

"Consensus statement on screening, diagnosis, classification and treatment of endemic (Balkan) nephropathy."

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Abstract

Currently used diagnostic criteria in different endemic (Balkan) nephropathy (EN) centers involve different combinations of parameters, various cut-off values and many of them are not in agreement with proposed international guidelines. Leaders of EN centers began to address these problems at scientific meetings, and this paper is the outgrowth of those discussions. The main aim is to provide recommendations for clinical work on current knowledge and expertise. This document is developed for use by general physicians, nephrologists, urologist, public health experts and epidemiologist, and it is hoped that it will be adopted by responsible institutions in countries harboring EN. National medical providers should cover costs of screening and diagnostic procedures and treatment of EN patients with or without upper urothelial cancers.

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Consensus statement on screening, diagnosis, classification and treatment of endemic (Balkan) nephropathy

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ABSTRACT

Currently used diagnostic criteria in different endemic (Balkan) nephropathy (EN) centers involve different combinations of parameters, various cut-off values and many of them are not in agreement with proposed international guidelines. Leaders of EN centers began to address these problems at scientific meetings, and this paper is the outgrowth of those discussions. The main aim is to provide recommendations for clinical work on current knowledge and expertise. This document is developed for use by general physicians, nephrologists, urologist, public health experts and epidemiologist, and it is hoped that it will be adopted by responsible institutions in countries harboring EN. National medical providers should cover costs of screening and diagnostic procedures and treatment of EN patients with or without upper urothelial cancers.

INTRODUCTION

This paper represents updated recommendations developed during the 'International workshop on diagnostic criteria on Endemic Nephropathy' held in Brač, Croatia, in 2008. The final comments were made at a meeting organized in 2012 (Skopje, Macedonia). The original aims of the workshop were to provide recommendations for the screening, diagnosing and therapy of patients with endemic (Balkan) nephropathy (EN) based on current knowledge. Leading experts were invited to address specific issues in the fields of public health, epidemiology, basic science, nephrology, urology, pathology, oncology and radiology. Data were presented and discussed in several expert sessions and at the end all panelists met in the final executive session to prepare the consensus statement. PubMed was searched using terms Balkan endemic nephropathy, aristolochic acid (AA) nephropathy and urothelial cancers, and systematic literature

review was circulated with specific questions prepared in advance to define the scope of the meeting. The Consensus statement is intended to serve as the scientific document of the conferences. It is developed for use by general physicians, nephrologists, urologist, public health experts and epidemiologist, and reflects the current state of knowledge and will need to be modified according to the development of new data and evidence.

It is hoped that the criteria proposed here will be adopted in all countries harboring EN. Importantly, by using same diagnostic criteria and clinical guidelines, it will become feasible, for the first time, to compare results obtained in countries where EN and associated upper urothelial cancers (UUC) remain a major medical problem.

Endemic (Balkan) nephropathy

EN, a chronic tubulointerstitial nephropathy characterized by an insidious onset and gradual progression to end-stage renal disease (ESRD), was first described 50 years ago and remains an important medical, social and economic burden for all countries harboring this devastating disease [1]. The number of patients undergoing dialysis remains unchanged [1–4]. However, a shift to older ages has been recorded among newly diagnosed cases pointing to lower exposure. High prevalence of UUC is an important characteristic of EN [1, 4, 5].

A variety of environmental agents have been investigated [1, 6–8]. The most widely studied ochratoxin A was rejected as an important factor for EN/UUC by the *EU Committee on Food Safety* [9]. Recent studies strongly suggest that AA is a major risk factor for EN/UUC most likely ingested via home-baked bread prepared from flour contaminated by seeds of *Aristolochia clematitis* [10–12]. Identified deoxyadenosine-aristolactam (dA-AL) DNA adducts in the renal cortex of patients with UUC and EN but not in patients with other forms of chronic kidney disease (CKD) and the predominance of 'fingerprint' A: T→T: A mutations in the p53 gene strongly support the

authors' conclusion that AA was the environmental mutagen involved [10, 11]. AA nephropathy (AAN) and EN are very similar in their clinical manifestations and pathophysiology [13–15]. EN- and AA-related nephropathy have the same etiology and depending on exposure dose and duration, one can develop relatively rapid progressing renal disease as was the case in the Belgian cohort, or a more slowly phenotype as was found in EN [6, 10–15]. Observed differences between neighboring villages in the prevalence of EN could reflect varying levels of exposure based on differences in the microenvironment, agricultural practices or dietary habits. In affected households, both genetically related and non-related family members are at risk, supporting the argument that household aggregation is more important than heredity [16]. EN reflects interactions between environmental factors and genes. EN patients could have the same genetic variant, which if combined with the common 'household' exposure could result in disease. EN affects both genders with slight female predominance.

Thus far, specific biomarkers for EN have not emerged [1, 17, 18]. Diagnostic criteria for EN were described more than 40 years ago but those currently used by EN centers involve different combinations of elements, various cut-off values and many of them are not in agreement with proposed international guidelines [19–24]. Leaders of EN centers began to address these problems at scientific meetings and this paper is the outgrowth of those discussions.

Methodological issues in epidemiology of EN

Position Statement 1: Early detection of EN/UUC is important. Subjects who screen positive for EN should be subjected to a diagnostic algorithm. Screening for EN is not justified for children and teenagers, and screening out of EN villages should be limited to sporadic forms of EN and family members who moved from the endemic areas.

Ethical considerations should be taken into account in screening surveys. Identification of new EN foci is not a priority.

Determination of kidney impairment in EN

The Kidney Disease: Improving Global Outcomes (KDIGO) guidelines recommend reporting estimated glomerular filtration rate (eGFR) in adults using the 2009 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation [24]. Serum creatinine should be measured using assays with calibration traceable to the international standard reference materials and minimally biased compared with isotope-dilution mass spectrometry. Albumine-to-creatinine ratio (ACR) was suggested for initial testing of proteinuria in untimed urine samples [24]. ACR ≥ 30 mg/g should be confirmed on a random untimed urine with a subsequent early morning urine sample.

Position Statement 2: eGFR and albuminuria (UAE) in EN should be determined and interpreted as in all other CKD patients according to the KDIGO 2012 recommendations (24).

Proximal tubule dysfunction is a hallmark of EN. $\beta 2$ -microglobulin ($\beta 2M$) has been used in screening of subjects for EN [1, 17, 25], but certain disadvantages were realized. Studies of cadmium nephropathy and EN suggested that alpha1 microglobulin ($\alpha 1M$) is more reliable than $\beta 2M$ as a biomarker of proximal tubule damage [17, 25]. In the KDIGO 2012 guideline, $\alpha 1M$ was the mostly discussed protein in evaluation of tubular proteinuria [24]. Ikeda *et al.* concluded that single determination of $\alpha 1M$ is acceptable for screening purposes, provided that samples have adequate urine density [$0.5 < \text{urine creatinine (g/L)} < 3.0$; or $1.010 < \text{specific gravity} < 1.030$] [25]. A variety of cut-off values have been used for $\alpha 1MCR$. Based on the analysis of urine samples of 939 subjects, values of 23.5 and 31.5 mg/g were recently proposed as cut-off values for screening and confirming purposes, respectively [26]. Bergon *et al.* analyzed the utility of $\alpha 1M/UAE$ ratio and concluded that an $\alpha 1M/UAE$ ratio of 0.91 could be used as a discriminating value [27]. Above this threshold, proteinuria could be defined as tubular in the presence of albuminuria. However, to avoid possible bias caused with calculating ratios with small numbers, it was proposed that in addition to $\alpha 1M/UAE > 0.91$, $\alpha 1M$ should be above the cut-off value.

Position Statement 3: $\alpha 1M$ is a reliable indicator of proximal tubular dysfunction in EN. A repeat sample should be obtained from subjects with inadequate urine density. In subjects with albuminuria, the ratio $\alpha 1M/UAE$ might help in distinguishing whether proteinuria is dominantly of tubular origin.

Criteria for screening and confirmation

UUC was for the first time introduced as one of the criteria for screening of EN. The other novelty in screening procedure is recommendation for the screening for EN out of known endemic villages. Based on observations and the fact that out of established endemic villages farmers might have been exposed to AA those who should be screened are listed in Position Statement 5.

Position Statement 4. *Criteria for screening in endemic villages are:* Residency in known endemic village and/or in households with cases of EN/UUC for more than 20 years; proximal tubular damage; decreased eGFR; UUC.

Position Statement 5. *Subjects who should undergo selective screening for EN out of endemic areas ('sporadic EN' cases) are:* (i) Patients where there is no alternative explanation for their CKD or patients with UUC living in farming villages outside of endemic regions not restricted only to Balkan region; (ii). Family members of: (a) patients named in (i); (b) EN patients undergoing dialysis in non-endemic dialysis units; (c) transplanted EN patients living in non-endemic areas (emigrants).

The diagnosis of EN can be confirmed by pathologist. However, it is not ethically justified or cost-effective to perform renal biopsies on everyone suspected of contracting EN. As for iatrogenic AA nephropathy, if no alternative cause of CKD is identified, using elements listed in Position Statement 6, we make a diagnosis of EN (Recommendation D).

Position Statement 6. Elements for establishing diagnosis of EN:

1. Evidence that proximal tubular impairment is a dominant feature.
2. Anemia adjusted to age and gender. Other causes of anemia should be excluded.
3. Other kidney diseases should be excluded. Kidney biopsy should be advised to patients where overlapping with other CKD is suspected and to subjects suspected of having 'sporadic' EN.
4. Environmental exposure to nephrotoxic agents other than AA should be excluded.

Morphologic criteria of EN. There are no diagnostic features which are pathognomonic of EN but the pattern of injury, in the absence of other disease is highly suggestive of this entity [28, 29].

Extensive hypocellular interstitial fibrosis associated with tubular atrophy involving medullary rays, the attending outer medulla and the cortical labyrinth where it decreases typically from the outer to the inner cortex is the consistent renal histologic finding characterizing the chronic progressive nephropathy affecting members of the same household in EN villages. In approximately one-third of the cases, these changes are accompanied by chronic interstitial inflammatory cells mainly in medullary rays and/or outer medulla, usually less than that might be expected in other renal diseases. About 40–46% of the patients will develop multifocal urothelial cancer of the transitional cell type usually in the upper urinary tract.

Glomerular and vascular lesions are associated with periglomerular fibrosis, glomerular lesions including ischemic, microcystic, obsolescent (collaptic type) glomeruli, occasional thrombotic microangiopathy-like lesions (glomerular basement membrane duplications or subendothelial fluffy widening) and focal segmental sclerosis-like lesions. Vascular lesions include arteriolar hyalinosis, intimal fibrous hyperplasia, occasional mucoid arterial intimal fibrosis and by electron microscopy multifocal thickening and splitting up of peritubular capillary basement membranes. These additional lesions likely are secondary to the progressive kidney destruction by the tubulointerstitial fibrotic process. At end-stage, the kidneys are extremely small, symmetrically contracted weighing only 20–30 g, each with smooth outlines. Histologic documentation of incipient lesions in these patients are absent. All potential causes of interstitial fibrosing nephropathy must be ruled out on the basis of morphologic and clinical data. This type of interstitial fibrosis is not seen in common end-stage renal failure

like nephrosclerosis, chronic pyelonephritis or reflux nephropathy and chronic glomerulonephritis. In contrast, it shares remarkable similarities with the type of renal fibrosis found in AAN which, in turn, is associated with a similar prevalence of UUC as observed in EN. Nevertheless, a similar interstitial fibrosis has been reported following exposure to cadmium, lead, cyclosporin A, ifosfamide, pamidronate, lithium, nitrosoureas and some herbal tea. Exposure to these agents should be ruled out on the basis of clinical and anamnestic data. This list is not exhaustive as other substances may induce a similar type of renal fibrosis. Of note, a similar pattern of renal fibrosis has been reported in pigs, but not in humans, exposed to OTA.

Although the pattern of renal interstitial fibrosis in analgesic nephropathy is markedly different from AAN, it is associated with urothelial malignancy in 5–24% of patients. Thus, consumption of non-steroidal antiinflammatory drugs (NSAIDs) should be ruled out. Clinician, in conjunction with the pathologist, should make a best diagnosis.

Diagnostic algorithm and guidelines for treatment and follow-up (Figs 1 and 2)

Recommendation A: screening and monitoring

- 1A. The entire adult population of EN villages should be screened. Diseased should be referred to local nephrologists.
- 2A. Suspects and members of EN households with no signs of either proximal tubular damage or UUC should be monitored on a yearly basis.
- 3A. Mass screening procedure should be repeated in the EN villages after five years.
- 4A. Tubular proteinuria, eGFR, red blood cell count, and urine cytology should be determined.
- 5A. Renal function should be checked in all UUC patients from farming villages. Renal cortex excised during surgery (distant from tumor) should be analyzed for the evidence of EN, and if possible, should be frozen at -20°C for subsequent determination of the level of AA-DNA adducts. p53 mutational spectrum should be determined on tumor tissue.
- 6A. Patients with ESRD of unknown origin from non-EN villages and members of such households should be screened to identify cases of sporadic EN/UUC.

Recommendations B-1: Diagnostic methods for EN.

- 1B. Only adequate random urine samples should be utilized.
- 2B. Cut-off values for screening for UAE and α -1MCR are 10 mg/L and 23.5 mg/g, respectively. α -1M/UAE should be used to evaluate patients with albuminuria. Cut-off value is ≥ 0.91 . α -1MCR should be used for confirmation of diagnosis and for follow up. Cut-off value is 31.5 mg/g.

- 3B. 24-h protein excretion should be measured in subjects with: reduced eGFR; with albuminuria and $\alpha 1\text{M}/\text{UAE} < 0.91$.
- 4B. GFR should be estimated using the 2009 CKD-EPI equation.
- 5B. Patients with EN should be classified according to the KDIGO 2012 Guideline.
- 6B. Anemia is defined as a hemoglobin level < 120 g/L for men and women > 50 years, and < 110 g/L for women ≤ 50 years.
- 7B. Renal biopsy should be considered in subjects with: proteinuria ≥ 1.0 g/dU; with suspected other coexisting kidney disease.
- 8B. Patients with EN in CKD stages $\geq 3\text{A}$ and patients with histopathological findings associated with chronic aristolochic acid nephropathy should be monitored for UUC.

- Recommendations B-2: Diagnostic methods for UUC
- 9B. Subjects at high risk for developing UUC (patients with: histopathological findings indicative of EN; EN patients in CKD stages $\geq 3\text{A}$ or undergoing dialysis) should be monitored every 6 months using urine cytology, ultrasound, and other imagine techniques.
 - 10B. Members of EN households should be examined every year.
 - 11B. Patients with previous UUC, bladder cancer or having hematuria should be examined every three months. Hematuria should be evaluated by cystoscopy.
 - 12B. Available imagine techniques (contrast CT and ureteropyeloscopy) should be used to visualize UUC [30].
 - 13B. In EN transplant patients and in EN patients undergoing dialysis cystoscopy should be performed every 6 months.

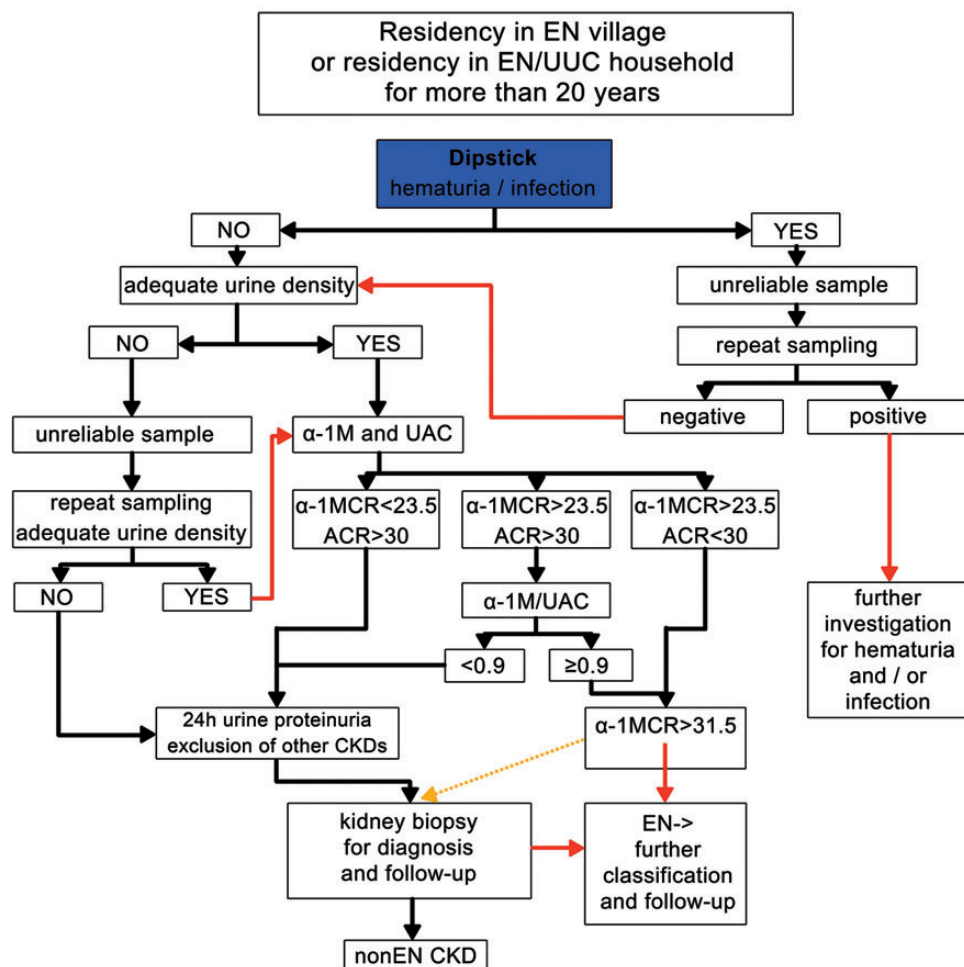


FIGURE 1: Diagnostic algorithm for endemic (Balkan) nephropathy. $\alpha\text{-1M}$, alpha1 microglobulin (mg/L); UAC, albumin (mg/L); $\alpha\text{-1MCR}$, alpha1 microglobulin/urinary creatinine (mg/g), ACR, albumin to creatinine ratio. Orange arrow denotes optional; decision may be made by nephrologist.

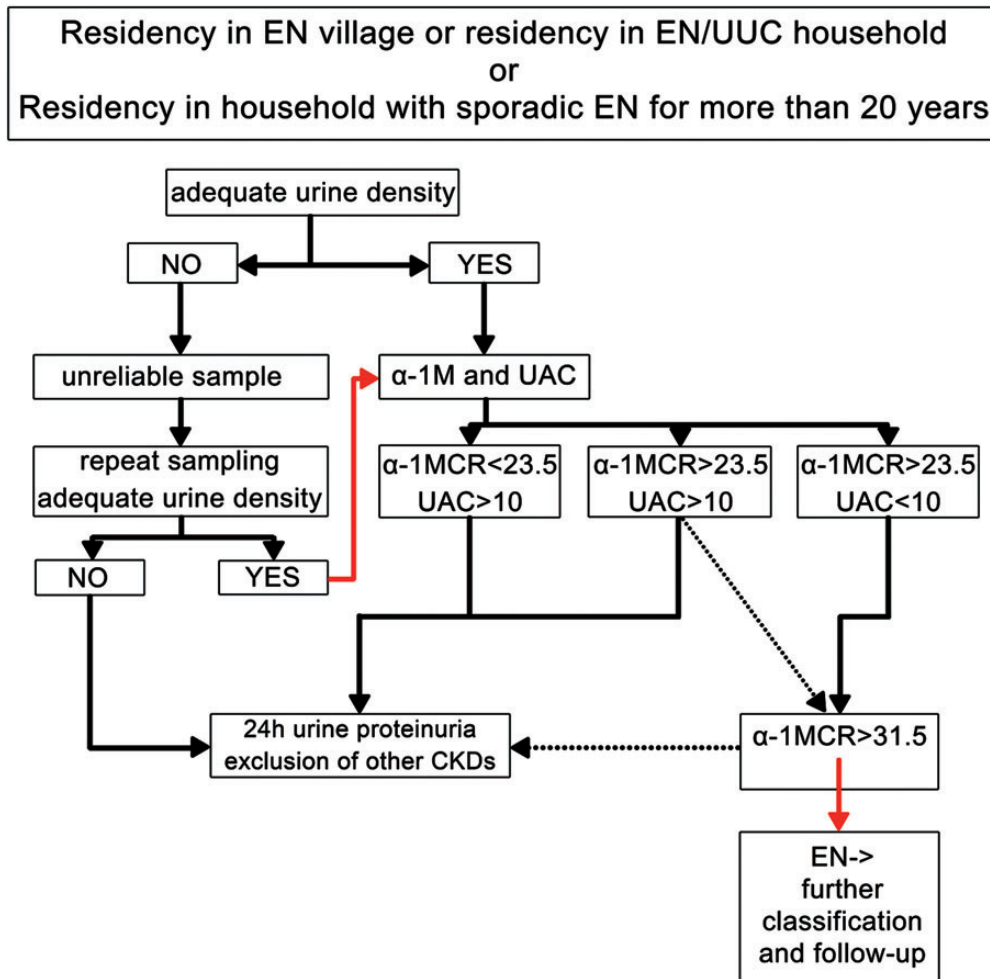


FIGURE 2: Diagnostic algorithm for patients with upper urothelial cancer and the risk for endemic (Balkan) nephropathy. For abbreviations, see legend in Figure 1. Histopathology of renal cortex (distant from tumor) obtained during surgery of patient with UUC may be sufficient for making definitive diagnosis of EN. The proposed algorithm will help to establish the degree of renal impairment, and it is also recommended for patients with bladder cancer. If the diagnosis of EN is established, further examination of upper urinary tract is warranted. As hematuria is frequently present in patients with urothelial cancer, dipstick is unreliable.

Treatment and prevention of patients with EN

Recommendation C: Prevention and treatment

- C1. Villagers should be informed that: (a) EN has insidious onset but invariably progresses to ESRD and that early diagnosis enables implementation of treatment that may slow down the progression of CKD; (b) EN and UUC are not hereditary diseases, but also should know that genetic predisposition is likely, but relevant only after long-term exposure to environmental nephrotoxins.
- C2. Villagers should be strongly encouraged to participate in screening programs and to visit local nephrologists.
- C3. Public health authorities should educate exposed populations and the role of AA in EN should be emphasized.
- C4. Public health and agricultural authorities should initiate efforts to eliminate *Aristolochia* from fields where grain is harvested.
- C5. Patients should be informed by family general practitioners as to the importance of primary and secondary

prevention of CKD starting with the importance of lifestyle changes, and about high risk of developing UUC and the need for regular monitoring.

- C6. Patients with established EN should be treated like other patients with CKD and ESRD, by peritoneal dialysis, hemodialysis or by renal transplantation.

Recommendation C: Treatment of patients with UUC

- C10. Standard therapy is total nephroureterectomy with excision of a cuff of bladder around ureteral ostium and regional lymphadenectomy. This is supported by the observation that recurrent tumors were observed in EN patients more frequently than in other patients with UUC.
As for iatrogenic form of aristolochic acid nephropathy routine cystectomy is not recommended in pre renal replacement therapy patients because the incidence of bladder cancers was not found to be

higher than of UUC. Cystectomy should be advised if AA-DNA adducts are detected in bladder tissue.

- C11. A conservative surgical approach should be reserved only for the highly selected patients with bilateral tumors in whom nephron sparing is essential. Patients treated with a conservative approach are at increased risk of local recurrence and require frequent and careful follow-up including imaging procedures and endoscopies.
- C12. All patients with UUC should be monitored carefully for recurrent or new tumors.
- C13. Systemic chemotherapy for unresectable and metastatic disease is indicated and all other oncologic treatment and monitoring of EN/UUC patients should follow general recommendations [30].

Renal transplantation in EN

In recent years, the number of renal-transplanted patients in Croatia has significantly increased. It is hoped that a similar pattern will follow in other countries harboring EN. Transplanted AAN patients are at high risk of developing UUC [31]. For this reason, AAN patients underwent bilateral nephroureterectomy either before or after renal transplantation. In Croatian registry, in the group of 40 transplanted EN patients more than 40% developed UUC [32]. This fact argues strongly for bilateral nephrectomy of native kidneys in EN-transplanted patients. The level of AA-DNA adducts should be determined in renal cortical tissue of removed kidneys [10, 11]. It was proposed that an international registry should be established for renal transplant

recipients with EN. No recurrence of EN has been found after transplantation.

Recommendation C: Renal transplantation for patients with EN

- C15. EN patients should be placed on waiting lists for renal transplantation along with other patients with ESRD but should undergo appropriate examinations before to exclude urothelial cancers.
- C16. For living donor transplantation, additional investigations should be performed. If the donor lived in the EN region for more than 15 years, renal biopsy should be performed to exclude EN and/or presence of AA-DNA adducts. Finding of each of them would exclude the subject as a living donor.
- C17. Considering the reported high incidence of UUC in EN/AAN transplanted patients, all patients with EN should undergo bilateral nephroureterectomy before transplantation. Bilateral nephroureterectomy should be performed in all EN recipients younger than 65 years. In EN patients older than 65 years bilateral nephroureterectomy should be performed prior to renal transplantation if (a) UUC or bladder cancer were already diagnosed; (b) family history for UUC or EN is positive
- C18. After transplantation, EN patients who refused bilateral nephroureterectomy should be monitored closely for UUC.
- C19. Immunosuppression with mTOR inhibitors should be considered for EN-transplanted patients.

Recommendation D: Classification of EN

I. Diseased/affected	II. Suspected EN
(1) Biopsy proven/indicative of EN ¹ or (2) Residency in an EN household >20 years + tubular proteinuria ² + decreased eGFR + anemia ² or (3) Residency in EN village >20 years + UUC + tubular proteinuria ²	(1) Residency in EN household >20 years + reduced eGFR + anemia ³ or (2) Residency in EN household >20 years + tubular proteinuria ² or (3) Residency in EN village >20 years + UUC
III. High risk group for EN	IV. Sporadic EN ⁴
(1) Residency in EN households >20 years (2) Residency in households with sporadic/suspected EN cases >20 years	Biopsy proven/indicative of EN in patient with UUC outside of the endemic region or in member of their household

¹There are no diagnostic features which are pathognomonic of EN but the pattern of injury, in the absence of other disease is highly suggestive of this entity. Detection of AA-DNA adducts and p53 fingerprint mutation is diagnostic.

² α -1MCR >31.5 mg/g and α -1/UAC \geq 0.91.

³Hemoglobin <120 g/L for men and women >50 years, and <110 g/L for women \geq 50 years.

⁴Subjects with chronic interstitial nephropathies where other causes should be excluded (reflux nephropathy, chronic pyelonephritis, recurrent pyelonephritis, hypertensive nephrosclerosis, exposure to lead, cadmium, herbs containing AA, cyclosporin A, ifosfamide, pamidronate, lithium and nitrosoureas, heavy use of NSAID).

Classification of EN population

Population of EN villages should be classified as described. Patients with UUC living in non-EN villages and members of their households should be screened for sporadic EN. In EN patients and in those suspected of having EN, other causes of chronic tubulointerstitial nephropathies should be excluded as well as occupational exposure to nephrotoxic agents and the ingestion of herbs. The latter must be repeatedly asked to the patients as most of them do not even mention their consumption as they believe that herbal remedies are natural hence innocuous products.

Concluding statement

EN is a form of chronic AA nephropathy where environmental etiological agent, i.e. AA, was ingested over years in small doses via contaminated bread contrary to AA nephropathy described worldwide where the same agent was ingested in higher doses either intentionally or inadvertently as a part of a medical treatment. Renal community all over the world should become aware that Balkan endemic nephropathy and AA nephropathy are the same disease frequently associated with upper urothelial cancers and caused with the same toxin. Balkan endemic nephropathy and AA nephropathy differ only in the clinical course.

This document should be adopted by responsible institutions in countries harboring EN. National medical providers (state health insurance companies) should cover costs of screening and diagnostic procedures and treatment of EN patients with or without UUC. Diagnostic algorithm and guidelines should be updated when new evidence-based data is in hand.

DISCLAIMER

This consensus document represents views of the international workgroup of experts in endemic nephropathy and AA nephropathy and this position statement is not an official view of ERA-EDTA.

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CONFLICT OF INTEREST STATEMENT

Authors do not have any financial interest with any company or any source for the purpose of writing the manuscript and any other financial connections, direct or indirect, or other situation that might raise the question of bias in the work reported or the conclusions, implications, or opinions stated, including pertinent commercial or other sources of funding for the individual authors or the associated departments or organizations, personal relationships, or direct academic competitions.

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