstrated comparable antiproteinuric effects between MMF and iv cyclophosphamide [31]. The American College of Rheumatology recommends MPA in conjunction with 0.5 mg/kg/d of prednisone as first-line treatment in patients with pure membranous lupus nephritis and nephrotic-range proteinuria [19].

Treatment of lupus nephritis in pregnancy

Almost all immunosuppressive agents, except Aza, as well as high-dose and pulse steroids are contraindicated in pregnancy. Immunosuppression with MPA or CY should ideally be stopped 3 months before gestation, whereas biological agents should not be used for at least 4 months preceding pregnancy. RAAS-blockade is also prohibited during pregnancy.
Hydroxychloroquine (HCQ) should not be stopped as it prevents renal flares in pregnant women with mild systemic lupus activity. Low dose acetyl-salicylic acid is recommended in order to decrease the risk of pre-eclampsia. In cases of severe lupus nephritis flares, Aza ($\leq$ 2 mg/kg/d) and moderate-dose steroids are the only treatment options. In very severe cases, early delivery after the 28th gestation week may be indicated [5,32].

Treatment of relapses

In general, initial management of moderate-to-severe renal flares requires re-initiation of induction therapy for at least 3-6 months. Various agents, including biologicals as Rituximab, have been used for the treatment of relapses in order to minimize toxicity for longer period, but the small number of patients and the limited duration of most studies require caution in the interpretation of the often promising results.

Treatment of refractory disease

Since for most cases of LN the treatment of choice consists either of MPA or CyC, for patients failing to respond partially in 6-12 months or completely in 24 months [5], treatment may be switched from the first drug to the other, or Rituximab may be added [19]. For cases of resistance or toxicity to conventional therapy additional alternatives may include calcineurin inhibitors, iv immunoglobulin or plasma treatment. The evidence for the use of calcineurin inhibitors (cyclosporine and tacrolimus) mostly comes from open-label and some recent prospective trials [33-35]. They can both be used as maintenance therapy in cases of persistent or relapsing proteinuria with good preserved renal function, in conjunction with low-to-moderate-dose steroids with or without MPA according to the clinicians' judgment (level C evidence). Additionally, IVIG has been used as a rescue therapy in several uncontrolled trials. Although results were satisfactory, they could be attributed to the pharmacologic action of the concomitant immunosuppressants the patients were receiving [36]. Plasma exchange techniques are applied in cases of rapidly progressive glomerulonephritis or treatment failure [37,38].
However, repeat kidney biopsy is mandatory for the management of the cases of nephritic or nephrotic flares, since transformation from one class of LN to another or to mixed classes are common.

Conclusions

Recent evolutions in the treatment of lupus nephritis

include the proven efficacy of either low dose CYC or MPA for induction as well as the use of MPA or AZA for maintenance treatment. They all contribute to long term lower toxicity of therapy that is essentially important for this young patient population. Emerging therapies, mainly those targeting B-cells seem also promising.

Conflict of interest statement. None declared.

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Review

Podocytes and Proteinuric Kidney Disease

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Abstract

Glomerular disease is the most common cause of end-stage renal disease (ESRD), accounting for almost two thirds of cases. In glomerular disease, alterations of podocytes are of particular importance. Podocyte loss represents a central mediator of glomerular sclerosis. Toxic, genetic, immune, infectious, oxidant, metabolic, hemodynamic, and other mechanisms can all target the podocytes. These mechanisms provide new insight into the unique dynamic microenvironment that each individual podocyte inhabits and how it can turn hostile to survival. At the same time, they raise new therapeutic challenges to preserve glomerular function by containing podocyte injury and limiting its spread, both in podocytopathies and in other progressive glomerular diseases. Treatment strategies should aim at enhancing podocyte survival. The renin-angiotensin axis blockade, apart from its antifibrotic and intraglomerular hemodynamic effects, has an important role in preventing podocyte loss. However, only long-term observational studies can clarify if many patients will benefit from podocyte-targeted treatment such as abatacept or similar agents.

Key words: podocytes, glomeruli, proteinuria, angiotensin blockade

Introduction

Glomerular disease is the most common cause of end-stage renal disease (ESRD), accounting for almost two thirds of cases [1]. Glomerular sclerosis represents a common finding in the ongoing progression of the glomerular disease. Nowadays constant efforts are ongoing to identify reliable noninvasive biomarkers for acute and chronic kidney injury. These biomarkers will contribute to identification of kidney injury not only at early stages, but classification of kidney disease according to its severity, prediction of disease outcome and monitor response to therapeutic interventions [2].

In glomerular disease the alterations of podocytes are of particular importance. The podocytes are the key organizers of glomerular development and maintenance [3]. They are the largest cells in the glomerulus and have a highly specialized three-dimensional structure with a molecular profile closely linked to the critical functions they perform [4]. The podocytes are postmitotic cells whose function depends on their highly specialized and unique architecture. They are believed to serve at least four distinct functions:

1. Regulation of glomerular permselectivity;
2. Structural support of the glomerular capillary, cooperating with mesangial cells to resist the distensive force of intracapillary hydraulic pressure;
3. Remodeling of the glomerular basement membrane in cooperation with endothelial and mesangial cells;
4. Endocytosis of filtered proteins [5].

Podocyte loss is a central mediator of glomerular sclerosis. Toxic, genetic, immune, infectious, oxidant, metabolic, hemodynamic, and other mechanisms can all target the podocyte. Whatever the initial insult to the glomerulus results in podocyte depletion, remains to be elucidated. The outcome depends on whether the normal mature podocytes become depleted or not. Because podocytes are very well-differentiated cells that lack the potential to proliferate, they are particularly vulnerable to attrition in response to critical levels of cell stress, leading to detachment, necrosis, or apoptosis [6]**.** Beyond that, glomerular enlargement leads to relative podocyte depletion. Also a switch of the podocyte phenotype can occur making impossible the preservation of normal glomerular structure and function [7].

The first indications of the importance of podocyte loss in the progression of kidney disease came from animal models and from cross sectional studies of human disease. The concept of "podocyte insufficiency" developed until the current canonical model for the development of glomerular sclerosis: a loss of podocytes leads to "bare areas" of glomerular basement membrane, which in turn leads to the formation of synechie to Bowman's capsule and then to segmental and finally global glomerular sclerosis [8]. Just how much podocytes loss is necessary to generate an initial sclerotic lesion and whether injury can propagate to other podocytes remain controversial.

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The concept of podocyte depletion as a cause of glomerulosclerosis originates with the seminal ultrastructural studies by Nagata and Kriz in the ablation model of FSGS produced by uninephrectomy in the young rat [9]. As glomeruli become hypertrophic in response to loss of functioning nephrons, the well-differentiated podocytes must stretch to provide cover for the enlarged glomerular tuft. The podocyte‘s capacity to hypertrophy is limited and sites of tuft denudation caused by individual podocyte failure and detachment become covered by parietal epithelial cells, forming a nidus for the development of segmental scars.

Fukuda, *et al.* have shown that ongoing loss of podocytes destabilizes the glomerulus, leading to glomerular sclerosis, but progression can be prevented by combined renin-angiotensin axis blockade (with enalapril and losartan) [10]. In fact, it has long been known that angiotensin blockade is "unreasonably effective" in preventing progression of many renal diseases. Actually it has been proved that the prevention of autonomous progression seems to be closely tied to amelioration of ongoing secondary podocyte loss. The angiotensin blockade, apart from its antifibrotic and intraglomerular hemodynamic effects, has an important role in preventing podocyte loss.

A number of intraglomerular mechanisms could explain the progressive autonomous loss of podocytes. Ichikawa, *et al.* consider that "podocytes damage damages podocytes" [11]. What mechanisms underlie the local propagation of podocyte injury? Such mechanisms envisage a podocyte to podocyte spread of injury, possibly due to the loss of antiapoptotic cell-cell signaling between adjacent, interdigitating podocytes via the slit diaphragm, autocrine danger or death signals coming from injured podocytes or deleterious effects of local protein leakage [12]. Hypothetical mediators include loss of pro survival factors such as nephrin signaling and vascular endothelial growth factor production or enhanced noxious factors such as TGF-β, angiotensin II, shear stress, or cell death gap junction signaling, none of which is mutually exclusive [11]. A functional consequence of podocyte loss, unremitting proteinuria itself, has also been shown to cause podocyte dedifferentiation and upregulation of TGF- β [13]. Loss of favorable nephrin signaling after disruption of cell-cell contacts is an especially attractive mediator, not only because immunoreactivity for nephrin in this model was more readily lost than podocalycin, but also because nephrin is known to serve as a signaling platform for a host of vital cellular functions such as maintenance of polarity, cell-cycle regulation, and cytoskeletal organization [14]. Podocytes interdigitate with other podocytes located within the same lobular unit of the glomerulus, which represents a major subdivision of the incoming afferent arteriole as it branches during glomerulogenesis. Loss of interdigitating podocyte partner, by disrupting the physical integrity of the slit diaphragm itself could propagate injury to neighboring podocytes, like a domino effect, until the entire lobule is captured. This scenario would explain the exqusite segmentality of the sclerotic lesions, which seems to respect lobular boundaries early in the disease.

These mechanisms provide new insight into the unique, dynamic microenvironment that each individual podocyte inhabits and how it can turn hostile to survival [15]. At the same time they raise new therapeutic challenges to preserve glomerular function by containing podocyte injury and limiting its spread, both in podocytopathies and in other progressive glomerular diseases. Treatment strategies should aim at enhancing podocyte survival.

One such strategy is rennin-angiotensin axis blockade as a good alternative for preserving the remnant podocytes in glomerular diseases. As podocyte number decreases either segmental parts of glomerular tuft are lost to sclerosis, or a decreased number of podocytes must "stretch" to cover the filtration surface, leading to broadening of foot processes. Either factor will lead to a decrease in the single nephron ultrafiltration coefficient, lowering the single-nephron glomerular fitration rate (SNGFR). An attendant decreased delivery of NaCl to the macula densa will lead both to a decrease in afferent arteriolar resistance via tubuloglomerular feedback (in order to increase intraglomerular capillary pressures and support SNGFR) and to an increase in local renin release from the juxtaglomerular apparatus. Release of renin from the afferent arteriole results in local activation of the renin-angiotensin system within the glomerular tuft. Increases in angiotensin II concentration within the glomerulus may affect the podocyte actin cytoskeleton (perhaps via activation of TRPC6 channels), increasing podocyte stress fibers and affectively counterbalancing the increased intracapillary pressures, thereby preserving glomerular capillary structure. It is a known fact that podocyte cytoskeleton is altered in patients with glomerular disease [16]. Although transient local activation of the renin angiotensin system may allow adaptive alterations in the podocyte cytoskeleton in the face of short-term increases in filtration pressures, long-term local angiotensin effects probably contribute to an ongoing loss of podocytes and this can occur via a number of other possible mechanisms. This may explain the 2-week lag period before angiotensin blockade seems to protect against podocyte loss into the urine. These mechanisms raise the possibility that if initial therapy in human disease can limit early podocyte losses to less than a certain threshold, inexorable progression to renal failure may be avoided [17]. But if this is not possible, it will always be the case that aggressive enough angiotensin blockade (possibly, targeted to minimize urinary podocyte excretion) will be sufficient to preserve glomerular architectural stability, protect remnant podocytes, and assure long-term renal survival.

Podocytes are normally absent or seen in small numbers in urine of normal individuals or those with inactive kidney disease. The number of podocytes in urine

increases with active kidney disease even before proteinuria appears and seems to improve with treatment. Also podocyturia seems to be confined to active disease, in contrast to proteinuria which is present with both active and chronic phases of glomerular damage [18]. So, podocyturia as a marker of subclinical early renal damage can be detected in glomerular disease before the occurrence of overt proteinuria. Since loss of podocytes is well associated with glomerulosclerosis, monitoring the podocyte loss by measuring podocytes or their products in the urine is a clinically useful tool in this time. Some authors detected podocytes and their fragments in the urine of humans with a variety of glomerular diseases using antibodies to the podocytes proteins: podocalyxin, podocin, nephrin, and synaptopodin [19,20]. In many glomerular diseases, including focal segmental glomerular sclerosis (FSGS), membranous nephropathy, membranoproliferative glomerulonephritis, amyloid nephropathy and diabetic nephropathy, podocytes are injured and then detached from their basement membrane are shed into the urinary space. In diabetic nephropathy, for example podocyte detachment is associated with the degree of proteinuria whereas podocyturia represents a useful marker of disease activity [21]. An association of urinary podocytes with toxemia of pregnancy has also been reported [22]. Podocyturia was shown to be present in patients with preeclampsia even at the time of delivery. Women with normotensive pregnancies and women with either hypertension or proteinuria but without clinical syndrome of preeclampsia had no podocyturia, a finding suggestive that podocyturia is not merely a result of hypertensive kidney damage or a marker of proteinuria [2]. Measurement of podocyte products in the urine as a potential non-invasive technique monitoring accelerated podocyte loss holds good potential for clinical application. So, podocyte replacement by stem cells may prove to be a useful strategy [7].

Some authors have introduced a new classification for proteinuric kidney disease, by dividing it into B7-1 positive and B7-1 negative, according to the presence or not of immunostaining for this costimulatory molecule [23]. Patients with B7-1 positive staining in their renal biopsy specimens have podocytes expressing B7-1, which is normally absent. The B7-1 positive podocytes show morphological and functional changes leading to detachment of podocyte foot processes from the glomerular basement membrane and proteinuria. It was found that administration of abatacept, an inhibitor of B7-1, appears to cure patients with severe nephrotic syndrome due to primary focal segmental glomerulosclerosis (FSGS) or recurrent FSGS after transplantation [1].

Podocytes are capable of expressing B7-1 under abnormal conditions [24,25]. After years of careful experimental studies on cultured podocytes and transgenic mice, the authors were able to apply these basic observations to the clinical field [1].

These observations may signal the start of a new era in the treatment of patients with proteinuric kidney disease. However, only long-term observation will clarify whether many patients will benefit from the podocyte-targeted treatment with abatacept or similar agents. On the other hand, any plan aimed at reducing the cost of health care will need to focus basic scientific effort on understanding podocyte biology and clinical research on learning how to prevent and monitor podocyte injury and depletion as major targets for intervention.

Conflict of interest statement. None declared.

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Review

Anderson-Fabry Disease in Females

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Abstract

Anderson-Fabry disease (AFD) is the second most common lysosomal storage disease. This is an X-linked disorder due to lysosomal enzyme deficiency of α-galactosidasae A, that results in accumulation of globotriaosylceramide in various tissues leading to organ damage, and resulting in a variety of cardiovascular, renal, neural, dermatological, psychological signs and symptoms. Despite being X-linked, heterozygous females can suffer from symptoms equally severe as male hemizygotes. This paper presents signs, symptoms, specific diagnostic approach and treatment possibilities of AFD in female patients.

Key words: Anderson-Fabry disease, α-galactosidasae A, female patients

Introduction

Anderson-Fabry disease (AFD) is an X-linked disorder due to lysosomal enzyme deficiency of α-galactosidasae A (GLA). After Gaucher's disease, it has been reported to be the second most common liposomal storage disease. Meikle, *et al*. reported the incidence of Fabry hemizygotes in Australia to be 1 in 117 000. No data on heterozygotes have been obtained, but the incidence determined in hemizygotes could be extrapolated to give a combined incidence of 1 in 58 000 [1]. In the UK, based on notifications of patients with low GLA activity the prevalence of Fabry disease was reported to be 1 in 366 000 [2]. The prevalence in Netherlands was estimated at 1 in 476 000 [3]. As it can be seen, the data on AFD incidence and prevalence are diverse [2-4]. AFD results from mutations in the GLA gene. More than 400 mutations have been indentified (mainly missense mutations but also nonsense and single amino acid deletions and insertions). The majority of these mutations have been identified only in individual families, while mutations of CpG dinucleotides account for most of the recurrent point mutations found in unrelated families with AFD [5].

Patients with this disorder are unable to effectively degrade membrane glycosphingolipids containing a terminal α-glycosidic galactose, especially globotriaosylceramide (Gb3). Gb3 accumulates in various tissues as the primary insult, followed by secondary cellular dysfunction, ischemia and fibrosis, which eventually lead to tissue damage and finally organ dysfunction [6]. The process of Gb3 accumulation, starting in the unborn child, can be subclinical until organ dysfunction appears. Anderson-Fabry disease affects all major organ systems in the human body, resulting in a variety of cardiovascular, renal, neural, dermatological, psychological signs and symptoms. In untreated patients death occurs typically in the late fifth to early sixth decade due to kidney failure, strokes and cardiac events [7,8]. As AFD is an X- linked disorder most females were thought to be asymptomatic through a normal life span or to develop only minor manifestation of the disease. Several studies have shown a severe and aggressive presentation indistinguishable from that seen in males [9]. Diversity of AFD clinical manifestations in female patients was demonstrated by Lukas, *et al.* in an extended Spanish family where related female AFD patients presented with severe neurological, cardiac and renal symptoms to only acroparesthesia [10]. Here we present a review of literature on clinical presentation, diagnostic specifics and treatment of Anderson-Fabry disease in women.

Clinical manifestations

The wide variability in clinical presentation is thought to be partly due to lionization [11], a process that occurs in embryos where one copy of the X-chromosome is randomly inactivated in all of its cells. This process happens in females that have essentially a "mosaic" of normal and mutant cells in varying proportions.
According to Fabry Registry (global clinical database that records longitudinal data on AFD), out of 1077 enrolled females in the registry, 69.4% had symptoms and signs of AFD. The median age at symptom onset among females was 13 years, which was significantly later in life than in male patients (9.0 years). Family history was positive in 84.1% of female patients, but

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the diagnosis was made at the median age of 31 years, with males being diagnosed at median age of 24 years. Twenty percent of female AFD patients experienced major cerebrovascular, cardiac or renal events at the median age of 46 years [7]. Signs and symptoms of AFD, as well as time of onset are shown in Table 1.

Table 1. Signs and symptoms of Fabry disease

Typical time at onset	Signs and symptoms
Childhood and adolescence (≤16 years)	Neuropathic pain
	Ophthalmological abnormalities (cornea verticillata and tortuous retinal blood vessels)
	Hearing impairment
	Dyshidrosis (hypohidrosis and hyperhidrosis)
	Gastrointestinal disturbances and abdominal pain
	Lethargy and tiredness
	Angiokeratomas
	Onset of renal and cardiac signs, e.g. microalbuminuria, proteinuria, abnormal heart rate variability
	Extension of any of the above
	Proteinuria and progressive renal failure
Early adulthood (17–30 years)	Cardiomyopathy
	Transient ischaemic attacks, strokes
	Facial dysmorphism
	Worsening of any of the above
Later adulthood (age >30 years)	Heart disease (e.g. left ventricular hypertrophy, angina, arrhythmia and dyspnoea)
	Stroke and transient ischaemic attacks
	Osteopenia and osteoporosis

Signs and symptoms of Fabry disease according to age (adapted from Mehta, *et al*. *Q J Med* 2010; 103:641-659 [5])

Cardiovascular manifestations

Cardiac manifestations of AFD may be due to the involvement of any of the cardiac structures, including myocardium, conduction system and valves [12-14]. Data in the Fabry Registry showed that cardiovascular disease was the most common cause of death [15]. In both female and male AFD patients arrhythmia is common and its frequency increases with age [16]. Left ventricular hypertrophy (LVH) is detected in ~ 50% of patients but is less frequent and occurs nearly 10 years later in life among females than in males. In female patients ejection fraction is preserved, and in the absence of myocardial infarction, or arrhythmia, with only palpitations being more common in female FD patients [17], they can have silent but progressive cardiac disease [18,19].

Renal disease

Among all AFD patients renal injury is reported in approximately 50% of patients, proteinuria being the most common renal symptom [20,21]. Data in the Fabry registry show that female patients exhibit significant kidney involvement as manifested by proteinuria and reduced estimated glomerular filtration rate (eGFR). Among female patients with eligible eGFR data 62.5% had eGFR<90ml/min/1.73m2, and 19% had eGFR<60 mL/min/1.73m2. Proteinuria ≥300 mg/day was present in 39.0% of females [7]. Also, female AFD patients have high proteinuria during pregnancy, and a slightly increased prevalence of hypertension and pre-eclampsia in comparison to non-AFD females [17]. Renal biopsy studies have shown that glomerular and vascular changes are present before progression to overt proteinu-

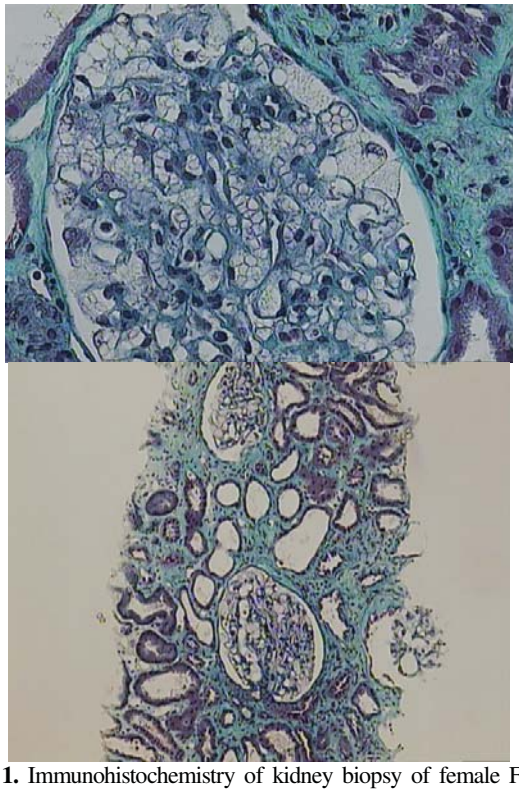

Fig. 1. Immunohistochemistry of kidney biopsy of female Fabry patient. Kidney was used for transplantation as described previously [22]

ria, which makes renal biopsy a potential useful tool for early detection of renal disease. Due to the late onset of renal involvement in female patients and proteinuria being present in less than a quarter of patients, it has been reported in literature that kidneys from a deceased female donor with undiagnosed Fabry disease were used for transplantation [22]. The histological changes from this transplanted Fabry graft are shown in Figure 1. End-stage renal disease (ESRD) is less common in females with AFD than in males. However, female patients develop ESRD approximately at the same age (median 38 years) as male patients [7].

Cerebrovascular and neurological manifestations

Transient ischemic attack (TIA) and stroke are frequently observed in Fabry disease [12,23]. Onset in female patients is later in life than in males. Stroke, mostly ischemic type, occurred in 4.2% of the females in the Fabry Registry, at a median age of 43.5 years. Fabry Outcomes Survey (FOS) registry data indicate that female Fabry patients have higher prevalence of stroke or TIAs of 16% compared to 11% in males [24]. According to Sims, *et al*. most patients (76.9% females and 70.9% males) did not experience renal or cardiac disease before their first stroke and 38.3% of females had their first stroke before being diagnosed with Fabry disease [25]. Isolated hyperintensity in the pulvinar on MRI T1 weighed images, the so-called "pulvinar sign" was shown as a characteristic manifestation of Fabry disease [26,27]. This sign was not found in any female patients [27]. The importance of the pulvinar sign was challenged by Fellgiebel, *et al*. whose study showed that basilary artery diameters were superior to all other MR measures for separating Fabry disease from control with the accuracy of 87% [28].
Neurological symptoms are the most frequently reported symptoms in Fabry disease (occurring in ~ 80% of patients). Pain in hands and feet, acroparesthesia, is a common symptom in AFD females, as well as joint pain [7,17]. Neuropathic pain usually occurs at a mean age of 16-20 years in females [12,29]. Overall prevalence of acroparesthesia is approximately 60% [17]. Hypohidrosis is considered to be a classic feature of Fabry disease; it was present in 29% of female AFD patients and was significantly more common than in control female patients [17]. Data from the Fabry Outcome Survey showed that hyperhidrosis was more prevalent among females than males (11.9% of females vs. 6.4% of males), and is an increasingly recognized feature of AFD [30].

Gastrointestinal symptoms

High prevalence of gastrointestinal symptoms was found in 82% of Fabry females in comparison to 51% controls in a case control survey that was performed in the Netherlands [17]. However, none of the individual symptoms were significantly more prevalent in AFD group in comparison to control group, which was also observed by Wilcox in the Fabry Registry data [17]. Nevertheless, nausea, swallowing difficulties, abdominal pain, diarrhea and constipation were more common in AFD females ($p<0.05$, OR 3 to 5) than in control patients in the case control survey published by Bouwman, *et al.* [17].

Other symptoms

Skin manifestations in AFD include angiokeratomas, teleangiectasias and abnormal sweating which have been previously elaborated. Angiokeratomas are a hallmark of Fabry disease (but are not specific for Fabry disease), 36% of female patients have angiokeratomas [8,31,32]. Angiokeratomas are usually diffuse and located in the lower part of the abdomen, but can also be discrete patches that are evident only by careful clinical examination.
Cornea verticillata is the most common ocular symptom in AFD, which occurs in over 70% of males and females [33].
According to Hegemann, *et al*. hearing in AFD patients is significantly impaired with respect to age-matched general population and this leads to clinically relevant hearing impairment in 16% of cases. Hearing loss is mostly sensorineuronal. Women are affected later in life and less severely than men [34]. There is also evidence in the literature of a positive association between hearing loss and the degree of peripheral neuropathy and cerebrovascular and renal damage [35].
In the case control study published by Bouwamn, *et al*. AFD female patients reported fatigue as the most common symptom (9%), which was in agreement with previous reports [8], although a substantial proportion of normal controls also experienced chronic fatigue (57%), which was significantly prevalent in the AFD females [17].

Diagnosis

A general algorithm for diagnosis of AFD in females is shown in Figure 2. In literature, serum and urine Gb3 concentrations, as well as globotriaosylsphingosine (lyso-Gb3) have been proposed as potential biomarkers [36-38]. By some authors only detection of elevated plasma lyso-Gb3 levels is a reliable biomarker for diagnosis of AFD in female patients [10]. Female patients with a positive family history or with clinical suspicion of AFD should be genetically tested for GLA gene mutations. Sequencing of the entire GLA gene including exon-intron boundaries is considered as the method of choice in detecting GLA mutations by some authors [39,40]. According to Lukas, *et al.* mere sequencing of GLA gene is insufficient in finding some gene mutations, and should be rounded up or replaced with multiplex ligation-dependent probe amplification (MPLA) [10].

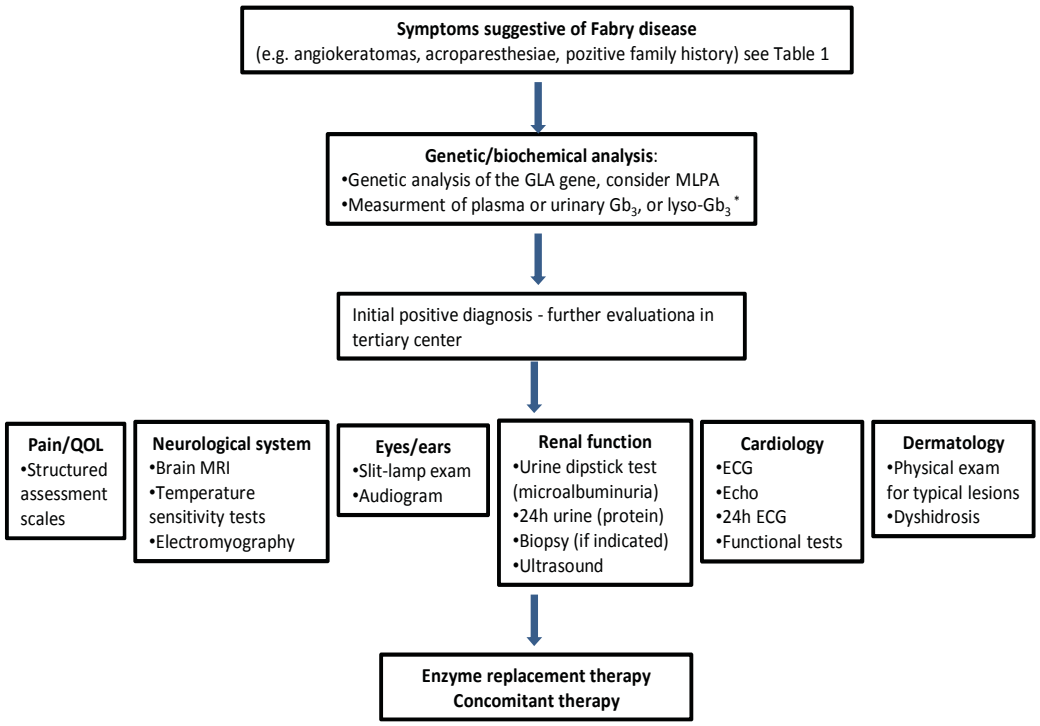

Fig. 2. Algorithm for the diagnosis and assessment of female patients with Fabry disease *some authors do not consider this to be specific for female patients, see text for further details. MLPA multiplex ligation-dependent probe amplification, QOL-quality of life; Adapted from Mehta, *et al. Q J Med* 2010; 103: 641-659

Enzyme replacement therapy (ERT)

Introduction of enzyme replacement therapy in 2001 marked a turning point in clinical management of AFD patients as this was a disease-specific treatment. Before ERT era clinical management was symptomatic and consisted in treatment of pain, cardiac and cerebrovascular complications and ESRD [6].

Two types of ERT are available. Agalasidase beta is produced in Chinese hamster ovary cells and was infused at a dose of 1.0 mg/kg every 2 weeks [41,42]. Agalasidase alfa is purified from a stably transfected human cell line and was infused at a dose of 0.2 mg/kg every 2 weeks. No differences in clinical outcomes could be determined between the two forms of agalasidase in a 24-month treatment [43]. ERT normalizes Gb3 in many different organs in most patients and may be associated with symptomatic benefit.

Treatment should be initiated early, as soon as clinical symptoms and signs are observed [44-46]. United Kingdom and USA guidelines have proposed the following criteria for initiation of ERT, based on evidence of renal disease: persistent proteinuria (>300 mg/24h), and clinically relevant reduction in eGFR (to <90 ml/min/1.73m2) [47,48].

Prospective trials show that initiation of ERT for mild Fabry nephropathy in which GFR is still normal or slightly impaired stabilizes GRF [45,49]. In patients whose GFR was already been compromised, the goal of treatment is stabilization or reduction of GFR rate decline. Observational reports of 8 stage 3 CKD patients (including 4 females) treated with agalasidase alfa showed stabilization of kidney function after 1-2 years of treatment, but in year 3 and 4 GFE decreased by more than 5 ml/min/1.73 m^2 per year in male patients [50]. A prospective, randomized, placebo controlled clinical trial showed no reduction in overt proteinuria with agalasidase beta ERT in patients with advanced Fabry nephropathy (stage 3 CKD) with overt proteinuria and continued decline eGFR [44,45]. Also in men with LVH at base line, it has been reduced after ERT treatment [51,52].

The largest and longest examination of agalasidase alfa therapy on women was described by Whybra, *et al*. They performed a longitudinal study of 36 female Fabry patients treated with agalasidase alpha for four years. This study showed that in female patients "pain at its worst" described by Brief Pain Inventory was significantly reduced after 12 months of therapy and remained reduced through 4 years. Mean left ventricular mass decreased from 89.4±29.3 g/m2 at baseline to 66.5±29.3 g/m2 after 12 months and remained reduced through 4 years. Average kidney function (eGFR and proteinuria) remained constant during the study [51].

Adjuvant therapy

Renal disease

Patients that develop chronic kidney injury require blood pressure, anemia and proteinuria management. Angiotensin converting enzyme (ACE) inhibitors and angiotensin

receptor blockers (ARB) are recognized as mainstay in the therapy of all forms of proteinuric kidney disease. The use of ACE inhibitors and ARBs has been associated with improved outcomes in Fabry disease [53]. Patients that develop end-stage renal disease require renal replacement therapy. Renal transplantation is therapy of choice. Ojo, *et al*. showed that patients with AFD demonstrated equivalent 5-year patient and graft survival compared with controls [54].

Cardiovascular and cerebrovascular disease

Patients with systolic impairment due to cardiovascular manifestations of AFD should be treated with conventional therapy (ACE inhibitors, or ARBs, diuretics). In advanced stages of the disease many patients may require pacemaker implantation due to conduction abnormalities. Those with malignant ventricular arrhythmias may benefit from implantable cardioverter- defibrillator implantation. Patients with advanced heart failure may be candidates for heart transplantation [55].
In primary prevention of stroke along with lifestyle changes, lipid control and blood pressure control, acetylsalicylic acid and other antiplatelet drugs are used.

Neuropathic pain control

Patients should change their lifestyle and take preventive measures to avoid exposure to individual provocateurs of pain. According to available data, there is no randomized controlled trial of analgesic for the treatment of painful peripheral neuropathy in Fabry disease. The treatment of other types of painful neuropathy may serve as a guide for treating the AFD patients. Multiple medications targeting different aspects of the complex pathways might be considered in patients with advanced disease. One should start pain medication(s) at low dose, and evaluate the tolerability and effectiveness of a change in medication(s) after 2-3 weeks. Carbamazepine alone or in combination with pregabalin is recommended as the first-line treatment in Fabry neuropathic pain [56].

Gastrointestinal symptoms

Delayed gastric emptying and dyspeptic symptoms in patients with AFD should be managed with metoclopramide and histamine 2 receptor blockers [47,57].

Prognosis

Without treatment, lifespan is typically reduced by 15 years in women with Fabry disease [2]. Surprisingly according to Fabry Registry data only 53.1% of females with LVH and 46.7% of females with stage 3 CKD or greater are treated with ERT [7].

Conclusions

Clinical presentation in female Fabry patients may range from minor manifestations to a major aggressive form very similar to clinical presentation in male patients. The cardiovascular and renal symptoms are less frequent in female FD patients than in males and they develop later in life. Interestingly, female patients have a greater prevalence of TIA and stroke than male patients, and many of these patients are not diagnosed with Fabry disease at the time of their first stroke. Early diagnosis of Fabry disease is essential. The diagnosis of the disease in female patients is based on genetic analysis of the GLA gene. Screening for Fabry disease should include unexplained end-stage renal disease, hyperthrophic cardiomyopathy and cryptogenic stroke in women. Enzyme replacement therapy and other adjunctive therapy is beneficial for female patients and should be started timely and in a large number of female FD patients.

Conflict of interest statement. None declared.

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Original Article

Antibiotic Sensitivity and Resistance Among the Most Common Uropathogens in Kidney Transplant Recipients

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Abstract

Introduction. Urinary tract infection (UTI) among kidney transplant recipients (KTRs) is one of the most common complications after transplantation. The aim of our study was to analyze the antibiotic sensitivity and resistance of the most common agents causing UTI in Bulgarian KTRs followed up in our Transplant Center.
Methods. We analyzed the antibiotic resistance and sensitivity of the most common strains of bacteria causing UTI in the Bulgarian KTRs, namely class Enterobacteriaceae and Enterococcus spp. We used conventional biochemical methods to identify different strains of uropathogens-miniApi (bioMerieux, France) and BBL Crystal (BD). The antibiotic sensitivity was determined via disc-diffusing method, according to the accepted Bulgarian CLSI standard. We used WHONET, version 5.6 to analyze the antibiotic resistance data.
Results. The total number of tested patients was 366 [males 228, females 138]. The total number of tested urine samples was 829 [positive ones-203), negative samples 606, contaminated 20]. The most commonly detected uropathogens in Bulgarian KTRs were Gram /-/ negative bacteria (63.80%). Of these, 93.28% belonged to the Enterobacteriaceae group, with E. coli, K. pneumoniae and the PPM /Proteus, Providentia, Morganela/subgroup being the most common (54.5%, 19.20% and 16%, respectively).
Gram /+/ positive bacteria were detected in 28.09% of the patients, Enterococcus spp being the most commonly isolated-67.79%. In the Enterococcus group, the strains of E. faecalis and E. faecium were the most commonly detected.
The bacteria belonging to Enterobacteriaceae group were most sensitive to carbapenems and aminoglycosides, with sensitivity peaking to almost 100%, whereas they were least sensitive to aminopenicillines [sensitivity below 20%]. The PPM subgroup revealed very high sensitivity to beta-lactamase protected broad spectrum penicillins (Piperacillin/Tazobactam, sensitivity - 90%).
Gram /+/ positive uropathogens were mostly sensitive to Linezolid, Vancomycin, Teicoplanin (100%). These strains were least sensitive to Erythromycin and Tetracicline (17.50%).
Conclusions. Our results were similar to previous studies. The differences detected can be explained with the characteristics of the bacterial strains and the specific practice of each transplant center. Having in mind the possible complications of UTIs, further studies are needed to clarify the problem with antimicrobial resistance in uropathogens and the use of antibiotics after KT.

Key words: kidney transplantation, urinary tract infections, antibiotics, antibiotic resistance and sensitivity

Introduction

Urinary tract infections (UTIs) are the second most common inflammatory disease in humans, coming after the inflammatory diseases of the lungs [1,2]. This is a non-specific, destructive disease of the renal interstitium, pelvis and the urinary tract due to direct bacterial, viral or mycoplasmal invasion, associated with inflammatory reaction from the patient [1].
The most commonly detected etiologic agents are Gram (-) negative bacteria (E.coli, Klebsiela, Proteus, Pseudomonas, Acinetobacter, Serratia), Gram (+) positive agents-Enterococcus spp., Staphylococcus spp., C. trachomatis; fungi (Candida spp.), viruses, tuberculosis [1-4].
UTIs are one of the most frequent complications of kidney transplantation (KT) with prevalence ranging from 30% to 80%. Urinary infection may cause graft dysfunction and may increase the risk for acute rejection [2-4]. Chronic UTIs in kidney transplant recipients (KTRs) have the same characteristics as those in the early post-transplant period, with a slow and chronic development of the symptoms. The treatment is based on similar principles. However, in KTRs there are certain peculiarities. There is a permanent immunosuppressive therapy in-

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creasing the risk for incomplete remission of the UTIs, especially in association with other risk factors [3-5]. Additional risk factors are female gender, elderly patients, urethral catheter (used routinely within 2 to 3 days after KT) and anatomical abnormalities of the native kidneys or the graft, diabetes mellitus, urologic procedures [4-6]. The etiologic agents are similar to those in the native kidneys in the common population. However, there is a difference in the prevalence of certain bacterial strains. UTIs in the first months after transplantation are associated with severe pyelonephritis, sepsis, and frequent transformation to chronic UTI. Therefore, they should be treated aggressively. Aggressive therapy means not only the bacterial sensitivity, but the equally important treatment duration. These infections are associated with graft dysfunction and increase the risk of acute rejection [4,7,8]. In cases of rapidly developed urinary infections in the early posttransplant period, associated with sepsis or pyelonephritis, intravenous antibiotic treatment must be initiated, followed by oral antimicrobials according to the bacterial sensitivity for 2 to 6 weeks [8-10]. Long-term therapy is also indicated in patients with predisposing factors for UTI (anatomical abnormalities, neuropathic bladder etc.). Outpatient UTIs developed within 3 months after transplantation should be treated with long oral course-up to 6 weeks [6,11,12]. Short antibiotic courses (10 to 14 days) are associated with high incidence of recurrence. Benign UTIs 3 to 6 months after KT have similar prognosis to those in the general population, therefore they can be treated with shorter oral courses (10 to 14 days). Single-dose therapy is not recommended in KTRs as it leads to unsatisfactory results (usually relapse). The presence of predisposing factors for UTI, especially hydronephrosis and urologic manipulation, may be an indication for prophylaxis [9,13,14].
The choice of the most adequate antibiotic is of utmost importance for the outcome of the UTI treatment [2,3,15]. Therefore, the study of the etiologic agents and their sensitivity to the most commonly used antimicrobials are major factors determining the plan of the treating physician. The aim of our study is to present the sensitivity/resistance of the most commonly detected etiologic agents for UTIs after KT to the most frequently used antibiotics.

Material and methods

A total of 366 KTRs, followed-up in our Transplant Center, were enrolled in our study from 1.01.2012 till 31.08.2012. Males predominated (n=228), and 138 were females. The total number of urine samples was 829, positive were 203 (24.29%); 606 were negative samples (73.10%); contamination was detected in 20 samples (2.41%).
The most commonly detected bacteria in Bulgarian KTRs were Gram /-/ negative (63.80%). Of these, 93.28% belonged to the Enterobacteriaceae group; with E. coli, K. pneumoniae and the PPM /Proteus, Providentia, Morganela/subgroup being the most common (prevalence of 54.5%, 19.20% and 16%, respectively). Gram /+/ positive bacteria were detected in 28.09% of the patients, Enterococcus spp being the most commonly isolated-67.79%. In the Enterococcus group, the strains of E. faecalis and E. faecium were the most commonly detected.
The immunosuppressive agents used in the KTRs were steroids, mycophenolate mofetil (MMF), Mycophenolate sodium (M-Na), cyclosporine A, Tacrolimus, Everolimus, Sirolimus in different combinations.
Urinary tract infection was defined as the presence of significant bacteriuria, combined with dysuria, pyrexia, hydronephrosis. Other routine tests were performed in order to optimize the therapy. The treatment was performed in out-patient or in-patient setting according to the individual characteristics of each patient. Additional laboratory and imaging studies were performed in order to assess kidney graft function, viral status (testing for hepatatis B, hepatitis C and cytomegalovirus infection).
Around 210 bacterial strains were isolated. In the cases where a given strain was detected more than once in a given patient the doubling results were excluded from the study.
We used conventional biochemical methods to identify different strains of uropathogens-automatic and semi-automatic biochemical identification systems-miniApi (bioMerieux, France) and BBL Crystal (BD). The antibiotic sensitivity was determined via disc-diffusion method, according to the accepted Bulgarian CLSI standard. We used WHONET, version 5.6 to analyze the antibiotic resistance data [16].
Microsoft Excel was used for statistical analysis. Variation and correlation analyses were applied, together with tables to compare the results and rank analysis to identify outliers.

Results

The total number of tested patients was 366 [228(62.30%) males, 138(37.70%) females]. The total number of tested urine samples was 829 [positive-203(24.29%), negative-606(73.10%), contaminated-20(2.41%)]. The most commonly detected bacteria in KTRs in our study were Gram /-/ negative (63.80%). Of these, 93.28% belonged to the Enterobacteriaceae group, with E. coli, K. pneumoniae and the PPM /Proteus, Providentia, Morganela/subgroup being the most common (prevalence of 54.5%, 19.20% and 16%, respectively).
Gram /+/ positive bacteria were detected in 28.09% of the patients, Enterococcus spp being the most commonly isolated-67.79%. In the Enterococcus group, the strains of E. faecalis and E. faecium were the most commonly detected.

Enterobacteriacee group

The most commonly isolated bacteria in our study (Enterobacteriacee) showed the highest sensitivity (S)

to Imipenem (99.20%), with resistance (R) rate of only 0.80%. Similar results were found for Meropenem (S 99.20%, R 0.80%), and for aminoglycosides (Amikacin, S 93.60%, R-6.40%).
High sensitivity was also detected for β-lactamase protected broad spectrum penicillins (Piperacillin-Tazobactam, S 84.80%, R 15.2%). For the cephalosporins tested (Ceftazidime, Cefotaxim, Cefoxitin) the results were the following: sensitivity 73.6%, 69.6%, 76%, whereas for the resistance rates the results were: 26.4%, 30.4%, 24%, respectively.
The sensitivity to Trimetoprim/Sulfametoxasol was 50.40%, and resistance rate was 49.60%. For ciprofloxacin the figures were 57.6% (S) and 42.40% (R). The least sensitivity in this group was detected for aminopenicillins (S 19.20%, R 80.80%).
The results for Enterobacteriaceae class are summarized in Figure 1.

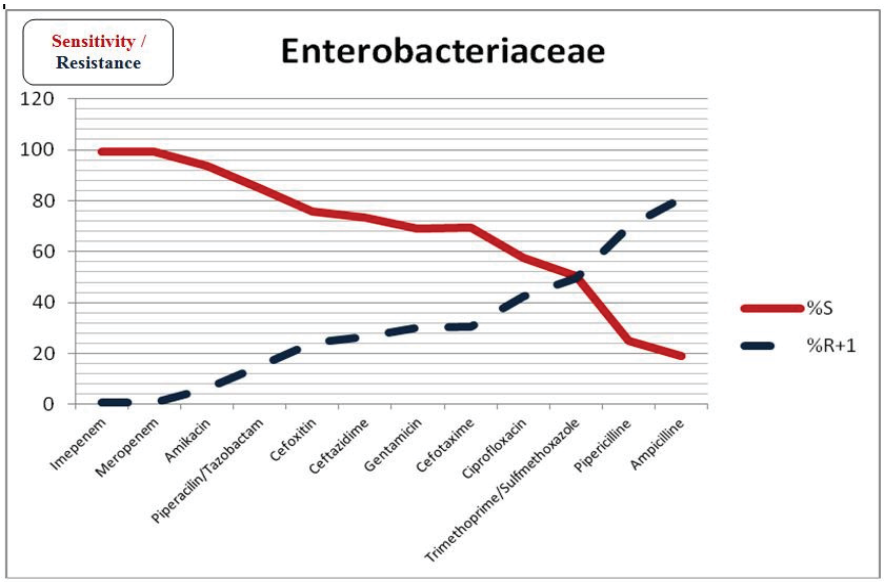

Fig. 1. Antibiotic sensitivity and resistance of the Enterobacteriaceae class

E. coli

E. coli were the most commonly detected members of the class Enterobacteriaceae. These microorganisms revealed 100% sensitivity to carbapenems (Imipenem amd Meropenem). Sensitivity to Amikacin was 97.1%, with resistance rate close to 3%.
High sensitivity was also found for β-lactamase protected broad spectrum penicillins [(Piperacillin/Tazobactam), S 92.6%].
The sensitivity rate of E. coli for second generation cephalosporins (Cefoxitin) peaked to 97.1%, with R of 3%. For Ceftazidime and Cefotaxim the figures were: S 85.3% and 80.9%; R 14.7% and 19.1%, respectively. Trimetoprim/Sulfametoxasol revealed S 64.7%, R 45.6% whereas for Ciprofloxacin E.coli's sensitivity was 64.7% (R 35.3%). The lowest sensitivity rate was established for aminopenicillins [(Ampicillin), S 27.9%, R 72.15].
The results for E. coli antibiotic resistance are depicted in Figure 2.

K. pneumoniae

We established 100% S of K. pneumoniae to carbapenems and 87.5% sensitivity to aminoglycosides (Amikacin). High S was detected also for Cefoxitin (75%, R 25%), moderate S for Piperacillin/Tazobactam (62.5%, R 37.5%). For Ceftazidime and Cefotaxim the sensitivity dropped to 45.8% for both antimicrobials. Similar findings were found for Ciprofloxacin (S 45.8%, R 54.2%) and Trimetoprim/Sulfametoxasol (S 37.5%, R 62.5%). Aminopenicillins (Ampicillin) showed the lowest sensitivity (S 0%, R 100%). The results for K. pneumoniae are shown in Figure 3.

The PPM (Proteus, Providencia, Morganella) subgroup

Again the highest S was detected for carbapenems and Amikacin (S peaking to 95%). Beta-lactamase protected broad spectrum penicillins (Piperacillin/Tazobactam) come second with S 90%, R 10%. For Ceftazidime and Cefotaxim sensitivity rate was 65% and 55%, respectively. Second generation cephalosporins had higher resistance among this subgroup (S 45%, R 55%) compared to third generation cephalosporins. The sensitivity rate dropped further for Ciprofloxacin (S 40%, R60%), Trimetoprim/Sulfametoxasol (S 35%, R 65%), Ampicillin (S 30%, R 70%). The lowest S in PPM subgroup was detected for Tetracycline (S 0%, R 100%). The results are summarized in Figure 4.

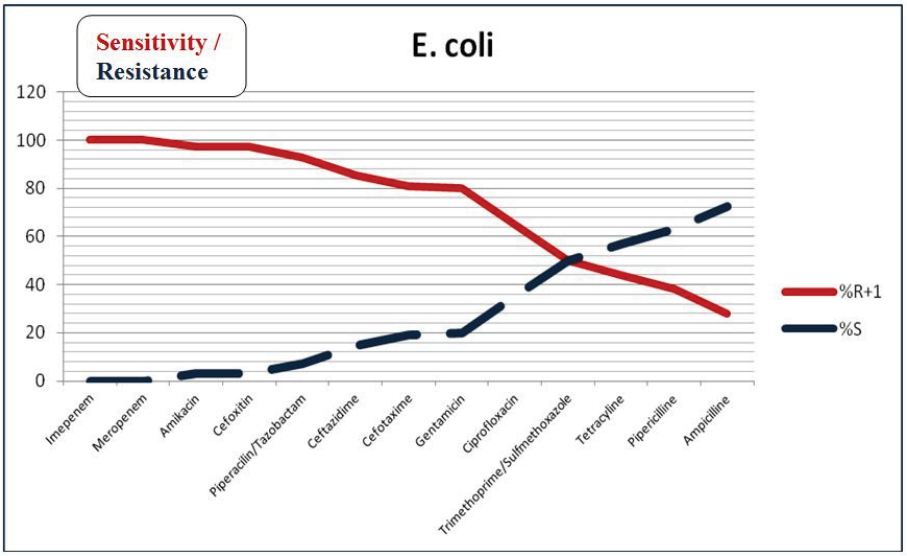

Fig. 2. Antibiotic sensitivity and resistance of E. coli

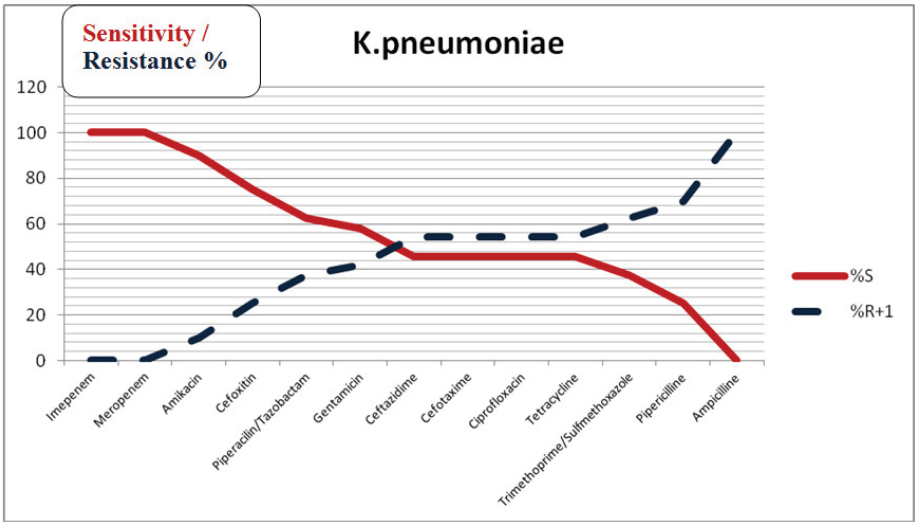

Fig. 3. Antibiotic sensitivity and resistance of K. pneumoniae

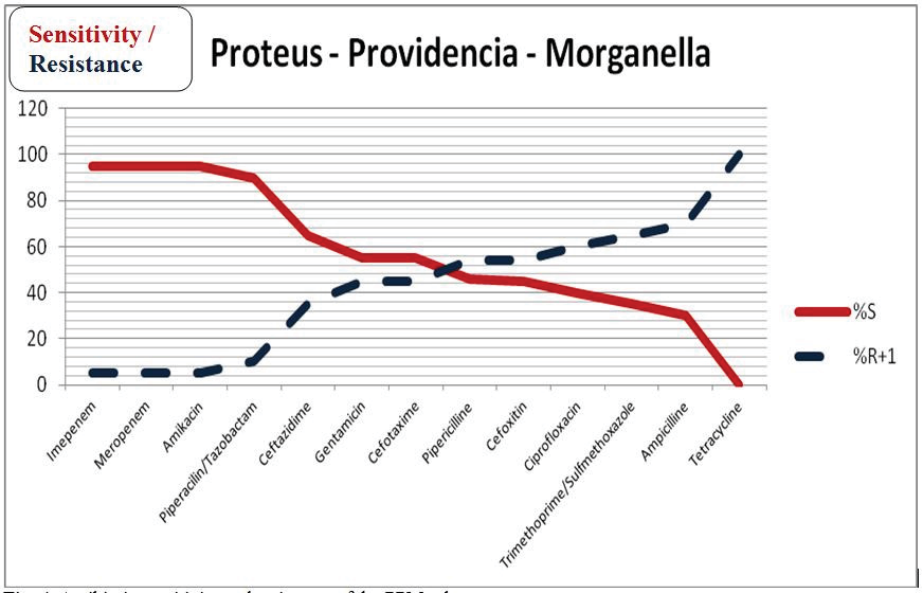

Fig. 4. Antibiotic sensitivity and resistance of the PPM subgroup

Gram /+/ uropathogens

The most commonly detected Gram positive bacteria causing UTIs in our cohort of patients were Enterococcus spp., presented by E. faecalis and E. faecium. They showed highest S to Linezolid, Vankomycin and Teicoplanin (S 100%). Ampicillin came second with S of 80%, R of 20%. Relatively low S was detected for Ciprofloxacin (S 35%, R 65%). The highest resistance was detected for Erythromycin (S 15%, R 85%) and Tetracycline (S below 17.5%). The results for Enterococcus spp are shown in Figure 5.

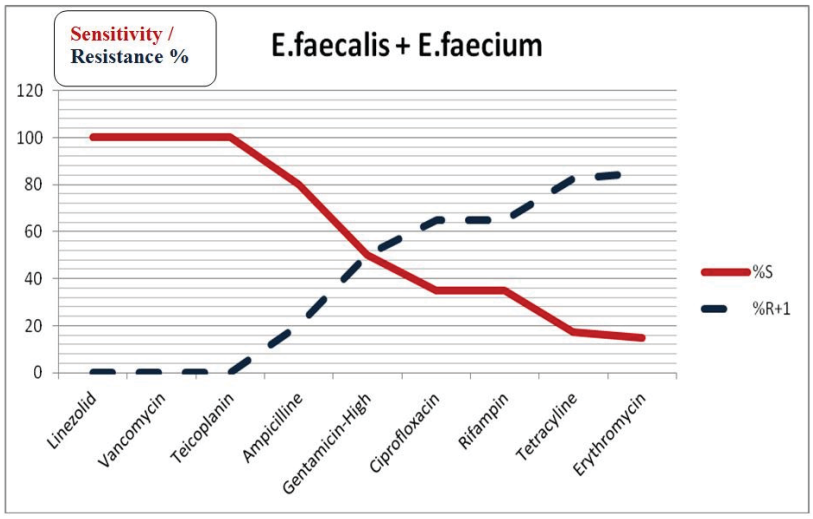

Fig. 5. Antibiotic sensitivity and resistance of E. faecalis and E. faecium

Choosing the best antimicrobial is of vital importance for the outcome in UTIs. Therefore studying of the uropathogens and their sensitivity to the most commonly used antibiotics is fundamental in the treatment strategy of the physician.

UTIs in KTRs may be asymptomatic due to the immunosuppressive treatment, and may evolve to acute pyelonephritis and sepsis, thus making UTIs one of the major factors for decrease in graft function and acute rejection. Therefore, the best choice and the most adequate antibiotic treatment are of utmost importance.

The use of long-term antimicrobial prophylaxis and treatment in KTRs is still under discussion.

Discussion

The urinary tract infections (UTIs) are the most common infections after kidney transplantation (KT). The high prevalence is due to a broad spectrum of factors: immunosuppressive agents, in-dwelling urinary catheters and stents, surgical manipulations, UTIs prior to KT, rejection episodes, cadaver donors.

Our findings confirm the high prevalence of UTIs-24.9% of our KTRs were detected with urinary infection. Our results, however, are lower compared to 30-80% rate established by other authors [2,3,8], which can be explained with the strict follow-up protocol, the prophylaxis and adequate treatment of this type of complication.

The high carbapenem sensitivity detected for the class Enterobacteriaceae bacteria (100%) indicated a low incidence of highly resistant strains. This might be explained by the adequate use of antimicrobials belonging to other classes, thus leaving carbapenems for life threatening, highly resistant UTIs.

The high sensitivity of second, third and fourth generation of cephalosporines coupled with their low cost and low nephrotoxicity [5,7] makes them antibiotics of choice in KTRs, and their utilizing in longer antimicrobial courses.

Gram-negative bacteria (E. coli in particular) had higher sensitivity to second generation cephalosporins than to third and fourth generation. This enables the transplant team to use these antibiotics for treatment of UTIs in the early post-transplant period as well as for oral prophylaxis.

We established medium E. coli sensitivity to Ciprofloxacin (64.7%), thus questioning the idea, that Ciprofloxacin is the antimicrobial of choice in E. coli-caused UTIs [7,8]. Having in mind the high cephalosporin sensitivity of E. coli in our study, we can assume that Ciprofloxacin/cephalosporins can be used in E. coli-UTIs, which definitely broadens our therapeutic armamentarium.

Due to their high nephrotoxicity, aminoglycosydes are used rarely in the treatment of UTIs. This can explain the high sensitivity of Gram-negative uropathogens to Amikacin, peaking up to 90-100%. We firmly believe that aminoglycosides can be used for the treatment of UTIs in KTRs with the adequate dose adjustment and strict follow-up of the kidney function. Our experience proves this approach to be a low risk one. Aminoglycosides offer additional options for antibiotic treatment, reducing further the risk of development of highly resistant bacterial strains.

Our study revealed medium to high resistance of the most common Gram-negative uropathogens to Trimetoprim/Sulfametoxazol (R peaking up to 50%) probably

due to its wide use for Pneumocystis jirovecii prophylaxis. The low sensitivity rate makes the use of Trimetoprim/Sulfametoxazol for UTI prophylaxis highly disputable, in contrast to the recommendations of other authors [9,14,17,18]. However, when indicated, Trimetoprim/ Sulfametoxazol can be used in the routine clinical practice.
The sensitivity to Linezolid, Vancomycin, Teicoplanin among Gram-positive bacteria reached 100%, thus making these antimicrobials the drugs of choice in severe and resistant UTIs after KT.
In addition, high sensitivity to aminopenicillins (80%) was detected in Gram-positive uropathogens. Having in mind their low cost, aminopenicillins can be used as an antibiotic of choice in Gram-positive UTIs. This group is widely used in our daily practice both intravenously and orally.

Conclusions

Our findings were similar to previously reported studies. The differences detected can be explained with the characteristics of the bacteria isolated and the specific practice of each transplant center. Creating protocols for the treatment of UTIs broadens the spectrum of antibiotics of choice and reduces the risk for antibiotic resistance. Further studies are needed to clarify the problem with antimicrobial resistance in uropathogens and the use of antibiotics after KT.

Conflict of interest statement. None declared.

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Original Article

Cystatin C versus creatinine-based GFR formula in CKD patients

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Abstract

Introduction. Glomerular Filtration Rate (GFR) is the main tool to assess kidney function. Some experts suggest cystatin C as a more precise and accurate indicator than creatinine to calculate GFR. This study is designed to assess if cystatin C is more helpful in early diagnosis and better follow-up of Chronic Kidney Disease (CKD) patients who may benefit more from appropriate and timely management.
Methods. We studied 312 patients in different stages of CKD and normal kidney function as control. GFR based on creatinine (Jaffe and enzymatic) and cystatin C were calculated and compared.
Results. A total of 146(46.8%) patients were male with a mean age of 53±17.5 years. The patients were divided into 3 groups based on GFR (>60 cc/min/1.73m^2, 30< GFR<60cc/min/1.73m^2, 15<GFR<30cc/min/1.73m^2). No significant differences in GFR estimation based on creatinine and cystatin C were found.
Conclusions. There were no significant differences between serum cystatin C-based formula and creatinine-based formula for GFR calculation. Therefore, they can be used interchangeably.

Key words: chronic kidney disease, estimated glomerular filtration rate, cystatin C, creatinine

Introduction

Chronic Kidney Disease (CKD) can occur due to different acute or chronic disease conditions. It can occur due to hypotensive attacks in patients undergoing chronic processes such as hypertension and diabetes mellitus [1]. The huge cost of renal replacement therapy (RRT) for health community system is the main reason that health care providers are keen on early detection programs of CKD [2]. Therefore, any more reliable tool than creatinine to assess kidney function, estimated glomerular filtration rate (GFR), to find CKD at an earlier stage to postpone end-stage renal disease and RRT is welcomed [3,4].

GFR is estimated routinely by different creatinine-based formulas like Cockroft-gault (CG) and modification of diet in renal disease (MDRD). Furthermore, other molecules like uthalamate and inulin have been introduced for GFR calculation. However, they have limited popularity because of the expensive and time-consuming process. Recently, serum cystatin C (s-CysC) has been suggested as a more reliable marker than serum creatinine to evaluate GFR [5-7].
Cystatin C is a cystein proteinase inhibitor that is constantly synthesized by all nucleated cells. It can be freely filtrated through glomerulus and then be absorbed without secretion [6,8,9]. There are some unrelated conditions to renal function that may cause serum cystatin C to rise, such as malignancy, thyroid disease, pregnancy and chronic infection [10]. Hejes, *et al*. found that-CysC-based GFR to be more accurate than creatinine-based GFR in patients with GFR <60cc/min/1.73m2 [11]. It is well-known that serum creatinine level is affected by muscle mass, catabolic state, age, gender, diet and medications. Some researchers believe that cystatin C is a better parameter than creatinine for GFR estimation [5,6].
This study was designed to assess the correlation of creatinine-based formula and s-CysC-based formula of GFR calculation in different stages of kidney function and to see if it has a significant impact on timely CKD diagnosis.

Material and methods

The study included patients who were admitted in the Nephrology Ward or the Clinic for CKD management and to other wards or clinics in Imam Khomeini Hospital Complex. They had normal creatinine in 2013 and were consecutively visited and enrolled in the study if they did not have thyroid disease, current infection and malignancy.
Blood samples were collected in order to determine creatinine (Jaffe), creatinine (enzymatic), cystatin C (enzymatic), cholesterol (CHOD Manner with autoanalyzer), triglyceride (PAP manner with auto analyzer), albumin (BCG manner with autoanalyzer), hemoglobin and blood glucose.

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GFR was calculated based on Cys C (CKD-EPI equation) if cys ≤0.8:133× (Scys/0.8)-0.499×0.996-age [0.932 if female] and if cys>0.8: 133× (Scys/0.8)-1.328×0.996-age [0.932 if female]. MDRD formula was used for calculating creatinine:

$$GFR=\text{"}{}^{cc}/_{min/1.73m^2}\text{"} = 186\times[serumCr(^{mg}/_{dl})^{-1.154}]\times[age(year)]^{-0.203}\times$$

[1.212 if Black] × [0.742 if female] and CKD-EPI equation: 144× (S Cr/0.7) $^{-1.209}$ × $(0.993)^{age}$ in females and 141× (S Cr/0.9) $^{-1.209}$ ×$(0.993)^{age}$

Patients were divided into three groups based on their GFR: >60 cc/min/1.73m^2, between 30-60cc/min/1.73m^2, between 15-30cc/min/1.73m^2 and correlation between s-CysC-based formula GFR with creatinine-based formula (Jaffe and enzymatic) GFR were assessed. All statistical analyses were conducted with the software package SPSS, version 15. Student's t-test and ANOVA were used to analyze correlations between variables. P<0.05 was regarded as statistically significant.

Results

A total of 312 patients were enrolled into the study. The mean age of patients was 53±17.5 (14-94) years. Of these, 146 patients were male (46.8%). One hundred and four patients (33.3%) suffered from hypertension and 95 patients (30.4%) had diabetes mellitus. The laboratory data are presented in Table 1.

Table 1. Laboratory data of patients

Laboratory findings	Mean± SD
Cystatin C	1.76±0.53
Jaffe creatinine (mg/dl)	1.37±0.58
Creatinine (mg/dl) enzymatic	0.79±0.32
Triglyceride (mg/dl)	145±84
Cholesterol(mg/dl)	182±44
Albumin (g/dl)	6.7±2.4
TSH	1.2±3.2
Hemoglobin (g/dl)	8.6±5.13
FBS (mg/dl)	60±6.12

Table 2. Mean eGFR calculated by different equations in different stages (Cys-C versus creatinine-based formula)

CKD stages	Cystatin-based eGFR calculation	Creatinine-based eGFR calculation	
	CKD-EPI equation	MDRD standard	CKD-EPI
Normal	96.4±13.7	103.6±15.2	103.90±10.7
60<GFR<90	78.4±16.6	77.4±12.3	75.2±8.7
30<GFR<60	39.9±16.2	44.8±10.3	42.4±8.3
15<GFR<30	26.5±14.7	25.5±4.6	24.1±2.9

The findings showed there were no significant differences in GFR estimation based on each of the mentioned markers (Table 2).

Cystatin C showed positive correlation with age (r=0.420 P<0.001), creatinine (Jaffe method, r=0.694, P<0.001), enzymatic creatinine measurement (r=0.591, P<0.001), triglyceride (r=0.188, P<0.001), and it had negative correlation with cholesterol (r=-0.122, P=0.04). Correlation of GFR calculation based on creatinine and cystatin C was 0.816, p<0.001. The GFR calculated by the two methods of CKD-EPI and MDRD based formula correlated significantly (r=0.995, P<0.001).

Discussion

In this study, accuracy of GFR estimation based on cystatin C or creatinine-based formulas was assessed. The results obtained showed that cystatin C can be used similarly to creatinine for estimating GFR in different stages of kidney function. Kumaresan, *et al*. also investigated this issue. They analyzed CKD patients and measured GFR by ^{99m}TC-DTPA (diethylene triamine penta acetic acid) as the gold standard. They found a significant correlation with cystatin C based-formula GFR calculation (r=0.8, P<0.001). Furthermore, they showed a significant correlation between serum creatinine and serum cystatin C (r=0.6, p>0.001) [12]. Sakaguchi, *et al*. showed correlation of cystatin C with MDRD GFR estimation in CKD patients (r=0.85, P<0.001) [13]. Khorgami, *et al*. found no significant differences between cystatin C-based GFR and creatinine-based GFR in hemodialysis patients. Their study included only hemodialysis patients with mean GFR of about 4-8 cc/min/1.73 m^2. They also showed the importance between the two formulas for GFR estimation: cystatin C (CKD-EPI) and creatinine-based GFR estimation (MDRD), (r=0.51, p<0.001). We studied a larger number of patients and compared GFR in normal and different stages of CKD. Our study also confirmed the previous studies with more solid data in wider range of kidney function [14]. However, Hejes, *et al*. insisted that cystatin C-based GFR formula was more accurate than creatinine-based GFR formula in patients with GFR<60cc/min/m^2 [11]. Soleimani, *et al*. found that creatinine and cystatin C was correlated on the third, seventh and 14th day after kidney transplantation and they showed higher accuracy

of serum cystatin C in renal function assessment the first week after kidney transplantation [15]. It seems that the situation of kidney transplantation looks like an acute process, which is not comparable with stable situation in CKD patients. Shlipak, *et al*. showed serum cystatin C to be more accurate than creatinine to predict cardiovascular accidents, especially when GFR was higher than 60cc/ min/m^2 [16]. We did not include children in our study, but Filler, *et al*. found that cystatin C was as much useful for children under dialysis as was for adults [17].

Conclusions

There were no significant differences between serum cystatin C-based formula and creatinine-based formula for GFR calculation. Therefore, they can be used interchangeably.

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Conflict of interest statement. None declared.

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Original Article

The Fast Peritoneal Equilibration Test First and Second Hour Results

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Abstract

Introduction. The Peritoneal Equilibration Test (PET) is employed to assess peritoneal membrane transport function. The purpose of the test is to determine the optimal peritoneal dialysis regimen. The performance of the test, which is conducted over 4 hours, is time consuming both for the nurses and the patient. There have been studies to validate an approved short version of the original PET protocol, and all have yielded different results. We evaluated the concordance between the 1-hour, 2-hour and 4-hour (classical) test results of the fast PET.
Methods. The study included 32 patients (20 males and 12 females). The patients underwent the 4-hour fast PET test, and the dialysate-to-plasma ratio of creatinine concentration (D/Pcrea) was determined. The standard deviation was added to or subtracted from the mean D/Pcrea ratios at hours 1, 2, and 4 to determine transport groups.
Results. The mean age of the patients was 51.4±16.7 years. Mean D/Pcrea ratios at hours 1, 2, and 4 were 0.41±0.07, 0.54±0.10, and 0.69±0.12, respectively. There was a strong correlation between the 4-hour D/Pcrea ratio and 1-hour ($r=0.756$, $p<0.001$) and 2-hour ($r=0.867$, $p<0.001$) D/Pcrea ratios. Seventeen patients (53%) were in the same transport group at hours 1, 2, and 4. Eighteen patients (56%) at 1 hour and 24 patients (75%) at 2 hours fell into the same transport group at 4 hours. The patients that fell into different transport groups at different time points showed a shift to a lower or higher transport group.
Conclusions. Two-hour fast PET gives promising results for clinical assessment purposes.

Key words: dialysate/plasma creatinine ratio, fast peritoneal equilibration test, peritoneal dialysis, peritoneal transport groups

Introduction

The peritoneal equilibration test is performed to determine peritoneal membrane transport functions in patients on peritoneal dialysis. The test was performed for the first time by Twardowski, *et al*. The aim of the test is to determine the most appropriate peritoneal dialysis regimen [1]. Standard PET is performed using 2.5% glucose peritoneal dialysis solution for a dwell time of 4 hours, and transport ratio of glucose in the dialysate, and transport ratio of creatinine in the blood is calculated. According to the dialysate-to-plasma creatinine ratio and end dialysate-to-initial dialysate ratio of glucose, the patients are divided into four categories of peritoneal permeability as high, high-average, low-average, and low [1,2]. Repeat testing is recommended after an episode of peritonitis, change in the treatment regimen, or in the presence of suspicion for insufficient dialysis, and at least once a year [2,3].
After the introduction of standard PET protocol, fast PET, short PET and modified PET protocols (using 3.5% glucose) have been described for the assessment of PET, and insufficiency of UF [3-6]. Standard PET is the most widely used testing method, and none of the other testing methods has been found superior to the other [2].It is very important that each patient should be followed on the same testing method in the future. Standard PET requires 4 hours to be performed, and it is a time consuming procedure necessitating nursing support and multiple sampling from the dialysate. This consumes nursing time for the patients and brings about loss in the work force. The fast PET requires the analysis of dialysate and plasma samples only at 4 hours [4]. The fast PET protocol therefore becomes less laborious (less work force), requires less sampling and nursing time, and limited use of medical processes without changing the total procedure time.

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There have been studies to validate the approved short version of the original PET protocol, and all have yielded different results. In the present study, the aim was to evaluate the concordance between the 1-hour, 2-hour, and 4-hour test results of the fast PET.

Material and methods

The study was conducted in 32 patients (20 males and 12 females) on peritoneal dialysis who were followed-up in the Peritoneal Dialysis Unit of Adnan Menderes University Faculty of Medicine and who remained stable for the last two months. The patients who sustained an episode of peritonitis within the last 3 months were excluded from the study. The patients were informed of the procedure, and their consent was obtained.

Fast PET Protocol

All patients underwent 4-hour fast PET protocol using 2.27% glucose-containing peritoneal dialysis solution. The fluid remaining in the peritoneal cavity after night-time peritoneal dwell for 8 hours was drained off in the Peritoneal Dialysis Unit. Later, 2 liters of 2.27% glucose dialysis solution was administered into the peritoneal cavity within 10 minutes. Ten ml of dialysate and simultaneous blood samples were obtained at hours 1, 2, and 4 for the analysis of urea, creatinine, and glucose. Dialysate/Plasma creatinine ratios (D/Pcrea) were calculated at hours 1, 2, and 4. Standard deviation (SD) was added to or subtracted from mean D/Pcrea ratios at hours 1, 2, and 4 to determine transport groups. The patients with a D/Pcrea ratio +1 SD higher than the mean value were considered high; patients with a ratio between +1 SD and mean value were considered high-average; patients with a ratio between mean value and -1 SD were considered low-average; and patients with a ratio -1 SD lower than the mean value were considered low-permeable (Table 1).

Table 1. Patients peritoneal transport groups according to 1. 2. vs 4. hour D/P creatinine ratio

Transport groups	1. hour D/Pcrea	2. hour D/Pcrea	4. hour D/Pcrea
Low	<0.34	<0.44	<0.56
Low-average	0.34-0.41	0.44-0.54	0.56-0.69
High-average	0.42-0.49	0.55-0.65	0.70-0.82
High	>0.49	>0.65	>0.82

Statistical analyses

Statistical analyses were performed using the Statistical Package for Social Sciences for Windows, version 17 [SPSS Inc; Chicago, IL, USA].
The Kolmogorov-Smirnov test was used to evaluate if quantitative data had normal distribution. Descriptive statistics included number (n, %) and mean ± standard deviation. Pearson's correlation coefficient was used to evaluate the correlation between the parameters. P values <0.05 were considered significant.

Results

The mean age of the patients was 51.4±16.7 years. The mean duration of peritoneal dialysis was 42.8 months. The most common cause of end-stage renal failure was hypertension (34.4%). The clinical and demographic features of the patients are presented in Table 2.
According to the fast PET results, 4 patients were in the high permeability category, 13 patients were in the high-average permeability category, 11 patients were in the low-average permeability category, and 4 patients were in the low permeability category. The mean D/Pcrea ratios at 1, 2, and 4 hours were 0.41±0.07, 0.54±0.10, and 0.69±0.12, respectively.
There was a strong correlation between 4-hour D/Pcrea ratio and 1-hour (r=0.756, p<0.001) and 2-hour (r=0.867, p<0.001) D/Pcrea ratios (Figure 1). Seventeen patients (53%) remained in the same transport group at hours 1, 2, and 4. Eighteen patients (56%) at 1-hour and 24 patients (75%) at 2-hours fell into the same transport group at 4-hours. The patients that fell into different permeability categories at different time points showed a shift to a lower or higher permeability category (Figure 2). Of the patients that fell into different permeability categories, 6 were male and 2 were female according to the 2-hour test results.

Table 2. Clinical and demographic features of patients

Parameters	Female	Male	Total
Patient number (n, %)	12(%37.5)	20(%62.5)	32(%100)
Age (year)	50.9±22.0	51.7±13.2	51.4±16.7
ESRD etiology			
Hypertension	6(%18.8)	5(%15.6)	11(%34.4)
Diabetes Mellitus	1(%3.1)	6(%18.8)	7(%21.9)
Glomeruloneph ritis	2(%6.3)	2(%6.3)	4(%12.5)
Unknown etiology	1(%3.1)	4(%12.5)	5(%15.6)
Post-renal	2(% 6.2)	3(%9.3)	5(%15,5)
Total	12(%37.5)	20(%62.5)	32(%100)
BMI (kg/m^2)	29.6±7.4	25.6±3.9	27.1±5.7
UF amount (ml)	159.6±202	261.8±229	223.5±221
Kt/V (week)	11.5±12.4	9.1±10.6	10.0±11.2
Peritoneal duration (month)	44.6±37.7	41.7±38.9	42.8±37.9
Treatment regimen (n, %)			
APD	8 (%25)	15 (%46.8)	23 (%72)
CAPD	4 (%12.5)	5 (%15.6)	9 (%28)

Abbreviations; ESRD; end-stage renal disease, BMI; body mass index, UF; ultrafiltration, APD; automated peritoneal dialysis, CAPD; continuous ambulatory peritoneal dialysis

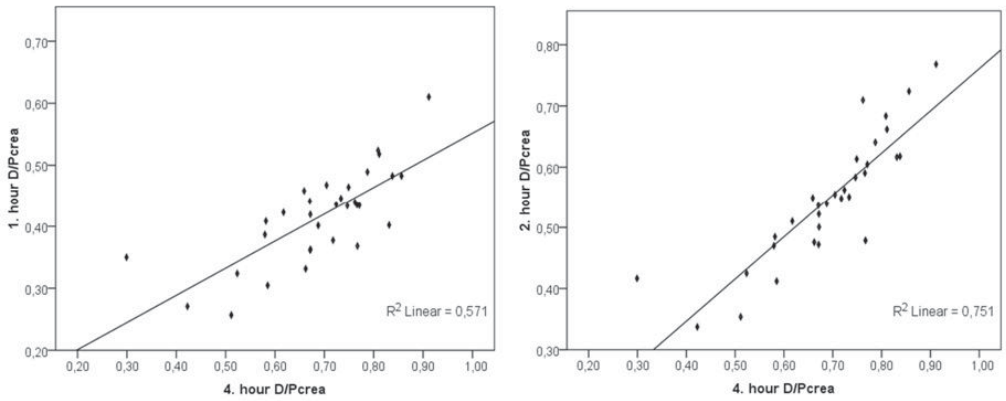

Fig. 1. Correlation between 1. hour, 2. hours and 4. hours D/P creatinine ratio results

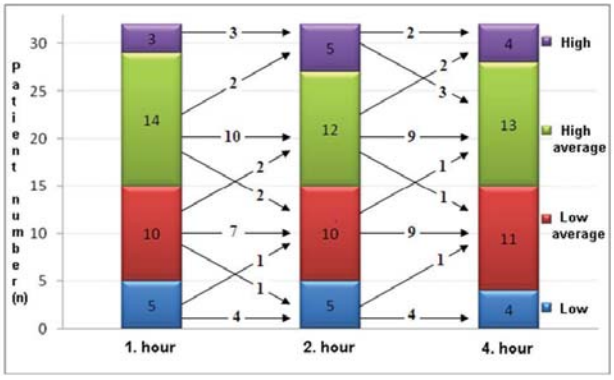

Fig. 2. Patients transport group distribution according to 1. hour, 2. hours and 4. hours D/P creatinine ratio

Discussion

Peritoneal dialysis is one of the renal replacement therapy options in patients with end-stage renal disease. Peritoneal dialysis is a safe and effective treatment modality even in elderly and patients transferred from transplantation or hemodialysis [7,8]. The fast PET was described in 1990 in order to decrease work load and to offer a more practical and easy-to-perform testing method and the test was later validated to assess peritoneal membrane functions [4]. One study comparing standard and fast PET reported a 94% similarity between the results of the two testing procedures, and they suggested that fast PET provided fast, practical, and ready assessment of the peritoneal permeability [9]. The studies comparing the results of the standard and the fast PET have reported a concordance rate of 80 to 100% between dwell times of 2 hours and 4 hours. In conclusion, dwell time of 2 hours provides reliable results and time-saving procedure for the health care personnel and it could be used for the clinical assessment of the patients [5,10-12]. In contrast, a study in children found no correlation between 2-hour and 4-hour dwell times and the authors reported that short testing procedure would not be reliable in pediatric population for the assessment of peritoneal membrane functions [13].

There is no study in the literature that validated the 2-hour fast PET protocol. In this study, we attempted to reduce the dwell time for the fast PET, which is routinely used at our Clinic. The fast PET is a simple and useful testing method and reducing the dwell time to 2 hours would be time-saving both for the patient and the operating nurses. In the present study, 2-hour test results showed 75% concordance with the fast PET. The patients that had a change in the permeability category showed a shift to a lower and higher permeability category. The current results are similar with the 2-hour results of the standard PET protocol.

Conclusions

In conclusion, in transport groups determined according to D/Pcrea ratio, 56% of the patients at 1 hour and 75 % of the patients at 2 hours fell into the same transport group at 4 hours. Two-hour fast PET gives promising results that have to be confirmed in the future in studies comprising a larger number of patients. Two-hour fast PET offers a simple and practical testing method, and it seems applicable to avoid loss of time.

Conflict of interest statement. None declared.

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Original Article

Low Serum Zinc Level May be Related to Higher Doses of EPO in Hemodialysis Patients

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Abstract

Introduction. Anemia is a complication of chronic renal failure observed in patients on hemodialysis (HD) affecting morbidity and mortality of these patients. It is associated with erythropoietin (EPO) deficiency and can be treated by human recombinant erythropoietin (hrEPO). Iron deficiency has been reported as the first line cause for inadequate response to this treatment. Zinc deficiency in hemodialysis (HD) patients was previously reported and the relationship between iron and zinc deficiencies has been known for years. The aim of this study was to find out the relationship between serum zinc level, anemia and rhEPO consuming in HD patients.

Methods. A total of 69 HD patients and 34 healthy individuals were included in the study. Serum zinc levels, clinical, hematologic, biochemical parameters and rhEPO doses were evaluated.

Results. Serum zinc levels were found to be lower in HD patients in comparison to those in the control group (29.92±12.94, 44.82±27.69μg/dL, respectively) ($p<0.001$). There was a positive correlation between serum zinc and hemoglobin (Hb) in the control group ($R^2=0.06$). In HD patients who needed less then 8000U/week rhEPO, Hb levels ($p<0.05$) and serum zinc levels were higher. Serum zinc and Hb levels were found to be higher in patients who were under HD treatment for more than 12 months (10.05±1.06 vs 10.69±1.29; $p=0.022$ and 26.96±13.29 vs 30.88±12.61; $p=0.178$).

Conclusions. HD patients who needed lower dose of EPO had higher serum zinc levels. Although the difference was not statistically significant, these results provide evidence that serum zinc level should be taken into consideration especially in HD patients resistant to EPO therapy.

Key words: serum zinc level, hemodialysis, anemia, erythropoietin

Introduction

The prevalence of anemia in patients with chronic kidney disease is reported to be approximately 50% [1]. In patients on hemodialysis (HD) treatment, a decrease of hemoglobin level by 1g/dl was found to be associated with left ventricular (LV) hypertrophy by 42%, which increases mortality and morbidity [1-3]. The most significant cause of anemia is erythropoietin deficiency due to decreased production of this hormone from kidney tissue [3-5]. The discovery of recombinant human erythropoietin (rhEPO) was a cornerstone in the management of patients with chronic kidney disease [4] and increased their quality of life tremendously. However, this therapy increases the medical expenses whereas some patients do not respond as expected. Iron deficiency, severe secondary hyperparathyroidism, hypo or hyperthyroidism, infectious diseases and some other comorbidities have been implicated as causes of inadequate response to rhEPO [6]. Nevertheless, malnutrition, hypoproteinemia, malabsorption and exposure to large a amount of dialysis solution which does not contain any trace elements might be related to zinc and other trace elements deficiency in HD patients [7]. The relationship between zinc deficiency and hypochrome microcytic anemia is well known since 1960's [8]. In this study, the potential relationship of zinc deficiency to the severity of anemia and to inefficient response to EPO treatment in HD patients has been investigated.

Material and methods

Ninety-six patients with End-Stage Renal Disease (ESRD) (46 males, 50 females, with mean age 56±14 years and HD duration for 44.52±38.00 months) undergoing maintenance HD three times a week, four hours each session, were included in this study. HD treatment was performed using Polysulphone FX60 and FX80 high-flux membranes in Fresenius trademarked 4008 S type machines. The bicarbonate-based dialysate did not contain any zinc supplement. Iron supplementation was performed routinely at

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least once a week to each patient unless a contraindication existed. EPO treatment to each patient was also performed to maintain hemoglobin level between 11-12 g/dL. Samples were drawn before the first HD session of the week. In addition to this group, 34 (14 males, 20 females; mean age 53±10 years) healthy persons were studied as a control group. In addition to demographic, clinical features of these patients and CBC (Combined Blood Count), other hematologic parameters, including serum iron, TIBC (Total Iron Binding Capacity), transferrin saturation, serum B12, ferritin, folate level and zinc levels in both groups were also obtained. Patients with known malignancy, infectious diseases, endocrine abnormalities, gastric problems as well as patients under the age of 18 were excluded from the study.

Appropriate methodology was used for the hematologic and biochemical parameters. The serum zinc level was determined quantitatively by using Inductive Comparable Plasma Optic Emission Spectrophotometric (ICP-AES) colorimetric method and on 206.200 nM wavelength [9].

Kocaeli University Ethicsl Committee approved that this study was in accordance with the ethical standards of the Committee on human experimentation with the Declaration of Helsinki and its revisions. Oral inform consents were obtained from patients and control subjects.

Statistical analysis

For the statistical analysis SPSS 15.0 program (SPSS Inc., Chicago Ill., USA) was used. All data are displayed as mean ± standard deviation. Student's t-test was applied to compare means of continuous data and data with a normal distribution, otherwise Mann-Whitney U test was used. Variables were compared using the Chi-square test, and Pearson's regression test was used to examine correlation between zinc concentration and hematologic data.

Results

Age, body mass index and gender were compared between HD patients and healthy subjects (Table 1). The mean zinc level was found to be lower in patients undergoing HD in comparison to that observed in the control group (29.92±12.94/ 44.82±27.69; p<0.001) (Table 1). In HD patients no statistically significant correlation was observed between serum zinc level and hemoglobin (Hb) (p>0.05) or hematocrit (Hct) levels (p>0.05). However, serum zinc and Hb levels were positively correlated in the control group (R^2=0.06) (Figure 1). When the patients were evaluated in terms of rhEPO doses, in the group who needed less than 8000IU rhEPO, mean Hb level was significantly higher and serum zinc level was also higher (Table 2), but this finding was not statistically significant. However, a positive but week correlation between Hb and serum zinc levels (R=0,003) was observed in this group (Figure 1). Patients undergoing HD treatment for more than 12 months had higher Hb, Hct and serum zinc levels than patients whose dialysis duration was less than 12 months (30.88±12.61 and 26.96±13.29, respectively) (Table 3).

Table 1. Demographic, clinical and biochemical features of patients and control subjects

	Patients (n=96)	Controls (n=34)	p
Age	55.98±13.95	53.15±10.56	0.290
Gender (F/M)	50/46	20/14	0.687
BMI(kg/m²)	26.88±5.22	28.78±5.07	0.320
WBC(X10³/uL)	6.69±2.47	7.184±1.87	0.297
Hb (g/dL)	10.51±1.25	13.92±1.64	<0.001
Hct (%)	33.49±3.77	41.50±4.60	<0.001
MCV (fL)	92.34±5.23	88.12±5.67	<0.001
PLT ($X10^3$/mL)	200.27±72.68	287.94±67.92	<0.001
Ferrum (mcg/dL)	46.64±17.61	78.50±34.98	0.006
Ferritin (ng/mL)	719.09±92.66	351.73±87.11	<0.001
TIBC (mcg/dL)	178.90±41.06	336.78±58.52	<0.001
Transferin saturation (%)	26.71±9.18	23.88±10.50	0.43
Vitamin B12 (pg/mL)	1497.81±2415.19	322.94±134.59	0.007
Folic acid (ng/mL)	48.14±79.75	14.84±35.69	0.066
ESR (mm/h)	34.70±13.91	16.64±12.54	<0.001
CRP (mg/dL)	1.67±1.46	1.22±2.44	0.565
Glucose (mg/dL)	136.99±87.47	103.64±68.62	0.051
Urea (mg/dL)	141.22±36.28	30.31±8.67	<0.001
Creatinin (mg/dL)	7.60±2.52	0.74±0.13	<0.001
Total protein (g/dL)	6.61±0.60	7.394 ± 0.50	<0.001
Calcium (mg/dL)	8.50±0.79	9.61 ± 0.56	<0.001
Phosphate (mg/dL)	5.27±1.41	3.38 ± 0.57	<0.001
Magnesium (mg/dL)	2.37±0.34	2.18± 0.17	0.021
Zinc (µg/dL)	29.92±12.94	44.82±27.69	<0.001

BMI=body mass index; WBC= white blood cell count; Hb= hemoglobin; Hct= hematocrit; MCV=mean corpuscular volume; PLT= platelet; TIBC= total iron binding capacity; ESR= erythrocyte sedimentation rate; CRP=C-reactive protein

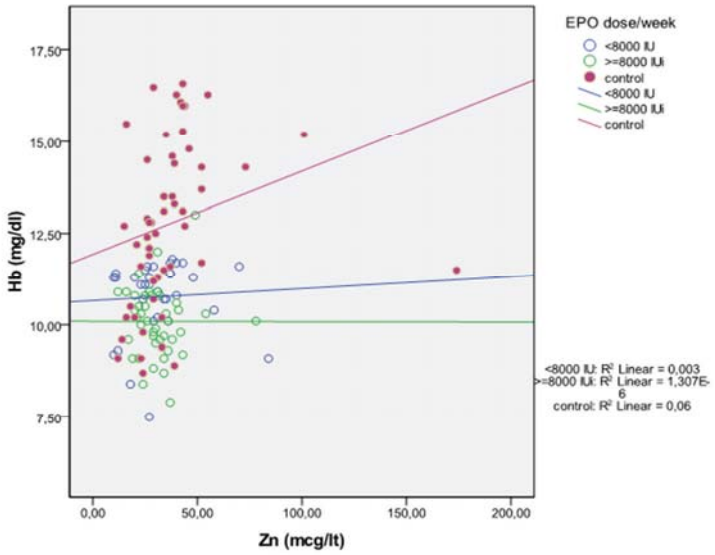

Fig. 1. Relationship between EPO dose, Hb and serum zinc level in control subjects and HD patients

Table 2. Differences between patients who needed higher and lower EPO dose

n	≥8000IU/week 42	<8000IU/week 33	p
Age	58,30±13,12	56,44±17,07	0,595
Dialysis duration (month)	46,69±29,96	41,69±33,64	0,409
Hb (g/dL)	10,09±0,94	10,77±1,03	<0,001
Hct (%)	32,64±2,80	33,93±3,41	0,078
MCV (fL)	91,16±5,63	94,08±4,80	0,020
Hb≤10g/dL	42/19(45%)	33/5(15%)	<0,001
Zn (µg/dL)	30,86±11,60	31,91±16,23	0,852
Ferrum (mcg/dL)	34.25±11.58	56.50±16.78	0.033
TIBC (mcg/dL)	151.00±40.14	195.33±37.74	0.201
Trasferrin saturation (%)	23.92±9.35	29.81±9.34	0.201
Ferritin (ng/mL)	831.95±512.02	639.07±268.49	0.522

Table 3. Hemodialysis duration shorter and longer than 12 months

	HD duration ≤12months	HD duration >12months	p
Zinc (µg/dL)	26.96±13.29	30.88±12.61	0.178
Hb (g/dL)	10.05±1.06	10.69±1.29	0.022
Hct (%)	32.25±3.51	34.01±3.81	0.038
rhEPO dose (IU/week)	7726±3240	8316±4668	0.533
T.protein (mg/dL)	6.69±0.63	6.56±0.59	0.358
Albumin (mg/dL)	3.68±0.57	3.78±0.46	0.422
Phosphate (mg/dL)	5.03±1.33	5.44±1.36	0.158

Discussion

Treating anemia in chronic kidney disease (CKD) is very important since cardiovascular mortality, morbidity and quality of life of these patients are very much related to better management of anemia. Human rhEPO has been used successfully for years in order to treat anemia in patients with CKD [10]. However, in some cases rhEPO failed to correct anemia for several reasons. The most important cause of inefficient response to rhEPO treatment is iron deficiency. The relationship between iron deficiency anemia and zinc deficiency was pointed out in 1961 by Prasad, *et al.* [11] in a child characterized by geophagia, iron deficiency anemia, hepatosplenomegaly, hypogonadism and dwarfism [12]. They claimed that consumption of a large amount of phythate rich grain could inhibit the absorption of both iron and zinc leading to iron deficiency anemia that does not respond to iron therapy unless if this is combined with zinc supplementation [12,13].

Although there is a variation in zinc levels depending on the geographic origin of patients, it has been reported that zinc level in the Turkish population is generally low. For example, zinc levels have been found to be 91.34 µg/dl in the south region of Turkey and 64.22 µg/dl in the east region of the country [14,15].

In this study, the mean serum zinc levels were 44.82± 27.69 µg/dl in the control group. The control subjects were from the northwest part of the country, which is well developped socio-economically. Thus, low zinc levels were not expected in this group of subjects. This result was considered to be related to their nutrition and/or the method used in this study for determination of serum zinc level.

It has been reported that trace elements, especially serum zinc levels were found to be lower in HD patients [15-17]. A marked decrease in serum zinc levels has been reported in CKD patients despite normal zinc levels in many tissues, which means that it may be due to re-distribution rather than total body deficiency [18]. Zinc deficiency in patients with CKD may be related to protein res-

tricted diet, malnutrition, hypoproteinemia, proteinuria, failure of tubular reabsorption, impairment in the formation of 1.25-dihydroxycholechalsiferol which plays a role in the intestinal zinc absorption. Furthermore, it has been discussed that HD patients are exposed to large amounts of highly purified dialysis solutions which do not contain zinc or other essential trace elements (manganese, copper, selenium). The removal of these elements with HD may lead to clinically relevant deficiency [19]. As a controversy to this theory, serum zinc level was lower in patients who were under HD treatment for less than 12 months and increased over time which was interpreted as zinc removal from these patients through the dialysis solution that is not significant in long term. Also, an efficient dialysis and rhEPO therapy corrects patient's nutritional status and mending zinc deficiency (Table 3).

Furthermore, it has been documented that red blood cell (RBC) survival is markedly reduced in patients with chronic renal failure [20,21]. There is extensive experimental evidence suggesting that uremia affects the mechanical properties of RBCs, such as deformability and fragility [20]. Zinc is an essential trace element, a structural and functional constituent of several enzymes which have important roles in the metabolism of nucleic acids, proteins and carbohydrates. It has been suggested that zinc also plays a significant role in the structure and function of biological membranes [21]. Therefore, it can be postulated that zinc deficiency in HD patients might contribute to some degree to the development of anemia in these patients.

In our study group, the serum zinc level of HD patients was 29.92±12.94 μg/dl, which was significantly lower than in the control group (Table 1, $p<0.05$). This finding is very similar to that reported in literature. It has been previously reported that there is a significant positive correlation between anemia parameters and serum zinc levels [22,23].

In this study HD patients were treated with a mean dose of rhEPO of 8077±33.73 U /week. We have observed that patients who used less than 8000 U/week dose of EPO had statistically significant higher Hb and serum zinc levels (Table 2). This finding was interpreted that the patient with a high serum zinc level would also have higher Hb levels. Thus, patients with higher serum zinc level would require less rhEPO doses to maintain the desired Hb level. Even though every patient received iron supplementation, those who needed EPO doses more than 8000U/week had a lower serum iron level as well as a lower serum zinc level. Consequently, serum zinc and iron levels were related to each other, and if there was a zinc deficiency, it would be difficult to correct iron parameters and to receive better results to EPO treatment [11-13].

There was a significant positive correlation between Hb and serum zinc level in the control group. In contrast, no correlation was detected among HD patients, which might be due to increasing Hb levels by using more rhEPO. Further studies should be planned to investigate the consequences of trace element deficiencies in HD patients and their effect on rhEPO and other treatments. Based on these observations, zinc deficiency may occur in HD patients more often due to their protein restricted nutrition and malnutrition because of uremic environment. When zinc deficiency develops, treatment of anemia becomes more difficult; patients need more amount of EPO to maintain the desired Hb level. In addition, treating the anemia complications increases the cost. When treating anemia in HD patients, it is important to take into consideration malnutrition and deficiency of trace elements, especially zinc along with many other factors.

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Conflict of interest statement. None declared.

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Original Article

Alexithymia Construct in Dialysis Patients

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Abstract

Introduction. The concept of alexithymia means dysfunction in emotional awareness, social attachment, and interpersonal relating. The study was performed to evaluate the alexithymia construct in patients treated with chronic maintenance haemodialysis.
Methods. TAS-20 was applied as a measure of alexithymia to a group of 230 patients, mean age 55.5±13.5 years, recruited from three dialysis centers.
Results. The results obtained showed that 50% of patients were alexithymic, and 18% had possible alexithymia. A small positive correlation was shown between age and obtained scores for alexithymia (r=0.025). Duration of dialysis also positively influenced the alexithymia scores (r=0.013). In addition, the duration of dialysis was significantly influenced by age (ANOVA 0.004, p<0.05). Factors analysis showed that F1 and F2 were not influenced by age or duration of dialysis. Only factor F3 (externally oriented thinking) was very perceptible and influenced by the age and the duration of dialysis (ANOVA p=0.016; <0.05).
No significant differences in scores between males and females were obtained. Only F1 was higher in males (p<0.05). The scores obtained for alexithymia were compared between healthy population and cancer and dialysis patients. Patients with chronic diseases were more alexithymic than healthy people (p< 0.05).
Conclusions. The alexithymia construct is a permanent personality trait related to neurobiological brain specifics. An alexithymia construct can influence the prognosis and outcome of dialysis patients as well as of other diseases. The psychological support for mediating alexithymia should be included in the therapeutic protocols, especially for end-stage renal diseases.

Key words: alexithymia, dialysis, end stage renal diseases

Introduction

Alexithymia (from the Ancient Greek word-e ɐɪlɛksə ‘θaɪmiə) refers to impairment of the ability to identify and describe one's own feelings and emotions. The core characteristics of alexithymia are marked dysfunction in emotional awareness, social attachment, and interpersonal relating. Furthermore, individuals suffering from alexithymia have difficulty in distinguishing and appreciating the emotions of others, which is thought to lead to unempathic and ineffective emotional responding. It is supposed that alexithymia is prevalent in approximately 10% of the general population and it is known to be comorbid with a number of psychiatric conditions.
The term and concept of alexithymia originally referred to a personality trait of psychosomatic patients. It was supposed that the poorer the capacity of a person to experience feelings and to express them verbally, the more the individual is liable to develop somatic symptoms in an emotionally stressful situation [1-3]. The construct was explicated on the basis of clinical observations of patients with classical psychosomatic diseases who manifested an externally oriented cognitive style and an inability to describe and differentiate feelings and to create fantasies. Many further studies confirmed that the clinical feature of the construct had been observed not only in psychosomatic patients but also among patients with post-traumatic stress disorders, patients with substance use disorders and patients with somatoform disorders [3,4].
Some cases of alexithymia are neurological, meaning that it is caused by a deficiency in the brain pathways that process emotions. Others develop psychological alexithymia as a self-defense measure against emotionally indigestible situations, such as terminal illness, or post-traumatic stress disorder. As a coping mechanism, the mind simply shuts down the pathways that process emotions, resulting in a stoic, emotionless state. This type of alexithymia could be reversible through psychotherapeutic interventions, and sometimes with the help of anti-depressants.
Since the formulation of the alexithymia construct in the mid-1970s, there has been controversy over its measurement. Several measures have been developed, including observer-rated questionnaires, self-report scales, projective techniques, etc. Although many investigators have used the Rorschach and/or the Thematic Apperception

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Test (TAT) to assess various facets of the alexithymia construct, there is little empirical support for the reliability and validity of these methods. The self-report Twenty-Item Toronto Alexithymia Scale [5-8] has become the most widely used test for the alexithymia construct.
One of the more challenging problems in the assessment of a personality trait, hypothesized to be a vulnerability or risk factor for certain medical or psychiatric illnesses, is to ensure that its measurement is not confounded by the state effects of the illness. Some investigators have argued that the presence of alexithymia may merely reflect a concomitant state reaction to an illness, which may be predicted by anxiety state, a depressed mood, or poorer quality of life, and lessens over time as the illness improves [9]. However, several longitudinal studies have yielded strong support for alexithymia being a stable trait that is independent of psychological distress or other effects of a medical or psychiatric illness.
Depression was used as the "disease" state on which the stability of the alexithymia construct was examined. Several studies with clinical or nonclinical populations have reported positive and significant relationships between the TAS-20 and measures of depression. It was hypothesized that depression and alexithymia would be correlated at both treatment initiation (baseline) and at follow-up (treatment completion), and that both constructs would show significant reductions from baseline to treatment completion [9,10].
In our previous article we discussed depression and personality profiles measured by the Beck Depression Inventory and MMPI-201 in dialysis patients [11,12] and concluded that depression is usually under-diagnosed even it is frequently present as comorbid in these patients.
The aim of this study was to evaluate alexithymia as a specific personality construct in patients treated with chronic maintenance haemodialysis.

Material and methods

In this study we evaluated 230 patients recruited from three state dialysis centers in the Republic of Macedonia (two in Skopje and one in Struga). The patients were randomly selected. The psychological evaluation was made during the process of dialysis (mainly within three hours/day). The 20-item Toronto Alexithymia Scale (TAS-20) was used to measure alexithymia.
The Twenty-Item Toronto Alexithymia Scale (TAS-20) was developed by Bagby, *et al.* (1994) and is a revised version of the earlier 26-item Toronto Alexithymia Scale (TAS; Taylor, Ryan, & Bagby, 1985). As a psychometric instrument, the TAS-20 has demonstrated good internal consistency and test-retest reliability. In the initial validation study, exploratory factor analysis of the TAS-20 yielded a three-factor structure congruent with the theoretical construct of alexithymia: (F1) difficulty identifying feelings and distinguishing between feelings and the bodily sensations of emotional arousal; (F2) difficulty describing feelings to others; (F3) externally-oriented thinking.
Items are rated using a 5-point Likert scale whereby 1=strongly disagree and 5=strongly agree. The TAS-20 uses cut-off scoring: equal to or less than 51 indicates non-alexithymia, equal to or greater than 61 indicates alexithymia. Scores of 52 to 60 indicates possible alexithymia.
The three-factor structure was found to be theoretically congruent with the alexithymia construct. In addition, it has been found to be stable and replicable across clinical and nonclinical populations. The TAS-20 has been translated into many languages, including Macedonian, using the method of back translation to establish cross-language equivalence [13].
The statistical evaluation was performed using the software package IBM SPSS Statistics 21. For descriptive purposes, mean values and standard deviation of continuous variables and percentage for categorical variables are presented. Correlations were calculated using the Student's t-test, and regression analysis (ANOVA) was calculated to determine significant differences. Statistical significance was taken at $p \leq 5\%$ level.

Results

Of the total sample comprising 230 patients, 110 were females (mean age 55.5±13.5 years), and 120 males (mean age 54.5±14.3 years).
The mean duration of maintenance haemodialysis was 8.3±5.8 years (Figure 1).

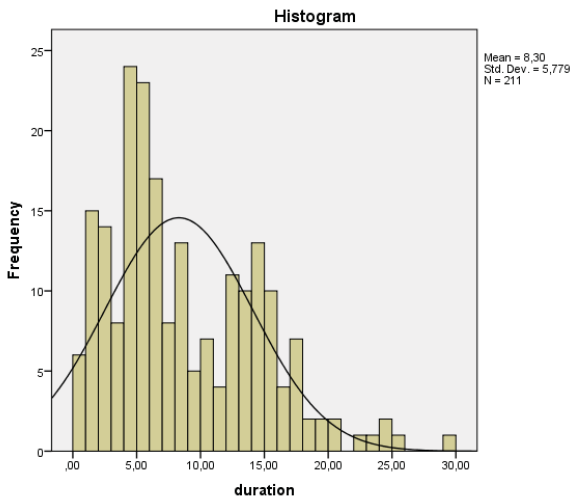

Fig. 1. Duration of haemodialysis in the analyzed group

The level of education was as follows: 51% had completed primary education; 43% had completed secondary school; 6% had a university degree. The TAS-20 was filled-in by patients themselves.
The results obtained for TAS-20 were as follows: 50% showed alexithymia, 18% possible alexithymia, while 32% were no alexithymic (Figure 2). This means that the alexithymia construct appeared to be a very frequent personality trait in patients treated with dialysis.

The duration of dialysis appeared to be slightly positively correlated with total scores for TAS 20 (r = 0.013) (Figure3).

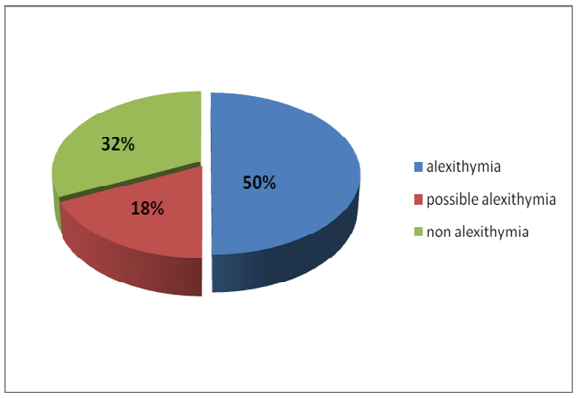

Fig. 2. Obtained results for alexithymia

Concerning the correlation between age and scores obtained for alexithymia, regression analysis showed a small positive correlation (r=0.025). ANOVA was p=0.721, (>0.05) which means that age could not be a predictor for scores obtained for TAS-20.

However, the duration of dialysis was significantly influenced by the age (ANOVA 0.004; p<0.05), as presented in Figure 4.

Factors analysis showed that F1 and F2 were not influenced by age or duration of dialysis. Only factor F3 (externally oriented thinking) was very perceptible and influenced by age and by duration of dialysis (ANOVA p = 0.016; <0. 05) (Figures 5 and 6).

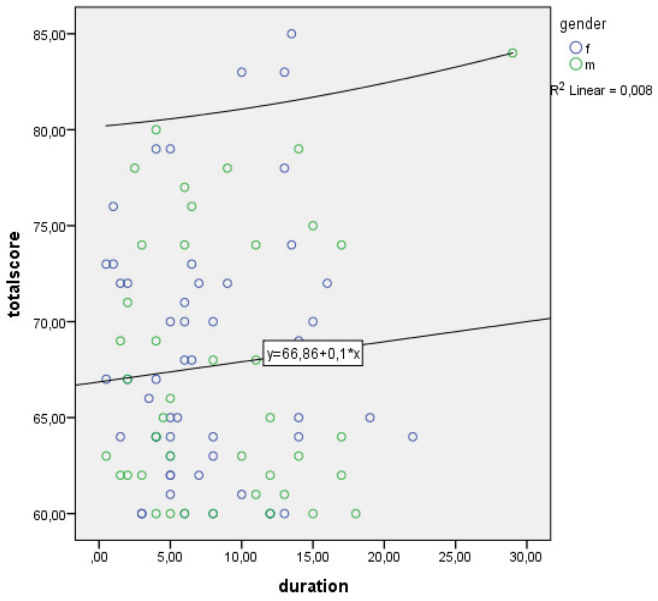

Fig. 3. Duration of dialysis and alexithymia scores

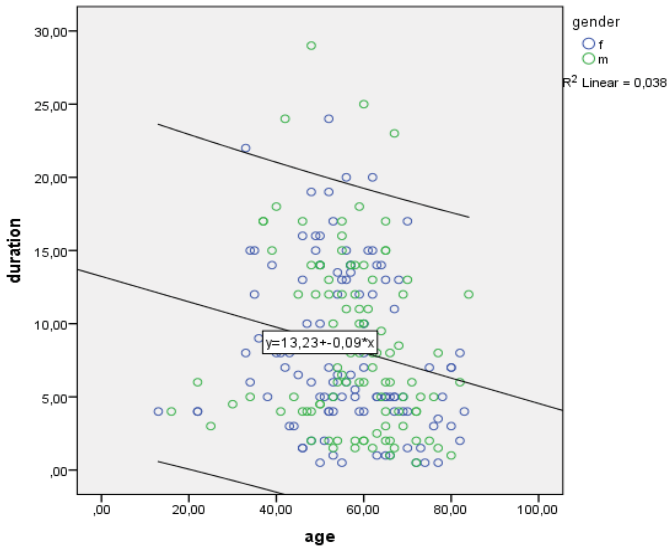

Fig. 4. Duration of haemodialysis and age

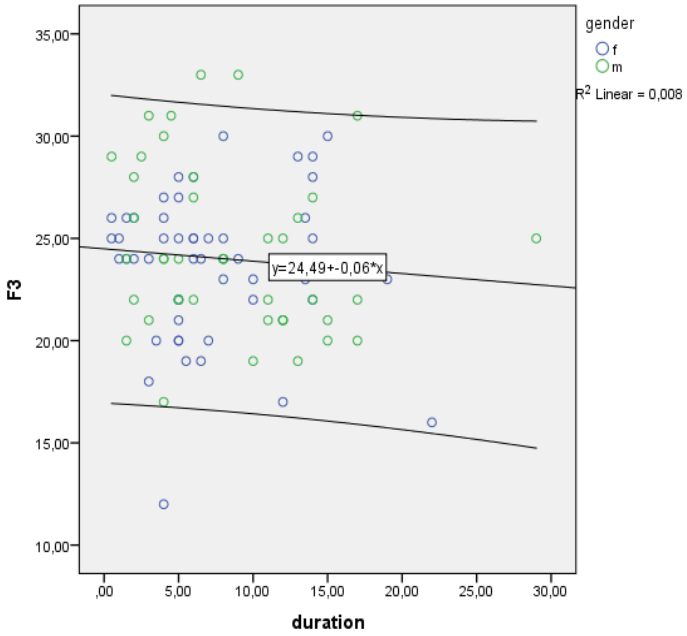

Fig. 5. Correlation between F3 and duration of dialysis

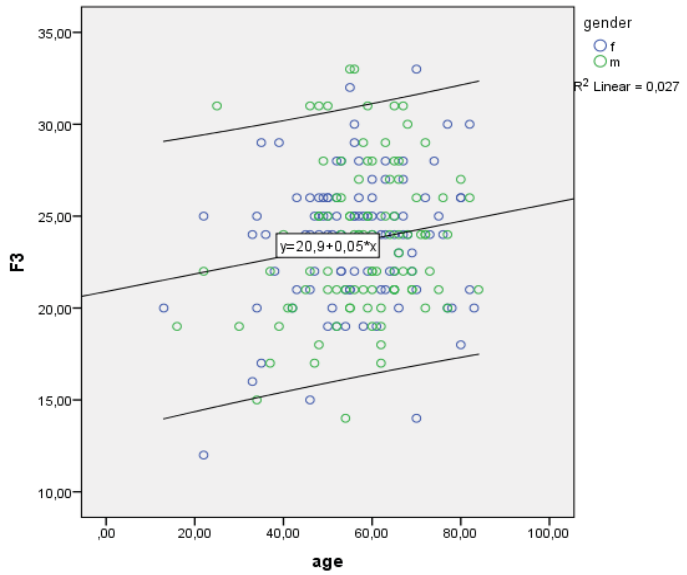

Fig. 6. Correlation between F3 and age

In addition, statistical analysis showed that no differences between gender and total scores for TAS 20, as well as gender and F2 and F3, were found. Only F1 (difficulty identifying feelings and distinguishing between feelings and the bodily sensations of emotional arousal) was significantly higher for males than for females ($p< 0.05$), which seems quite logical.

Only the results obtained for the group manifesting real alexithimia are presented in Figure 7 (total mean score, together with three factors).

If we compare obtained alexithymia scores for patients on dialysis with the control group (N=100) and patients with cancer (N=100) [14] it is clear that patients on dialysis showed the highest alexithymia scores (Figure 8).

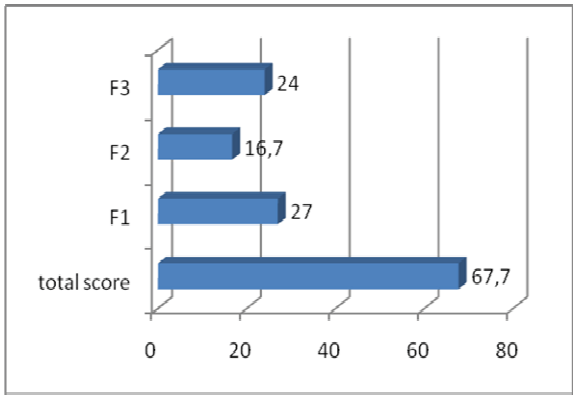

Fig. 7. Results obtained with TAS-20 for alexithymic group of dialysis patients

ANOVA confirmed the significance of the differences between groups on haemodialysis and control (t=18.35; p<0.05), as well as between groups of cancer and control (t=11.95; p<0.05). The analysis of differences in factors also confirmed significance between dialysis and control groups (for F1 t=10. 60; p<0. 05; for F2 t=23.21; p<0.05 and for F3 t=9.92; p<0.05).

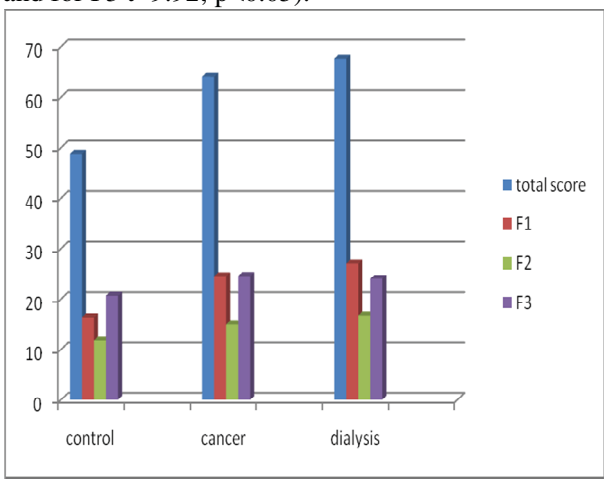

Fig. 8. TAS-20 obtained for control, cancer and dialysis patients

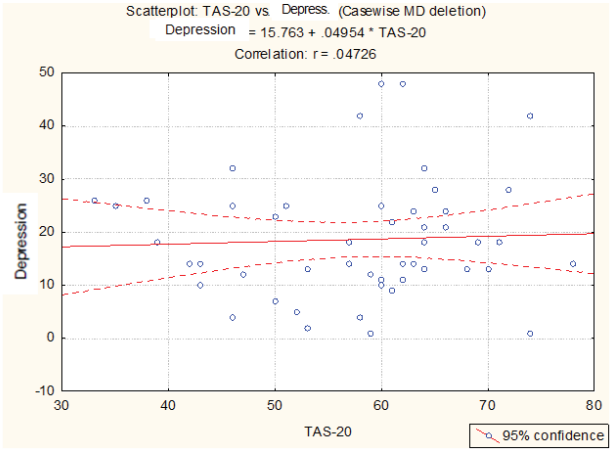

Fig. 9. Correlation between depression and alexithymia scores

Discussion

In our research we confirmed that alexithymia could be an important personality trait, which influences the adjustment, course and mortality rate in patients on haemodialysis. The results obtained showed that half of the examined patients on dialysis manifested a real alexithymia and 18% a possible alexithymia. Age of patients did not influence directly the scores for alexithymia, but the duration of dialysis was positively correlated with alexithymia scores. The alexithymia scores obtained in the study by Cabras, et al. [15] appeared to be directly correlated with the duration of dialysis. It was suggested that the prominence of the alexithymic phenomenon may be related to defense mechanisms against recurrent anguish about dying and to the tendency to assume certain characteristics of the particular therapeutic regimen, such as concreteness and rationality, so as to be able to tolerate the aggressive aspects of the treatment.

Obtained alexithymia scores are almost the same for male and female patients. Factor analysis showed that only F3 was sensible and influenced by both, age and duration of dialysis. Only F1 (difficulty identifying feelings and distinguishing between feelings and the bodily sensations of emotional arousal) was signifycantly higher in males (p<0.05).

Comparison of the alexithymia construct of patients on dialysis with healthy people and cancer patients showed significant differences confirming that alexithymia is much more specific for the dialysis group. The differrence between cancer and dialysis patients confirmed a significant difference not only for total scores, but also in two factors (F1 and F2).

Chronic kidney diseases, like other chronic illnesses, are related to many psychological characteristics [16-18]. Some characteristics are primary, but many of them could be secondary to the chronic disease. As a common comorbid psychological characteristics depression at different levels appeared to be present especially in patients treated by dialysis. In a recent review and meta-analysis Palmer, *et al.* (2013) [19] analyzed the prevalence of depression among these patients using MEDLINE and Embase articles. They concluded that the prevalence of interview-based depression was 22.8%, but it was much higher (39.3%) if the depressive symptoms were diagnosed with self-report or clinician-administered scales.

In our previous research concerned with the personality characteristics of patients treated by maintenance haemodialysis [11,12] we found a variable percentage of depression in examined patients checked with the Beck Depression Inventory: minimal in 21.43%, mild in 35.71%, moderate in 17.85% and severe in 14.28%. In addition, as specific characteristics of personality obtained with MMPI, we found hypersensitivity, depressive mood and withdrawal from friends and relatives. As more specific emotional traits we found accentuated anxiety, a low level of hostility, and high passive aggression which destroys their social communications. In many other studies the psychological factors are pointed out as very important in the course and prognosis of patients with chronic kidney diseases.

Bearing in mind our previous findings about personality characteristics of patients treated by haemodialysis, we supposed that the alexithymia construct could be another important personality characteristic in these patients. Results confirmed high alexithymia traits in these patients. However, alexithymia in our research was not related to the level of depression.

Alexithymia, as a poor ability to experience and express emotions, has been evaluated in many other studies of patients with chronic kidney diseases.

In the research of Jula, *et al.* (1999) [20] it was concluded that alexithymia appeared to be associated with

elevated blood pressure independent of sodium and alcohol intake, body mass index, and physical fitness. Kojima, *et al.* [21] in his research concluded that alexithymia had a stronger independent association with the increased risk of 5-year mortality than depression among patients on chronic haemodialysis.

Some findings suggest that cultural background may affect adaptation to chronic haemodialysis therapy and in this context could influence psychological problems [22]. The results may suggest the possibility that the differences in dialysis policy between different countries have secondary effects on alexithymia, which is one of the psychosomatic factors reflecting self-control ability in dialysis patients [23].

There are scanty data available on alexithymia in patients with end-stage renal disease, which point to an independent association with depression and social support [24-26].

Many individuals with alexithymia have somatic complaints. Considerable empirical evidence links prolonged states of emotional arousal, and the concomitant physiological arousal, with susceptibility to certain somatic disorders. Clearly, someone who cannot verbally express negative emotions will have trouble discharging and neutralizing these emotions, physiologically as well as psychically. All feelings, whether normal or pathological, are ultimately bodily feelings. Those with alexithymia lack a lived understanding of what they experience emotionally.

It was found that alexithymia was associated with somatization independently of somatic diseases, depression and anxiety and confounding sociodemographic variables. The TAS-20 factor scale "Difficulties Identifying Feelings" was the strongest common denominator between alexithymia and somatization [4].

From the perspective of development, alexithymia implies a glitch in the process that permits the expression of feelings in words that capture the body's involvement in these feelings. Perhaps the child's mother failed to sufficiently encourage a language of feelings (surely excluding her from the pantheon of Winnicott's "good enough" mothers). Alternatively, emotional trauma later in life may compromise the connection between what is felt and what can be grasped about this feeling and can be put into words, particularly if that link was tenuous to begin with.

Kojima, *et al.* (2010) [26] found that depression increases the risk of mortality in haemodialysis patients. Alexithymia, a disorder of affect regulation, has also been reported to be associated with mortality risk in the general population. They concluded that alexithymia is a strong independent risk factor for all-cause mortality in haemodialysis patients.

In addition, it was found that alexithymia scores were significantly positively correlated with anxiety scores, suggesting that alexithymia may be related to anxiety derived from the stress associated with dialysis therapy [27,28].

The influence of psychodynamic aspects such as the defense mechanisms for conflict on secondary alexithymia in hemodialysis patients was examined among dialysis patients and their family members. The results that have been obtained suggest that this defense mechanism strongly suppresses the manifestation of conflict, and that secondary alexithymia in dialysis patients may be derived from defense mechanisms such as denial [29].

Neuroimaging of alexithymia is in its infancy, and there are not so many functional brain imaging studies which have attempted to clarify brain mechanisms related to alexithymia.

The review of neuroimaging studies on alexithymia suggests that alexithymia is associated with reduced neural responses to emotional stimuli from the external environment, as well as with reduced activity during imagery, in the limbic and paralimbic areas (i.e., amygdala, insula, anterior/posterior cingulate cortex). Alexithymia is also known to be associated with enhanced neural activity in somatosensory and sensorimotor regions, including the insula. Moreover, neural activity in the medial, prefrontal, and insula cortex was lowered when people with alexithymia were involved in social tasks [30,31].

It is supposed that alexithymia is present in about 10% of the general population and generally is associated with a lower quality of life. However, alexithymia is a major risk factor for a range of medical and psychiatric problems, especially in chronic patients.

Conclusions

Generally, we can conclude that alexithymia is an important topic in the field of chronic end- stage diseases, such as patients on dialysis are. Alexithymia could have an effect on the progression of the disorders, as well as on the quality of life.

The alexithymia construct is a permanent personality trait related to neurobiological brain specifics. In our research alexithymia has been confirmed as an important personality trait in patients treated with haemodialysis. In any case, psychological support mediating alexithymia should be included in the therapeutic protocols of all chronic patients, especially in those treated with haemodialysis.

Conflict of interest statement. None declared.

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Short communication

Aspergillus Peritonitis in Chronic Peritoneal Dialysis Patients: Review of the Literature and Report of Two Cases

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Abstract

Although gram positive bacteriae are the most common causative microorganisms of chronic peritoneal dialysis (CPD) peritonitis, fungi are responsible for 1-15% of all cases. On the other hand, fungal peritonitis episodes may potentially cause serious consequences such as resistance to treatment, extended hospital stay and also a higher probability of death. Fungal peritonitis due to *Aspergillus spp* is relatively uncommon, but its mortality rate and severity is known to be even higher. It was our aim to conduct a review of the medical literature regarding the treatment and clinical outcome of *Aspergillus* related CPD peritonitis and to present two cases with *Aspergillus* related CPD events. Our current knowledge and the outcome of our two cases suggest that, despite the use of recommended therapeutic measures, *Aspergillus* induced fungal peritonitis in CPD patients may still be fatal. Therefore, there is a need for development of more efficient therapeutic approaches including the type, dose and route of antifungal therapy.

Key words: peritoneal dialysis, fungal peritonitis, *Aspergillus, Aspergillus flavus, Aspergillus niger*

Introduction

Peritonitis is one of the most important complications of chronic peritoneal dialysis treatment and is reported to be responsible for 40-47% of technical failure with mortality rates as high as 1-6% and frequent hospitalizations seen in that treatment modality [1,2]. Although gram-positive bacteriae are the most common cause of CPD peritonitis, fungi are responsible for 1-15% of all cases [3,4]. On the other hand, fungal peritonitis episodes may potentially cause serious consequences including resistance to treatment, extended hospital stay and also a higher probability of death [5]. Fungal peritonitis due to *Aspergillus spp* is relatively uncommon, but its mortality rate and severity is known to be even higher [6]. It was our aim to conduct a review of the medical literature regarding the treatment and clinical outcome of *Aspergillus* related CPD peritonitis and to present two cases. One of the patients died due to *Aspergillus flavus* and the other was with fungal colonization in titanium adapter proven by positive fungal culture for the presence of *Aspergillus niger*.

Cases

Our first patient was a 49-year-old man with end-stage renal disease secondary to type I diabetes mellitus and has been included into our continuous peritoneal ambulatory dialysis (CAPD) program since 2008. He had undergone an inguinal hernia surgery at the 18th month after commencing CAPD treatment. He had also an episode of bacterial peritonitis in 2010 which had been treated successfully.

In 2012, he was admitted to the Emergency Department with abdominal pain, nausea and cloudy dialysate. His blood pressure was 130/80 mmHg, pulse 82 beat/minute and body temperature 37.2°C. The physical examination revealed disseminated abdominal tenderness and he was positive for signs and symptoms of peritoneal irritation. No signs of infection around the exit site and catheter tunnel was observed. Laboratory test results of our patient were as follows: peripheral blood white blood cell (WBC) count: 18,450/mm3 (N=4.800-10.800), hemoglobin 9.5 gr/dl (12-16 gr/dl), erythrocyte sedimentation rate 120 mm/hour, C-reactive protein 46.1 mg/dl (0-0.5 mg/dl) and creatinine 12.6 mg/dl (0.7-1.2 mg/dl). His peritoneal effluent WBC count was found to be 3020 /mm^3 with a differential of neutrophils: 570/mm^3, lymphocytes: 280/mm^3, monocytes: 2080/mm^3. Based on his clinical picture and laboratory criteria, he was diagnosed as having CPD-related peritonitis. Because of drainage problems in his peritoneal dialysis catheter, after initial samples for cultures and peritoneal effluent cell counts were taken, peritoneal dialysis catheter was removed and hemodialysis was initiated. Direct microscopic investigation of peritoneal fluid with gram staining showed no microorganisms. Peritonitis treatment was initiated with cephtazidime intravenous 1 gr 2x1 and ampicillin/ sul-

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bactam 1 gr 4x1 empirically. But despite treatment, his abdominal pain and clinical condition have not improved. Cultures for aerobic and anaerobic bacteria and tuberculosis were negative. But eventually, *Aspergillus flavus* was isolated from his peritoneal fluid samples and also from his removed catheter tip cultures. Therefore, intravenous administration of liposomal amphotericin B 200 mg/day was initiated at the fourth day of his hospitalization. This treatment was continued for 26 days. At days 0, 7, 14, 21 and 26, his C-reactive protein (CRP) levels were 24.8 mg/dl, 18.4 mg/dl, 17.7 mg/dl, 21.7 mg/dl, 19.5 mg/dl and peripheral leukocyte counts were 9.180/mm³, 14.450/mm³, 11.820/mm³, 35.270/mm³ and 23.020/mm³, respectively. Unfortunately, no clinical and laboratory improvement was observed and our patient died the 30th day of his admission.

Our second patient was a 77-year-old man with end-stage renal disease secondary to hypertension, chronic obstructive pulmonary disease and cor pulmonale. He has been on automated peritoneal dialysis for 24 months with no history of peritonitis. The patient was seen in our Peritoneal Dialysis Outpatient Clinic because of his report of accidental cut in his Tenckhoff catheter at the distal tip over a point very close to titanium adapter approximately 15 hours ago. There were no signs and symptoms of peritonitis, including abdominal pain, fever or cloudy dialysate. His blood pressure was 90/50 mmHg, temperature 37°C, pulse 90 beats per minute. The physical examination did not reveal any acute health problem and he was also negative for abdominal tenderness and signs of peritoneal irritation. There was no evidence for any infection around the exit site and catheter tunnel. His basic laboratory tests were as follows: peripheral blood WBC: 8600/mm^3 (4.800-10.800/mm^3), hemoglobin: 12.7 gr/dl (N=12-16 gr/dl), hematocrit: 37 (N=35-52), sedimentation: 37 mm/hour, CRP: 0.2 mg/dl (N=0-0.5), BUN: 53 mg/dl (N=6-20 mg/dl), creatinine: 4.68 mg/dl (N=0.7-1.2 mg/dl); peritoneal dialysate was clear with a WBC count of 100/ mm^3 with 40% neutrophils. His titanium adapter was replaced with a new one and dialysate samples for aerobic, anaerobic, acid-fast bacteriae and fungal cultures were taken and the patient was initiated prophylactic oral amoxicilline/clavulonic acid 1000 mg BID. All cultures were reported to be negative except *Aspergillus niger* growth in his fungal culture. Considering no development of any peritonitis signs or symptoms at the fourth day from the accidental cut of his catheter, we have decided to observe the patient for development of any evidence of peritonitis and keep him on antibiotic propylaxis without initiating any antifungal therapy. During the follow-up, the patient has remained peritonitis-free and control cultures for fungi were negative.

Although fungi may be found in the regular flora of human skin and mucosa, long-term antibiotic usage [7], use of immunosuppressive drugs and diseases supressing immune system [8], possibly non-biocompatible high glucose containing dialysis solutions [9] and mechanical and/or chemical irritations caused by peritoneal catheters may be among the causes of fungal peritonitis. Transvaginal entrance of fungi into the peritoneal cavity may also occur. Intestinal perforations caused by diverticulitis have also been reported to cause fungal peritonitis.

Discussion

Fungal peritonitis episodes in CPD patients present as severe clinical form of peritonitis with high mortality rate of 20-30% [10]. *Candida spp* are known to be the most common cause of fungal peritonitis. But much less frequently, PD peritonitis may be caused by *Aspergillus spp* such as *Aspergillus thermomutatus* [11], *Aspergillus niger* [12,13], *Aspergillus flavus* [14], *Aspergillus fumigatus* [8], *Aspergillus terreus* [15,16], *Aspergillus oryzae* [5], *Aspergillus sydowii* [17]. In 2002, Matsumoto, *et al.* reviewed 20 *Aspergillus spp* peritonitis cases that have been published between 1968-2002. In our literature review covering the period from 2002 to 2013, we were able to find the records of 13 published cases of *aspergillus* peritonitis, including our two cases presented here (Table 1) [18-21]. Combined outcome results of two series reveal that, out of 33 cases presented since 1968, 11(33%) died and 13(39%) had to be transferred to hemodialysis. Only 8 patients (24%), including two patients with no signs and symptoms of overt peritonitis with culture proven *Aspergillus* colonization in the catheters, (Reference 19 and our second case presented in this report), were able to continue chronic peritoneal dialysis treatment suggesting a high risk clinical profile. Presence of severe abdominal pain, fever, delay in withdrawal of catheter, intensive antibiotic usage longer than three months and technical difficulties are reported to be related with mortality in fungal peritonitis [3,4]. On the other hand, as we have reported in our patient, *Aspergillus spp,* also seen in peritonitis episodes caused by other fungi, have a tendency to form adhesive fibrin plugs causing drainage problems and total obstruction of peritoneal catheter [10]. Interestingly, in one case, *Aspergillus niger* peritonitis was reported to be associated with eosinophilia, which is a clinical sign of pulmonary *aspergillosis* [22]. In our patient, both initial peripheral blood cell and dialysate eosinophil counts were within normal limits (0.9% and 0.4%, respectively).

If we analyze the fatal outcome in one of our patients, regarding the risk factors given above, the patient was neither on any immunosuppressive drugs nor there were any laboratory or clinical signs of hematologic or oncologic problems which may potentially affect his immune system besides known type I diabetes. Clinically, only abdominal pain was observed as one of the stated mortality risk factors. His peritoneal dialysis catheter was removed appropriately on the first day of admission and he was not on any long-term broad spectrum antibiotic therapy. But despite practicing the recommended general

therapeutic measures, our patient died the 26th day of the admission. *Aspergillus*-related peritonitis episodes can be treated with amphotericin B alone or in combination with azol derivatives such as ketocanazol, fluconazole or itracanozol. Because of serious side effects such as fever, chills, rigor, nausea and hypotension intravenous use of conventional amphotericin B is often limited. Therefore, lipophilic form of amphotericin B is recommended and it is reported to be equally effective [11]. On the other hand, intraperitoneal use of amphotericin B may induce a chemical peritonitis with severe abdominal pain and it is not recommended. Intravenous use of amphotericin B may not be sufficiently effective because of drug's high protein binding capacity and limited transfer to the peritoneal area [23]. Aspergillus terreus has been reported to be resistant to amphotericin B both in vivo and in vitro [24].

TABLE 1. Summary of reported CPD- related peritonitis cases caused by *Aspergillus spp 2003-2013*

Patient No	Gender	Species	Catheter removal	Antimicrobials	Outcome	Reference	Year
1	M	*Aspergillus terreus*	Yes	Amphotericin B	Death	17	2003
2	M	*Aspergillus fumigatus*	No	Amphotericin B, oral itracanazol	Death	15	2004
3	F	*Aspergillus terreus*	Yes	Amphotericin B, itracanazole	Death	16	2004
4	F	*Aspergillus fumigatus*	Yes	Amphotericine B	HD	6	2005
5	F	*Aspergillus sydowii*	Yes	No treatment	HD	17	2005
6	F	*Aspergillus fumigatus*	Yes	Amphotericin B	HD	18	2006
7	F	*Aspergillus terreus*	Yes	Itracanazole (Catheter colonization)	PD	19	2006
8	F	*Aspergillus terreus*	Yes	Voricanazole	Death	20	2007
9	M	*Aspergillus oryzae*	Yes	Amphotericin B+caspofungin, itracanazole	HD	5	2007
10	F	*Aspergillus nidulans*	Yes	Amphotericin B, voricanazole	HD	21	2011
11	M	*Aspergillus flavus*	Yes	Voricanazole	HD	14	2013
12	M	*Aspergillus flavus*	Yes	Amphotericin B	Death	Case I	2013
13	M	*Aspergillus niger*	No	No treatment (Catheter colonization)	PD	Case II	2013

Conclusions

In conclusion, despite the use of recommended therapeutic measures, *Aspergillus* induced fungal peritonitis in CPD patients may still be fatal. There is a need for development of more efficient therapeutic approaches including the type, dose and route of antifungal therapy.

Conflict of interest statement. None declared.

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Case report

A Stepwise Diagnosis of Sarcoidosis Presenting with Renal Impairment and Hypercalcemia

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Abstract

Sarcoidosis is a multisystem, immune-mediated, granulomatous disease. Clinical presentation of this disease may vary; in majority of cases (~90%) thoracic involvement is the leading sign. Although renal involvement is thought to be uncommon in sarcoidosis this entity may not be so rare. Hypercalcemia seems to be the most likely cause of sarcoidosis-associated renal disease, it can even cause acute renal failure in 1-2% of sarciodosis patients. Immediate treatment is appropriate whenever organ function is threatened or when symptoms are severe. We present a case of sarcoidosis with hypercalcemia excluding other clinical conditions, which may potentially confuse the diagnosis.

Key words: sarcoidosis, hypercalcemia, renal failure, primary biliary cirrhosis, biopsy

Introduction

Sarcoidosis is a multisystem, idiopathic, inflammatory granulomatous disease. The diagnosis is usually based on an appropriate clinical presentation, involvement of at least two organ systems, histologic evidence of non-caseating granuloma from at least one organ and exclusion of other granulomatous diseases [1]. Sarcoidosis can involve any organ system and the clinical course may be highly variable. Difficulties in diagnosis of the disease is caused by the varying forms and presentations, the lack of a single diagnostic test and high-quality randomized controlled trials. Renal involvement, especially hypercalcemia is the clinically severe presentation of sarcoidosis. Neurological, ocular, renal, cardiac, pulmonary hypertension and depression are the treated features of sarcoidosis [2]. We should suspect sarcoidosis in a patient with renal failure associated with hypercalcaemia.

Case report

A 54-year-old woman had a 9-year history of primary biliary cirrhosis (the diagnosis was made on the basis of high levels of alkaline phosphatase and γ-glutamyl transpeptidase, positive antimitochondrial antibody-titer 1:20-and MRCP findings) and 4 months of depression, with a chronic use of daily ursodeoxycholic acid of 750 mg and sertralin of 50 mg. She was admitted to our hospital for further examination of renal impairment and hypercalemia detected at a routine control. A week ago she was given an intramuscular injection of vitamin D. She was complaining of fatigue and loss of appetite. Family history of the patient was unremarkable. The patient did not have any history of cough, joint pains, dysuria, hematuria or any other symptoms. There was no history of tuberculosis or other granulomatous disorders. A physical examination upon admission revealed a poor general condition, body temperature of 36.8°C, blood pressure 135/80 mmHg and a regular pulse of 24 beats/ min. No superficial lymph adenopathy was evident. Heart auscultation was normal. Bibasilar crackles were heard in her lungs. Abdominal examination revealed no hepatomegaly or splenomegaly. Skin examination revealed no changes. Laboratory investigations showed ESR of 34 mm/first hour, fasting blood sugar: 96 mg/dl, urea: 98 mg/dl, creatinine: 2.91 mg/dl, uric acid: 4.4 mg/dl, calcium: 13.6 mg/dl, potassium: 4.1 mmol/l, sodium: 136 mmol/L, phosphorus: 5.1 mg/dl, total protein: 7 gr/dl, albumin: 3.4 gr/dl, globulin: 3.6 gr/dl, WBC 7830, hemoglobin: 11.6 gr/dl, hematocrit: 35.2%, platelets: 339000, CRP: 4.2 mg/dl and ALP: 110 U/L. Urinalysis revealed density 1010, pH 5.5, protein ++, leukocytes none, eryth-

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rocytes 1/each field. Arterial blood gas analysis were as follows: pH 7.37, TCO_2 21.9, HCO_3 20.8, lactate 0.8. Daily urinary protein excretion was 844.6 mg/day, detected in the twenty-four hour urine sample. Serum antinuclear antibody, anti-double stranded-DNA, rheumatoid factor, hepatitis B surface antigen, HIV antibody, hepatitis C virus antibody, antineutrophil cytoplasmic antibody were all negative. Serum complement (C3,C4) and immunoglobulin levels were in normal ranges except for total IgE 114.7 IU/mL(N:1-100). Glomerular filtration rate was estimated as 17.9 ml/min/ 1.73 m^2 according to the short MDRD formula. Ultrasonography of the right kidney was reported as 100x49 mm and parenchymal thickness of 16 mm with a Grade 1 echogenicity. Ultrasonographic size, parenchymal thickness and echogenicity of the left kidney were 101x51 mm, 15 mm and Grade 1, respectively. There was no sign of stone or ectasia. The posterior anterior X-ray of the lungs showed bilateral reticulonodular infiltration in all lung zones (Figure 1).

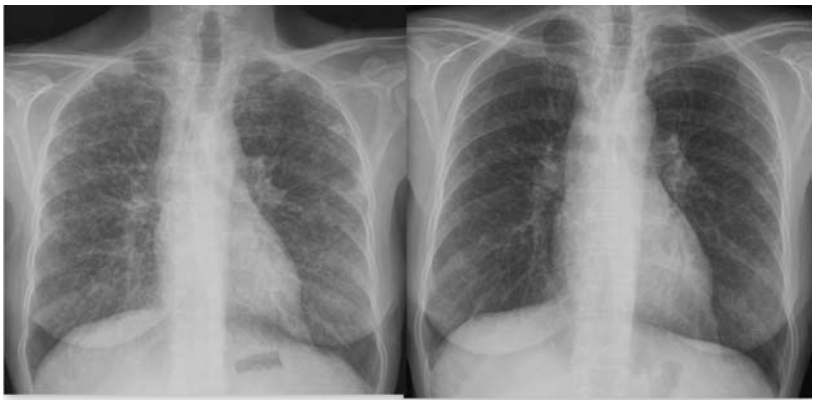

Fig. 1. Chest X-ray. Stage III sarcoidosis. Bilateral reticulonodular infiltration in all lung zones. Regression of radiological findings after 3 weeks steroid therapy

12-lead ECG was in sinus rhythm. Transthoracic echocardiogram revealed ejection fraction of 60%, mitral regurgitation 1^0, pulmonary arterial pressure 25 mmHg. Although the patient received a D vitamin injection (300.000/IU D3) 1 week ago that had been advised by her gastroenterologist due to the chronic liver disease, hypervitaminosis D was suspected at first. Serum level of PTH and 25-hydroxyvitamin D were 2.7 pg/ml (N: 16-88.3) and 6.9 ng/mL (N:88-963), respectively. With these findings, hypervitaminosis D and hyperparathyroidism were excluded. Tumor marker levels were not significantly high. Mammography and mammary ultrasonography were done to exclude malignant breast cancer and a 11x5 mm lobulated, solid, irregularly shaped hypoechoic nodule in the upper outer quadrant of the right breast was detected. Ultrasound guided needle biopsy of the breast was done and the biopsy result was a benign neoplasm. As she was found to have renal failure, hypercalcemia and additionally high serum levels of globulins, serum and urine immunofixation electrophoresis, peripheral blood smear, X-ray of the bones was performed and they were not significant for multiple myeloma (A bone marrow aspiration and biopsy was suggested but she did not accept the procedure)**.** Meanwhile, due to the chest X-ray findings (although not specific), renal failure and hypercalcemia sarcoidosis was suspected. Contrast-enhanced thoracoabdominal CT was performed and images showed mediastinal, bilateral hilar and right paratracheal nodal enlargement, bilateral reticulonodular infiltration and mosaic pattern of lung attenuation, hypodens areas within the spleen (Figure 2). Serum levels of angiotensin-converting enzyme (ACE) was elevated as 427 U/L (N:8-52). 24-hour urine level of calcium was 425 mg/day (N: 100-300). To investigate the lung involvement bronchoscopy was done. Bronchoscopy revealed diffuse mucosal nodularity and the biopsy specimen obtained from

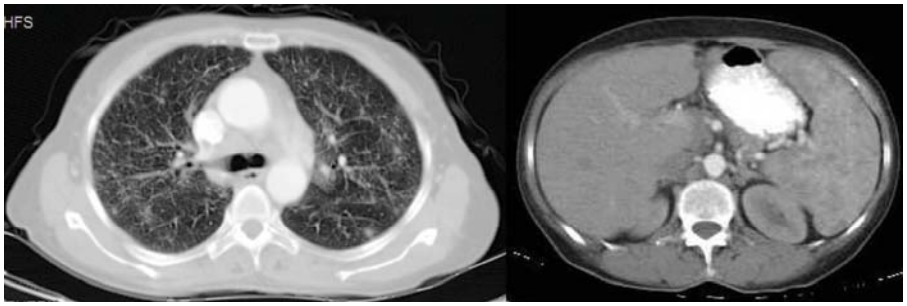

Fig. 2. Thoraco-abdominal CT. Bilateral reticulonodular infiltration and mosaic pattern of lung attenuation. Hypodens areas within the spleen

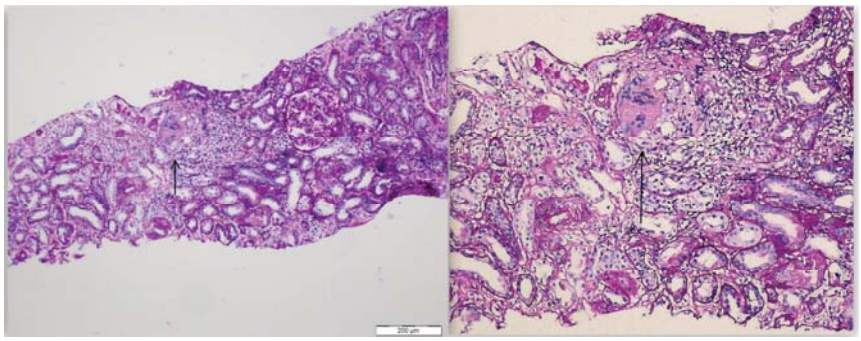

Fig. 3. Renal biopsy**.** Hematoxylen&Eosin and Periodic acid-Schiff stain. Diffuse interstitial inflammation, a noncaseating granuloma with a multinucleated giant cell (*arrows*)

these lesions later showed noncaseating granulomatous inflammation. No microorganisms were identified in specimens, visually or with special stains for fungi, mycobacteria, atypical mycobacteria and Nocardia spp. Cultures for fungi, bacteria, and acid-fast bacilli, as well as viral respiratory panel were all negative. A reduction in carbon monoxide diffusion capacity (DLCO*)* was detected (DLCO/VA:%53).

As soon as the possibility of urolithiasis-related renal failure was ruled out by ultrasonography and abdominal CT, ultrasound-guided renal biopsy was performed for better understanding the renal pathology. Light microscopy revealed 13 glomeruli, of which 2 had segmental glomerular sclerosis and others had an increase in the mesangial matrix. Diffuse interstitial inflammation, a noncaseating granuloma with a multinucleated giant cell, tubulitis and focal atrophy were apparent (Figure 3). Histochemically, periodic acid-Schiff, M. Silver, Masson's Trichrome staining was used. Congo red staining was negative. With direct immunofluorescence method, IgA, IgG, IgM, C3, C4, C1q, fibrinogen, kappa and lambda were applied and there was no specific deposition. The above findings indicated a diagnosis of sarcoidosis after clearly excluding other possible clinical entities potentially confusing the diagnosis. 18-FDG PET-CT (*Siemens, Biograph*™ *mCT, 5mCi IV FDG*) was also performed due to the multiorgan involvement. During the follow-up the patient's calcium levels stayed constantly high although she was treated with continuous saline infusion and a loop diuretic, under calcium restricted diet. Oral prednisolone therapy of 1 mg/kg/day was started. The calcium concentration levels decreased with steroid therapy. Serum creatinine was also in normal ranges after 3 weeks of steroid therapy (Figure 4).

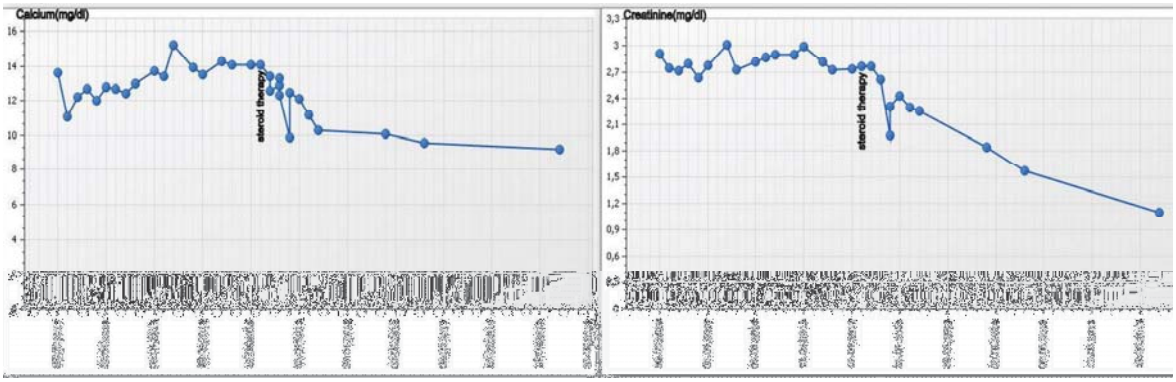

Fig. 4. Normalization of serum calcium&creatinine concentrations after steroid therapy

Discussion

Sarcoidosis is a multisystem, inflammatory granulomatous disease of undetermined etiology. Histopathology of the disease is characterized by T-lymphocyte infiltration, presence of noncaseating granulomas as pathologic hallmark and deformation of normal microarchitecture. It has a variable clinical presentation; in majority of cases (~90%) there is thoracic involvement whereas other commonly affected organs are the liver, skin, and eye. In our case multiorgan involvement was also present. Extrathoracic involvement can be an initial manifestation in one-half of symptomatic patients. The involvement of two or more organs for a specific diagnosis is needed. Skin lesions, lymph node enlargement, renal stone, bundle branch block can be the initial signs of sarcoidosis and these entities may stay unrecognized for a long time. Almost 30% of patients have a persistent disease, which may lead to significant organ impairment. It can occur at all ages, but it usually develops before the age of 40-50 years; incidence peak occurs at 20 to 40 years and it seems to affect women more frequently [3].

Constitutional symptoms such as fatigue, fever, night sweats, and weight loss may be seen frequently. In our patient these symptoms could partially or strongly be related to depression, malignancy, infections or primary biliary cirrhosis; therefore, the inital presentation of sarcoidosis can mimic various clinical situations. It is well-documented that the main presentation of sarcoidosis is pulmonary with a chest X-ray carried out by chance. Although our patient had no respiratory complaints, lung involvement was present and even showed regression after steroid therapy. Lung and lymphatic systems are the principal localizations [4]. Hilar lymphadenopathy in sarcoidosis is typically bilateral and symmetric, being the most common radiological finding. Overall mortality from sarcoidosis is reported to be about 1-5% (respiratory, cardiac or central nervous system disease) [5].

Some environmental/occupational exposures and microbial origins are speculated to be associated with the risk for sarcoidosis (musty odors, pesticides, propionibacter acnes, mycobacterial KatG protein, beryllium, IFNα/β, employment in the aerospace, automotive, ceramic or computer industries) [1]. The exact inciting stimulus/cause of sarcoidosis is unknown. HLA-DQB1*0201 and HLA-DRB1*0301 alleles are associated with acute disease and good prognosis. HLADRB1 *1501/DQB1*0602 haplotype predicts a chronic course and severe pulmonary sarcoidosis. We could not find a relationship between the disease and any of the exposures mentioned above.

Noncaseating epithelioid granulomas are the most important and the basic pathologic abnormality in sarcoidosis [6]. Multinucleated giant cells are frequently found among the epithelioid cells within the granuloma follicle and often have cytoplasmic inclusions, such as asteroid bodies, Schaumann bodies, and birefringent crystalline particles [7]. There was granuloma in our renal biopsy specimen. Sarcoidal granulomas produce angiotensin-converting enzyme (ACE). Elevated levels of ACE are reported in 60-75% of patients with acute/untreated disease [8]. In our patient the level of ACE was significantly high, supporting our diagnosis.

Symmetrical hilar adenopathy with or without parenchymal lung involvement is the most common thoracic manifestation. Dyspnea, persistent/mild/dry cough and wheezing can be the symptoms. A decreased diffusion capacity and a restrictive ventilatory defect, obstructive airways disease, pulmonary arterial hypertension, chest pain are the clinical features of sarcoidosis with lung involvement. Examination of the chest can often show nothing [1]. The CD4/CD8 ratio may be increased in bronchoalveolar lavage in about 50% of patients with sarcoidosis, but bronchoalveolar lavage findings are nonspecific and should not be used to diagnose sarcoidosis alone. Bronchoalveolar lavage findings also cannot predict prognosis or responsiveness to corticosteroid therapy. The diffusion of carbon monoxide (DLCO) is the most sensitive test for an interstitial lung disease and it was decreased in our case, too.

Depression is not seldom reported; its incidence was nearly between 13% and 66% in a large multicenter study [9]. Our patient was also under sertralin therapy for 4 months and it was important to differentiate chronic complaints of depression from another serious clinical entity.

Clinical manifestations of sarcoidosis include heart failure (restrictive cardiomyopathy), conduction abnormalities (atrioventricular blocks), atrial and ventricular arrhythmias (monomorphic VT), pericardial effusion, valvular dysfunction and sudden cardiac death. These entities may be induced by sarcoidal granulomas and patchy myocardial fibrosis. Although clinical cardiac involvement occurs in less than 10% of patients, in postmortem studies this rate may range up to 25%. Thus, cardiac monitoring and evaluation in these patients should seriously be done. In our patient, 18-FDG PET-CT (*Siemens, Biograph™ mCT, 5mCi IV FDG*) was performed for investigating the cardiac involvement and high myocardial uptake was detected (SUV max 5.1). The patient was called for frequent follow-up and cardiac monitoring although she did not have any cardiac symptoms (Figure 5).

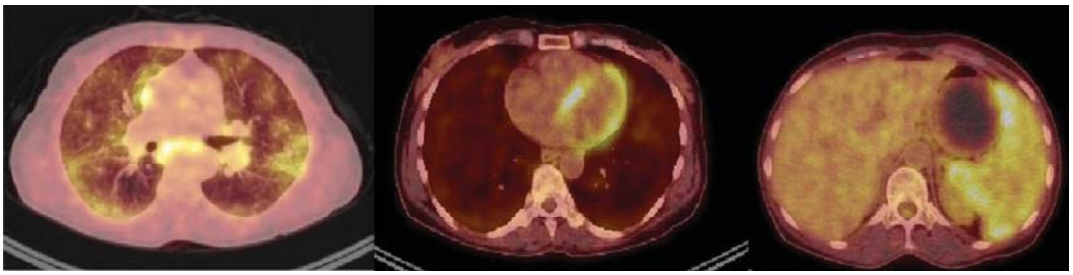

Fig. 5. 18-FDG PET/CT. High thoracic, myocardial, splenic uptake (SUV max 3.4, 5.1, 4.9, respectively)

Bone lesions are rare in sarcoidosis. The reported incidence of radiographically evident osseous involvement is between 1% and 13%, with an average of 5% [10]. We excluded the skeletal involvement by 18FDG-PET/CT in our patient showing high levels of calcium concentrations. Gallium scanning has a historical role in the diagnosis of sarcoidosis but its sensitivity is limited [11]. Recently 18FDG-PET/CT has been used more frequently with a high sensitivity among sarcoidosis patients [12-14].

The abdomen is the commonest extrapulmonary site of involvement with a frequency of 50-70% [15]. Autopsy studies have revealed splenic involvement in 38-77% of

patients with this disease [6]. The mean serum ACE levels were found to be higher in patients with splenic nodules than in cases without splenic nodules [6]. Our patient had similar presentation showing both splenic involvement and high levels of ACE. Association of primary biliary cirrhosis and sarciodiosis was reported before but the relationship between these two entities is still not clear. Kishor, *et al*. recently reviewed 17 patients with sarcoidosis and PBC and suggested that a common pathway contributes to granuloma formation in both disorders [16]. On the other hand, no significant association has been identified in a large database in the United Kingdom [17].

Although renal involvement is thought to be uncommon in sarcoidosis, this entity may not be so rare-about 10% of cases [18,19]. In another study, comparing clinical series of sarcoidosis, the incidence of renal involvement was between 9.8-18% [20]. Renal disease may include hypercalciuria (30-50% of cases), granulomatous interstitial nephritis, glomerular disease (membranous nephropathy, proliferative or crescentic glomerulonephritis, focal glomerulosclerosis), renal tubular dysfunction, polyuria (due to nephrogenic and/or central diabetes insipidus), hypertension, nephrocalcinosis-lithiasis (10-14% of cases), hypergammaglobulinimia-related disease, renal vascular disease and obstructive uropathy [21]. The occurrence of hypercalcemia (10-20% of cases) is correlated with disseminated sarcoidosis. Our patient also had multisystem involvement, supporting this finding. Hypercalcemia seems to be the most likely cause of sarcoidosis-associated renal disease, it can even cause acute renal failure in 1-2% of sarciodosis patients. The effects of hypercalcemia on the kidney are more common than direct granulomatous involvement or interstitial sarcoid inflammation [22]. Direct kidney involvement-granulomas in the kidney-occurs in <5% of sarcoidosis patients and this can lead to nephritis [23]. The prevalence of tubulointerstitial nephritis ranges from 7% to 27%, although chronic renal failure develops in less than 1% of cases [24]. In our case, renal failure was attributed to hypercalciuria and granulomatous interstitial nephritis (seen histopathologically).

Increased 1-α hydroxylase activity in macrophages within granulomas and the alveoli converts 25-hydroxyvitamin D to the biologically active form 1,25-dihydroxyvitamin D (calcitriol), therefore resulting in increased intestinal absorption of calcium leading to hypercalcemia with a suppressed parathyroid hormone (PTH) level. An increased exogenous vitamin D from diet or sunlight (during the summer months) exposure may exacerbate this problem. Patients with sarcoidosis may be sensitive to vitamin D therapy and even in small doses hypercalcemia can be apparent [25]. One dose of vitamin D injection seems to clarify our clinical vignette.

A breast lesion in a patient with sarcoidosis is generally more likely to be a granuloma than breast cancer. However, in a trial comprising 629-women with sarcoidosis it was shown that mammographic and physical findings could not distinguish between sarcoidosis in the breast and breast cancer [26]. Thus, it is recommended that routine screening including mammography be performed along with other imaging studies (ultrasound, MRI) or biopsy as clinically indicated. We also performed an ultrasound-guided needle biopsy from the hypoechoic nodule of the right breast. The biopsy confirmed the presence of dense fibrous (connective) tissue and granulomas were not seen. Therefore, we excluded malignant breast cancer in this patient.

Corticosteroids remain the principal treatment and may improve renal function by correcting hypercalcemia and/or hypercalciuria and by decreasing granuloma formation in the renal interstitium, along with the associated interstitial nephritis [27,28]. Immediate treatment is appropriate whenever organ function is threatened or when symptoms are severe. Oral prednisone at a dose of 20 to 40 mg per day for 3 months is usually the initial recommended therapy. Patients should be followed closely for 1-2 years after discontinuing treatment because of the risk of recurrence. Failure of serum calcium to normalize within 2 weeks should alert the clinician to an alternative diagnosis such as underlying malignancy. The granulomatous response of sarcoidosis can resolve with or without therapy. Initiating treatment, most patients with granulomatous interstitial nephritis regain renal function. However, in at least 20% of patients with sarcoidosis, a chronic form of the disease develops. Certain risk factors at presentation for a possible chronicity are fibrosis on chest X-ray, presence of lupus pernio, bone cysts, cardiac or neurologic disease (except isolated seventh nerve paralysis), and presence of renal calculi due to hypercalciuria. Hydroxychloroquine, methotrexate, azathioprin, anti-CD20 antibodies, statins, phosphodiesterase inhibitors, infliximab are the other potential therapies highlighted in the literature [2]. Our patient had serious organ involvement and hence we started oral steroid therapy of 40 mg prednisolone. During the close follow-up after starting the treatment, high levels of calcium and creatinine showed regression (Figure 4).

Conclusions

There are often delays in the diagnosis of the disease and care because there is no criterion diagnostic test. Therefore, being suspicious for sarcoidosis should be the first step of the diagnosis in our clinical practice. Novel algorithms and modern tools such as 18-FDG PET/CT can broaden the vision in diagnosis of sarcoidosis from "a disease of exclusion" to a more frequently seen entity.

Conflict of interest statement. None declared.

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Case report

Kidney Transplant in an Old Woman-A Case Report and Review of the Literature

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Abstract

The number of elderly patients with chronic kidney disease (CKD) as well as those with end-stage renal disease (ESRD) are increasing worldwide. Renal transplantation is now the treatment of choice for all ESRD patients, including those that are aged 65 or over. Namely, there is a growing evidence that elderly patients, in the absence of contraindications, have better outcomes after renal transplantation than alternative forms of RRT. Although survival, quality of life and economic advantages have been shown after transplantation, renal transplantation is still infrequently offered to older patients. Hereby, we present a case of an old woman who was transplanted in 1994 when "senior" program was still not established and when kidney transplantation at this age was rarity in many countries. She lived 16 years and 8 months with a well-functioning graft and died at the age of 89.

Key words: renal transplantation, elderly patients, survival

Introduction

The number of elderly patients with chronic kidney disease (CKD) as well as those with end-stage renal disease (ESRD) are increasing worldwide. According to the literature, nearly half of all ESRD patients are now over 60 years in the USA as well as in Europe. Furthermore, previous research highlighted that half of patients receiving renal replacement therapy (RRT) in the dialysis units are aged 65 or over. Renal transplantation is now the treatment of choice for all ESRD patients, including those that are aged 65 or over. Namely, there is a growing evidence that elderly patients, in the absence of contraindications, have better outcomes after renal transplantation than alternative forms of RRT. The success of solid organ transplantation is accompained by a severe shortage of available organs for those currently awaiting transplantation. In the recent years, there has been an increasing demand for organs but not a similar increase in the supply leading to a severe shortage of organs for transplant and increasing waiting times for recipients. This has resulted in expanded donor criteria to include older donors and donors with mild diseases. Kidney transplantation offers the potential for improved quality and prolonged length of life in elderly patients and reasonable outcomes have been reported for selected patients in their 70s and even 80s. Furthermore, the mean age of renal transplant recipients is rising, with advanced age no longer considered a contraindication to transplantation. Although survival, quality of life and economic advantages have been shown after transplantation, renal transplantation is still infrequently offered to older patients. This is mainly due to the fact that many clinicians view dialysis as a stable strategy with an acceptable survival and few short-term risks. On the other hand, transplantation is viewed as having significant risks of short-term morbidity and mortality [1-5].

To overcome these limitations, in January 1999, Eurotransplant established the Eurotransplant Senior Program (ESP) allocation scheme to match the functional capacity of organs from donors ≥65 years to the functional requirements of recipients ≥65 years. The organs are allocated by blood group and waiting time only. The main goals of this program are to increase the number of kidneys from elderly donors, shorten the long waiting time for elderly recipients, and not affecting negatively the graft and patient survival. ESP became fully implemented into the Eurotransplant Kidney Allocation System in January 2001. In contrast to Eurotransplant Senior Program, Croatia has developed its own program for elderly patients. Namely, Croatia joined Eurotransplant in 2007. Our "senior" program is based on the allocation of kidneys from donors >65 years to recipients at the same age, but we included human leukocyte antigen matching in the allocation scheme in contrast to the ESP [3,6].

Hereby, we present a case of an old woman who was transplanted in 1994 when "senior" program was still not

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established and when kidney transplantation at this age was rarity in many countries.

Case report

A 72-year old woman started a haemodialysis (HD) treatment as a method of choice of RRT in 1994. The primary renal disease that had led to the development of end-stage renal disease (ESRD) was polycystic kidney disease. Following proper pretransplantation screening, she was transplanted six months after she was started with HD. She was subjected to cadaveric kidney transplantation and she got a kidney from a 34-year-old donor. The number of HLA mismatches was 3, and cold ischemia time was 10 hours. The patient received immunosuppressive therapy consisting of cyclosporine (dose adjusted to trough levels), azathioprine (initial dose 100 mg per day) and corticosteroids, e.g. methylprednisolone. She had no delayed graft function (defined as the need for dialysis for >7 days after transplantation) or any surgical complications. On the twentieth day of hospitalization, she was discharged from the hospital. Onemonth following transplantation her graft function was good with serum creatinine values of 90 µmol/L. She did not experience any acute rejection crisis. During the next five years posttransplantation she was coming on regulatory ambulatory monitoring at our Department. She complained only of the pain in the back. In 1999 she was admitted to our Department due to high blood pressure. After the initiation of an anti-hypertensive therapy consisting of calcium-channel blocker and renninangiotensin inhibitor, her blood pressure levels were satisfactory and she was discharged from our Department. Her graft function was stable (serum creatinine was around 90 µmol/L). In 2008 she was hospitalized because of hydronephrosis grade I. During the hospital stay urological and nephrological diagnostic strategies did not find the cause of hydronephrosis. Her serum creatinine values were between 100 and 120 µmol/L. During that time her immunosupressive therapy was modified (azathiprine was switched to mycophenolate mofetil). She died in 2011 at the age of 89 years with a functional graft. Her last values of serum creatinine were between 120 and 130µmol/L. She lived 16 years and 8 months with a well-functioning graft (Figure 1).

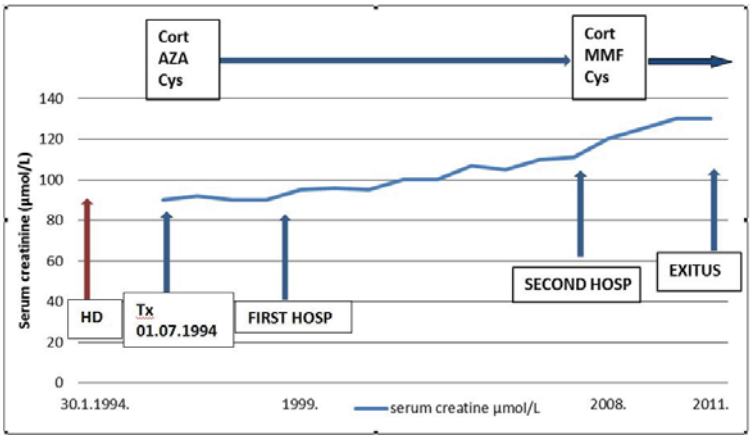

Fig. 1. Shematic view of the course of treatment in our patients
*Hemodialysis (HD); transplantation (Tx); hospitalization (hosp);corticosteroid (Cort); azathioprin (AZA); cyclosporine (Cyc); mycophenolate mofetil (MMF)

Discussion

It has been shown by numerous studies that there is less mortality in patients who receive a kidney transplant compared to those who remain on dialysis. For example, according to the study by Wolfe, *et al.* [7] who used data from the United States Renal Data System (USRDS) of more than 250.000 incident dialysis patients from 1991 to 1996, the survival of patients receiving a deceased donor kidney transplant was longer than that of patients on maintained dialysis. The survival was the best in younger ESRD patients, but patients of all ages gained additional years of life with transplant compared with dialysis. Furthermore, according to the recent study by Dempster, *et al.* [8], older patients experience good outcomes following renal transplantation. Similar results were reported by some other studies. Therefore, renal transplantation is the treatment of choice for ESRD patients. Although survival, quality of life and economic advantages have been shown after renal transplantation in elderly patients, including even those with co-morbidities, it is still limited for many old people with ESRD. We wonder why renal transplantation is still a limted treatment modality for many old people. We do not have clear reasons for this; many clinicians might view dialysis as a stable strategy with an acceptable survival and few short-term risks. Furthermore, it is possible that a perception among health care providers is that transplantation has significant risks for short-term morbidity and mortality. It is also possible that a decreased interest in kidney transplantation among older patients could be a contributing factor [1-6]. Our patient lived 16 years and 8 months with a well-functioning graft and had a good quality of life. It is noteworthy that our

patient received kidney transplant in 1994 when kidney transplantation at this age was rarity in many countries. Namely, according to the study that was published in 1999 [7], only about 1% of ERSD patients older than 70 years received a deceased donor transplant in the USA. In the approach to the elderly patients who are considered for renal transplantation, several factors are important. In addition to recipient comorbidities, donor quality, immunosuppressant, dialysis vintage, and strength of social support networks affect the success of transplantation. Older recipients are more likely to have comorbid diseases at the time of transplantation than younger patients and these conditions can be associated with higher posttransplantation morbidity. The most important comorbid conditions are cardiovascular diseases (CVD). As we know, the risk of CVD increase with the progression of chronic kidney disease and CVD are responsible for almost 40% of all deaths in the ESRD patients [6-8]. Furthermore, it is well-documented that the traditional risk factors for CVD development (arterial hypertension, diabetes mellitus, dyslipidemia and obesity) are common among renal transplant recipients (RTRs). This is mainly a consequence of immunosuppressive therapy that is used in these patients. Similar to the dialysis population, the most common cause of mortality in RTRs is CVD. But, studies showed that the risk of cardiovascular death for all age groups of patients is greater for wait-listed patients than for transplant recipients. Therefore, in comparison with dialysis population, transplantation is associated with a decreased risk of cardiovascular death [9-11]. Or we can say that mortality rate in older people with ESRD is lower when treated with transplantation than with alternative forms of RRT. Prior to transplantation, we have evaluated the cardiac status of our patient and it was satisfactory. She was treated with HD only six months before transplantation.

It is generally assumed that aging is associated with a progressive decline in immune function. But, a variety of co-factors, including co-morbidities, drug-drug interactions, diet, renal and liver function, and immunosenescence may influence on the overall effect of immunosuppressive drugs in elderly patients. On the other hand, studies showed the significantly reduced rate of biopsy-proven acute rejection of renal transplant in elderly patients. It is assumed that in most of the patients we can reduce dosage of immunosuppressive medications. This is important in older patients because it has been documented that immunosuppressive dose reduction has been associated with improved recipient and graft survival, reduction in CVD, reduced drug side-effect and economic benefits. But, we still cannot make generalizations about the effect of individual immunosuppressive drugs in older patients; therefore, there is a need for further studies that will give guidelines about choice and dosage of immunosuppression in older renal transplant recipients [6,8,12,14].

Conclusions

In conclusion we can say that some of the major challenges facing transplant programs are related to the evaluation, education, and list maintenance of elderly transplant candidates. In general, elderly ESRD patients benefit from kidney transplantation because mortality rate in older people with ESRD is lower when treated with transplantation than with alternative forms of RRT. It is important to recognize that risk factors may predict a complicated posttransplantation course. Therefore, after carefully weighing the risk and benefits of transplantation, elderly ESRD patients should be considered for kidney transplantation, with case-by-case individualization.

Conflict of interest statement. None declared.

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