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MINIREVIEWS

New antigens involved in membranous nephropathy beyond phospholipase A2 receptor

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Abstract

When the physiopathology of membranous nephropathy was first described, almost 30% of cases were recognized to be secondary to well-known diseases such as autoimmune diseases, tumors or infections. The remaining 70% cases were called primary membranous nephropathy as the exact mechanism or pathogenic factor involved was unknown. The discovery of the M type phospholipase A2 receptor and thrombospondin type 1 domain containing 7A as causative antigens in these "so called" primary membranous nephropathies provided new insights into the effective causes of a large proportion of these cases. Novel techniques such as laser microdissection and tandem mass spectrometry as well as immunochemistry with antibodies directed against novel proteins allowed the confirmation of new involved antigens. Finally, using confocal microscopy to localize these new antigens and immunoglobulin G and Western blot analysis of serum samples, these new antigens were detected on the glomerular membrane, and the related antibodies were detected in serum samples. The same antigens have been recognized in some cases of secondary membranous disease due to autoimmune diseases, tumors and infections. This has allowed examination of the relationship between antigens in primary membranous nephropathy and their presence in some secondary nephropathies. The aim of this study is to describe the characteristics of the new antigens discovered and their association with other diseases.

Key Words: Membranous nephropathy; Exostosin ½; Neural cell adhesion molecule 1; Neural epidermal growth factor like-1 protein; Protocadherin 7; Semaphorin 3B

Core Tip: The pathophysiological mechanisms of membranous nephropathy have been partially known for a long time. Novel techniques have allowed identifying several antigens and the corresponding antibodies as the main cause of a large part of these diseases. Therefore, a large part of membranous nephropathy, once called primary, are due to immune complexes whose components are now recognized. The same antigens have been recognized in a part of secondary membranous disease, which are due to autoimmune diseases, tumors and infections, diseases. This fact allows a relationship between antigens found either in primary membranous nephropathy or in some forms of secondary nephropathies.

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INTRODUCTION

Membranous nephropathy (MN) is a rare disease that affects podocytes and is characterized by the accumulation of immune deposits on the subepithelial side of the glomerular capillary wall. These immune deposits consist of immunoglobulin (Ig) G directed against antigens that have long remained unknown. MN is referred to as primary MN when there is no association with a known disease (70% of cases) or secondary MN when MN occurs in association with clinical conditions such as autoimmune diseases, tumors, infections and hepatitis B (30% of cases). Two studies in 2009 and 2014 allowed us to identify causal antigens involved in primary MN[1,2]. A related study reported the first human podocyte antigen in a rare subset of infants born with MN that developed because the mother was deficient in neutral endopeptidase (NEP) due to a truncating mutation in the *MME* gene coding for NEP [3,4]. The first antigen, identified in 2009, is the M type phospholipase A2 receptor 1. The antigen recognized in the 2014 study is thrombospondin type 1 domain containing 7A (THSD7A)[1,2]. Using Western blotting and mass spectrometry, THSD7A was identified in serum samples from patients with MN. Additionally, immunohistochemical analysis of biopsy samples from the same patients revealed that THSD7A localized to podocytes, and immunoglobulin G (IgG) eluted from these samples was specific for THSD7A.

Phospholipase A2 receptor (PLA2R) and THSD7A are involved in 70% and 5% of primary MN cases respectively (Figure 1).

These antigens were thought to be specific to primary MN, but were also later found in patients with MN related to hepatitis B infection and sarcoidosis[5-7].

Novel techniques

Recently, an approach using laser microdissection and tandem mass spectrometry (MS/MS) enabled the detection of novel proteins in glomerular diseases. MS/MS can identify approximately 1500-2000 proteins in glomerular extracts and allows semiquantitative measurements.

Briefly, these techniques were used to identify proteins with high spectral counts in PLA2R-negative MN patients and control patients with different nephropathies. This new protein was identified, using immunochemistry with antibodies directed against the new protein, which revealed membranous staining along the glomerular basement membrane (GBM), confirming a new antigen involved in MN.

Finally, using confocal microscopy to localize the new antigen and IgG and Western blot analysis of serum samples, we detected the new antigen on the glomerular membrane and the related antibody in serum samples[8].

The aim of this study is to describe the characteristics of the new antigens discovered principally thanks to these novel techniques and to clarify their association with other diseases.

Exostosin 1/ 2 associated MN

An examination of both serum samples and glomerular eluates from patients with the so- called idiopathic MN negative for PLA2R with these new techniques revealed the first novel antigens, namely exostosin 1 and exostosin 2 (EXT1 and EXT2, respectively).

EXT1/EXT2-positive MN cases were more common in females (80.9 with a mean age of 35.7 years) [8]. In the first report, the mean serum creatinine and proteinuria levels at presentation were 1 mg% and 5.9 g/24 h, respectively. A total of 70.8% of patients had abnormal laboratory values for antinuclear antibodies, double-stranded DNA antibodies, anti-Smith antibodies or anti-Sjogren syndrome-related antigen A or B[9]. Thirty-four percent of patients had a clinical diagnosis of systemic lupus erythematosus.

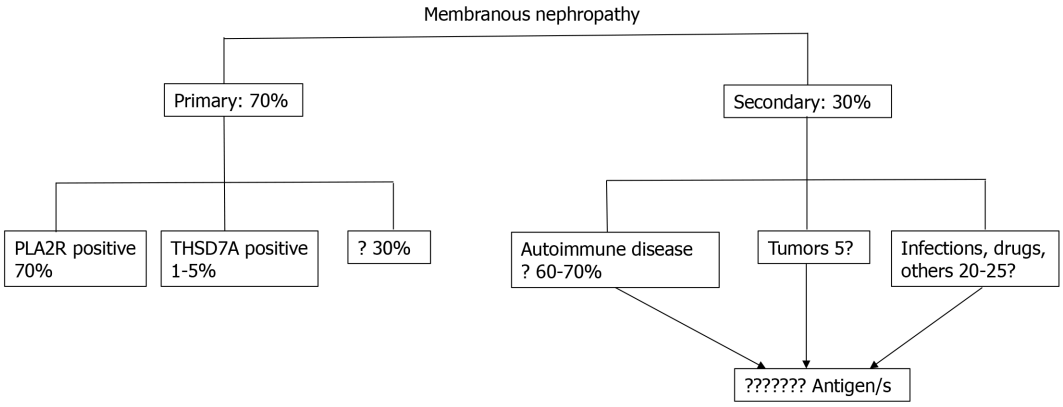

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Figure 1 Membranous nephropathy. Old classification of membranous nephropathy. PLA2R: Phospholipase A2 receptor; THSD7A: Thrombospondin type 1 domain containing 7A.

Based on MS analysis, all four classes of IgG were present in patients with EXT1/EXT2-positive MN with IgG1 the most abundant, followed by IgG2, IgG3 and IgG4. In addition to IgG and C3, 84% of patients exhibited staining for IgA or IgM. Seventy-three percent of patients showed staining for C1q on immunofluorescence, and all patients showed subepithelial deposits. Mesangial deposits were also present in 96% of patients. Subendothelial deposits were less frequently found.

Tubulointerstitial inclusions were present in 80% of patients.

The GBM is composed of mostly type IV collagen, laminin, nidogen and heparan sulfate proteoglycans. Agrin and perlecan are the main heparan sulfate proteoglycans in GBM. Heparan sulfate proteoglycans are present in the basement membrane and matrix and on cell surfaces.

EXTs are glycosyltransferases responsible for the synthesis of heparansulfate, through the addition of glycosaminoglycan residues to the core protein. The result is the generation of complex polysaccharides, which explains why these two proteins are found together[10,11].

EXT1 and EXT2 show structural similarities, and EXT1 and EXT2 can exist as a heterodimers and act as a copolymerases in heparan sulfate chain elongation. The EXT1/EXT2 heterodimer also has increased stability and activity compared to those of the individual proteins, which are transmembrane proteins.

EXTs are secreted into the extracellular medium in a truncated form[12]. Five genes encode EXT proteins: *EXT1, EXT2, EXTL1, EXTL2* and *EXTL3*[13]. Mutations in *EXT1* and *EXT2* generate hereditary multiple exostoses, one of the most common inherited skeletal disorders[14].

To date, it is still difficult to detect circulating anti-EXT1/EXT2 antibodies. This difficulty may be because serum antibodies target truncated EXT proteins or are present at a very low titer.

In a recent study, EXT1/EXT2 were present in 33% of a cohort of patients with membranous lupus nephritis[15]. Compared with EXT1/EXT2-negative membranous lupus nephritis, EXT1/EXT2-positive disease appears to represent a subgroup with favorable kidney biopsy findings with respect to chronicity indices. Indeed, cases of membranous lupus nephritis that are EXT1/EXT2 negative are more likely to progress to end-stage kidney disease (ESKD) than those that are EXT1/EXT2 positive.

In conclusion, using proteomics and immunochemistry, the authors found EXT1/EXT2 in the GBM of PLA2R-negative MN patients. Clinical and biopsy findings showed features of autoimmune disease, including lupus nephritis in 8% of patients[16].

Neural cell adhesion molecule 1

Neural cell adhesion molecule 1 (NCAM1) is a member of the IgG superfamily of proteins that was identified using the techniques described above[17]. NCAM1 colocalizes with IgG within glomerular immune deposits, and antibodies against NCAM1 could be detected in patient sera. NCAM1 was predominantly expressed in membranous lupus nephritis patients but was also found in 2% of primary MN patients. Many lupus nephritis patients with NCAM1 were also positive for EXT2. NCAM patients were also positive for IgA, IgM and C1q. Neuropsychiatric disease occurred in 40% of NCAM-positive patients, probably due to NCAM1 expression in the central nervous system[18].

Neural epidermal growth factor-like 1 protein

Neural epidermal growth factor-like 1 protein (NELL-1) is a secreted, 90-kDa protein expressed in osteoblasts that promotes bone regeneration[19]. The NELL-1 gene is named after its similarity to a gene called *Nel* that is strongly expressed in neural tissue and encodes a protein with epidermal growth factor (EGF)-like repeats (Figure 2)[20].

In the kidney, NELL-1 expression is increased in tubules and detectable in the glomeruli, as 20% of glomerular cells express NELL-1 at the RNA level[21,22].

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Figure 2 Structure of neural epidermal growth factor-1 protein. TSPN: TSP-1-like domain; C-C: Coiled coil domain; VWC: Von Willebrand factor type C domain; EGF: EGF-like domains E.

Sethi *et al*[23] suggested that NELL-1 is associated with MN.

The authors selected PLA2R-negative MN patients and identified this novel NELL-1 protein by laser microdissection and MS. Granular anti -NELL-1 GBM staining was documented using immunohistochemistry, and NELL-1 and IgG colocalization was observed by confocal microscopy. Finally, serum antibodies against NELL-1 were detected by Western blotting. Sethi *et al*[23] concluded that NELL-1 positive MN is a distinct type of MN. The authors suggested that NELL-1 is shed from podocytes rather than entrapped from circulating antigens or immune complexes[24].

Most importantly, Sethi's finding was confirmed by validation in a French cohort and a Belgian cohort.

Kidney biopsy specimens from patients with NELL-1-associated MN showed features of MN with a thickened GBM as well as IgG and C3 expression. IgG subtyping revealed predominantly IgG1. In a subset of the biopsy specimens, a segmental GBM distribution of immune deposits was observed by immunofluorescence and electron microscopy[25].

In particular, *Kudose et al*[25] examined 2003 MN patients without lupus. Fifty of them showed segmental MGN (sMGN) defined by subepithelial deposits involving 25%-75% of the GBM. Among these cases with sMGNs, NELL-1 staining was present in 25%. PLA2R, THSD7A and EXT 1 were negative in all cases evaluated.

Among 21 patients with sMGN at follow-up, 7/21 patients had received immunosuppression, 86% had stable improved renal function, and 60% had complete (45%) or partial (15%) remission of proteinuria. Accordingly, MGN is a rare PLA2R-negative variant of MN with NELL-1 positivity in 29% of patients and favorable prognosis, even in the absence of immunosuppressive treatment.

According to this study, NELL-1 appears to be the first antigen in segmental MN.

In a recent study, Caza *et al*[26] found that NELL-1 is a target antigen associated with MN malignancy. They found active or metastatic malignancy in 33% of patients with NELL-1-associated MN. Additionally, they found NELL-1 positivity within glomeruli and a tumor from the same patient affected by invasive ductal carcinoma of MN in the breast.

The authors concluded that NELL-1, a recently identified antigen in MN, is enriched in patients with malignancy-associated MN, and anti-NELL-1 antibodies can be detected within the serum of these patients.

Finally, a recent study by Spain *et al*[27] found that in patients administered lipoic acid for different conditions, high-grade proteinuria could appear. These patients may have NELL-1-associated MN, and the discontinuation of lipoic acid could result in proteinuria remission.

Semaphorin 3B-associated MN

Semaphorin 3B (SEMA 3B) is a recently discovered target antigen that has been principally detected in pediatric patients, particularly very young children[8]. The mean age of these pediatric patients was 6.9 years, and approximately half of SEMA 3B-associated MN was detected in children < 2 years. Among all

Table 1 Clinical findings in Semaphorin 3B-associated membranous nephropathy[8,28,29]

Case	Age (yr)/sex	UP (g/24)	Serum CR (mg/dL)	Remission	Serum CR/UP/24 h
1	41/F	7.9	0.74	Spontaneous	0.6/no proteinuria (16 mo)
2	26/F	6.2	0.4	Spontaneous	0.43/400 mg (18 mo)
3	2/M	5	0.5	Immunosuppressive	0.35/150 mg (24 mo)
4	40/F	17.3	0.9	Immunosuppressive	0.6/no proteinuria (10 yr)
5	19 mo/M	0.4	0.7	Immunosuppressive	0.9/400 mg (18 mo)
6	2/F	UP/CR ratio 6.81	0.21	Immunosuppressive	UP/CR ratio 0.23 (13 mo)
7	17/M	UP/CR ratio 0.78	0.6	Immunosuppressive	UP/CR ratio 0.1 (19 mo)
8	9 mo/M	UP/CR ratio 0.94	0.45	Immunosuppressive	UP/CR ratio 0.09 /14 yr)
9	2/M	UP/CR ratio 1.95	0.13	Immunosuppressive	UP/CR ratio 0.12 (5 yr)
10	14/M	3	0.64	Lost to follow up	n/a
11	16/M	12	0.83	Immunosuppressive	Dialysis

F: Female; M: Male; UP: Urinary protein; CR: Creatinine.

patients, SEMA 3B-associated MN is rare and accounts for 1%-3% of all MNs. In the pediatric group, it accounts for approximately 15% of MN cases.

After the initial identification of three pediatric patients, an additional eight cases of SEMA 3B-associated MN were identified in validation cohorts from France and Italy[8]. To date, 11 patients with SEMA 3B-associated MN, including three adults have been identified[28].

Using laser dissection and MS/MS, SEMA3B was detected in PLA2R-negative MN biopsies[29].

Semaphorins are a group of secreted and transmembrane/membrane-bound proteins containing a conserved extracellular semaphorin (sema) domain of approximately 500 amino acids that is characterized by highly conserved cysteine residues[30-32].

More than 20 semaphorins have been identified and divided into 8 subclasses. SEMA 3B is a secreted protein with a sema domain, a plexum-semaphorin-integrin domain, an Ig domain and a basic domain (Figure 3).

The semaphorin 3 family and its receptors have been detected in endothelial cells, podocytes and tubular epithelial cells[33]. In SEMA 3B-associated MN, bright granular capillary wall staining for SEMA 3B along the GBM have been documented, and SEMA 3B may be found using immunofluorescence microscopy. Confocal immune fluorescence microscopy analysis has shown the colocalization of SEMA 3B and IgG in glomerular immune deposits.

Using Western blot analysis, anti-SEMA 3B antibodies have been detected in patients with SEMA 3B-associated MN. The SEMA 3B autoantibody can recognize a cryptic epitope that is unmasked by disruption of disulfide bonds.

Clinical findings in SEMA 3B-associated MN are shown in Table 1, and proteinuria remission may be obtained either spontaneously or with immunosuppressive treatment.

Recurrence of anti-SEMA 3B-mediated MN after kidney transplantation was recently reported[34]. Kidney biopsy confirmed histological MN recurrence with the colocalization of SEMA 3B antigen and IgG. Treatment with rituximab was effective, and the disappearance of anti-SEMA 3B antibodies was noted 40 days after rituximab treatment.

Given the discovery of the antigen SEMA 3B, the distribution of podocyte antigens in patients with "primary" MN is presented in Figure 4.

Protocadherin 7-associated MN

Chauhan *et al*[35] performed laser microdissection and MS/MS in kidney biopsies from patients with PLA2R-negative MN and detected a unique protein, namely, protocadherin 7 (PCDH7) in glomeruli from 10 patients who were also negative for THSDF7A, EXT1/EXT2, NELL 1 and SEMA 3B. Additionally, in a validation cohort from the UCLouvain Kidney Disease Network in Belgium, four additional patients were identified[36]. In all patients, immunohistochemistry showed bright granular staining along the GBM. Confocal microscopy showed colocalization of PCDH7 and IgG along the GBM, and Western blot analysis using sera revealed antibodies against PCDH7.

Cadherins are a large group of transmembrane proteins that mediate cell-cell recognition and adhesion[37]. Cadherins are classified into subfamilies on the basis of the number and arrangement of extracellular cadherin (EC) domains. Therefore, cadherins are subdivided into classic cadherins, closely related cadherins, desmosomal cadherins and protocadherins[38]. PCDH7 is a 16-kDa glycosylated protocadherin with seven EC repeats. Its function is unknown, but it is likely important in cell signaling

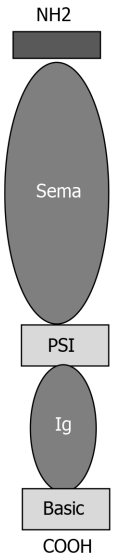

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Figure 3 Structure of Semaphorin. Sema: Sema domain; PSI: Plexin-semaphorin-integrin; Ig: Immunoglobulin.

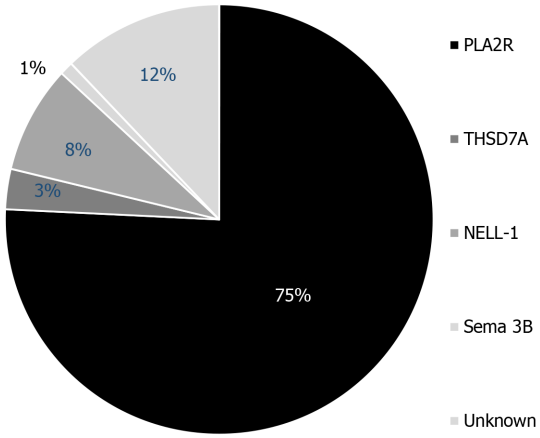

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Figure 4 Distribution of podocyte antigens in patients with 'primary' membranous nephropathy. PLA2R: Phospholipase A2 receptor; THSD7A: Thrombospondin type 1 domain containing 7A; NELL-1: Neural epidermal growth factor-like 1 protein; Sema: Sema domain.

[39]. PCDH7 is mostly present in older patients. Complement activation is minimal in these patients, and spontaneous remission frequently occurs without immunosuppressive treatment. In addition, the MS/MS complement profile of PCDH7-associated MN is lower than that of other antigen-associated MNs[40]. The clinical and pathologic findings in PCDH7-associated MN are described in Table 2.

In conclusion, PCDH7 has been identified as a novel antigen along with circulating anti- PCDH7 autoantibodies, in a subset of adult patients with PLA2R-negative MN.

Interestingly, Bobart *et al*[41] analyzed a total of 320 adult patients with MN in native kidneys between 2015 and 2020. Overall, they found three patients with PCDH7-associated MN. This study is interesting as the authors presented a patient distribution based on the antigen found and the presence of associated diseases (Figure 5). Similarly, the authors presented a table reporting the demographic and clinical characteristics based on the antigen involved (Table 3).

The new classification of MNs is shown in Figure 6.

Sethi[42] suggested that MN is not a single disease but rather a pattern of injury resulting from different diseases. Each antigen associated with the MN pattern should be considered as representative of a specific disease, and each disease results in an MN pattern of injury (Figure 7).

Newly discovered antigens

As described in Figure 6, other antigens and immune complexes are probably less frequently involved. Recently, Contactin 1 was shown to be a novel target antigen in MN associated with chronic inflammatory demyelinating polyneuropathy (CIDP)[43].

Table 2 Clinical and immunomicroscopy findings in protocadherin patients[35,36]

Patient	Age (yr)	Urinary protein (g/24h)	Serum creatinine (mg/dL)	Immunofluorescence	IgG
1	73	3.2	1.1	IgG 3+; C1q 1+	IgG1 2+; IgG3 1+; IgG4 1+
2	66	9.6	1.3	IgG 2+	IgG1 1+; IgG4 2+
3	68	NA	1.1	IgG 2+	IgG4 2+
4	59	3	1.1	IgG 2+; C3 1+	1gG 1+; IgG2 2+; IgG3 2+; IgG4 3+
5	61	7	1.9	IgG 3+; C3 1+	IgG1 2+; IgG3 2+
6	38	3	1	IgG 1+	NA
7	37	1.4	1.76	IgG3 +	IgG4 1+
8	67	4.3	1.2	IgG 3+	NA
9	75	7	1	IgG 3+; C3 1+	NA
10	70	1	1.6	IgG 3+; C3 1+	NA
11	64	8.4	1.2	IgG 3+; C3 3+	IgG3 2+; IgG2 2+
12	61	3.9	1	IgG 3+	IgG4 2+; IgG2 1+
13	66	23.3	3.8	IgG 3+; IgA 2+	IgG4 2+; IgG2 2+
14	72	21	1.3	IgG 1+	NA

NA: Not applicable. IgG: Immunoglobulin G; IgA: Immunoglobulin A.

Table 3 Demographic and clinical characteristics according to antigen

Total 270	PLA2R 220	EXT1/EXT2 11	NELL 1 6	PCDH7 3	THSD7A 1	NCAM-1 1	Septule negative 28
Age	54.0 (43.2-61.0)	40.0 (25.0-48.0)	57.0 (36.7-66.5)	73 (69.0-74.0)	67.0	46	52 (44.5-66.5)
Male sex % (*n*)	75.0 (165/220)	27.2 (3/11)	66.6 (4/6)	33.3 (1/3)	100 (1/1)	0 (0/1)	60.7 (17/28)
Serum creatinine (mg/dL)	1.1 (0.9-1.4)	1.0 (0.7-1.1)	1.9 (1.0-4.9)	1.1 (1.03-1.48)	0.9	0.6	1.2 (0.9-1.7)
eGFR (mL/min/1.73 m^2)	68 (49.9-91.0)	85.0 (65.2- 113.4)	38.3 (14.5-75.7)	57 (39.8-66.6)	89.5	114.0	67 (40.0-95.0)
Proteinuria (g/24 h)	8.0 (5.2-12.0)	5.6 2.6-9.3)	11.0 (6.8-16.1)	3.2 (1.55-6.05)	14.4	5.7	4.5 (3.2-9.9)
Malignancy %	5.0 (11/220)	9.0 (1/11)	33.3 (2/6)	-	100 (1/1)	-	25.0 (7/28)
Autoimmunity %	5.4 (12/220)	81.8 (9/11)	33.3 (2/6)	-	-	-	46.4 (13/28)
Paraproteinmemia %	4.0 (9/220)	-	-	-	100 (1/1)	-	35 (1/28)
Infection %	0.4 (1/220)	-	-	-	-	-	-
NSAID %	1.8 (4/220)	-	16.6 (1/6)	-	-	-	14.2 (6/28)
No associated disease %	84.0 (185/220)	18.1 (2/11)	16.6 (1/6)	100 (3/3)	-	100 (1/1)	21.4 (6/28)

eGFR: Estimated glomerular filtration rate; EXT: Exostosin; NCAM-1: Neural cell adhesion molecule 1; NELL-1: Neural epidermal growth factor-like 1 protein; NSAID: Nonsteroidal anti-inflammatory drug; PCDH7: Protocadherin; PLA2R: Phospholipase A2 receptor; THSD7A: Thrombospondin type 1 domain containing 7A.

In 2018, Hashimoto *et al*[44] described a patient with chronic inflammatory demyelinating polyneuropathy with concurrent MN. CIDP may be due to autoantibodies against paranodal proteins, such as neurofascin 155 (NF155) and contactin-1 (CNTN1).

Autoantibody assays revealed the presence of IgG4- and IgG1-reactive anti- CNTN1 in MN. The authors hypothesized that CIDP with MN, can be detected by anti-CNTN1 antibodies in some cases.

More recently, Xu *et al*[45] described a 57-year-old man admitted to the hospital for limb numbness, weakness and sensory disorder. This man had MN and was diagnosed with anti-CNTN1 antibody-associated autoimmune nodopathy. Reviewing the literature, the authors found 22 cases of CIDP with

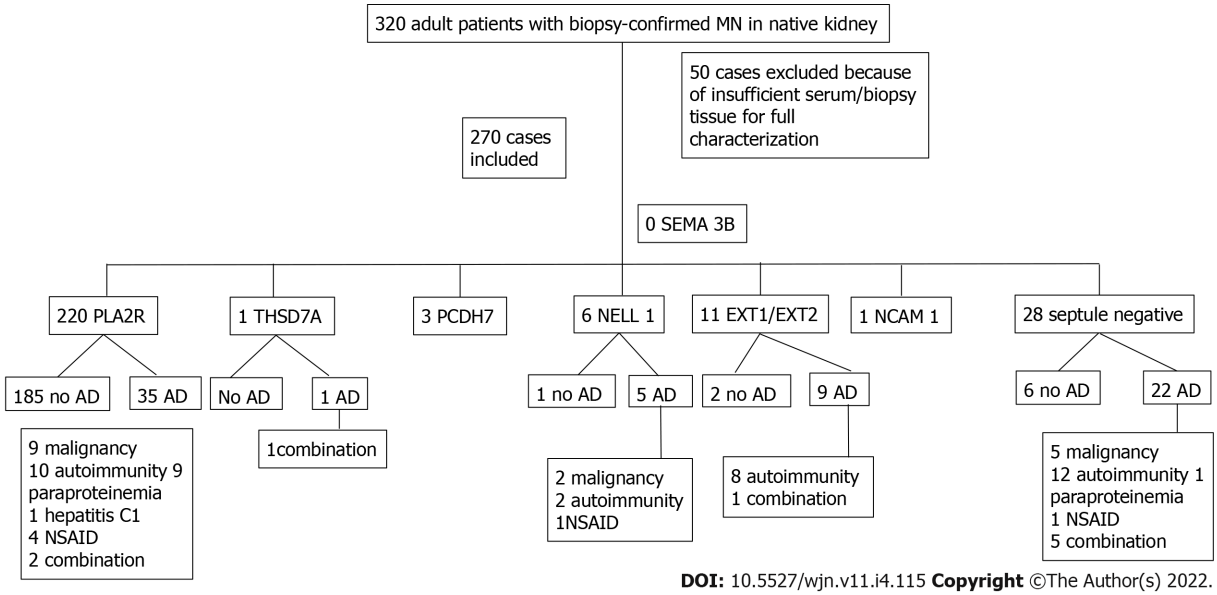

Figure 5 Patient distribution according to antigen and presence of associated disease. AD: Associated disease; NSAID: Nonsteroidal anti-inflammatory drug; EXT: Exostosin; MN: Membranous nephropathy; NCAM-1: Neural cell adhesion molecule 1; NELL-1: Neural epidermal growth factor like-1 protein; PCDH7: Protocadherin; PLA2R: Phospholipase A2 receptor; SEMA 3B: Semaphorin 3B; THSD7A: Thrombospondin type 1 domain containing 7A.

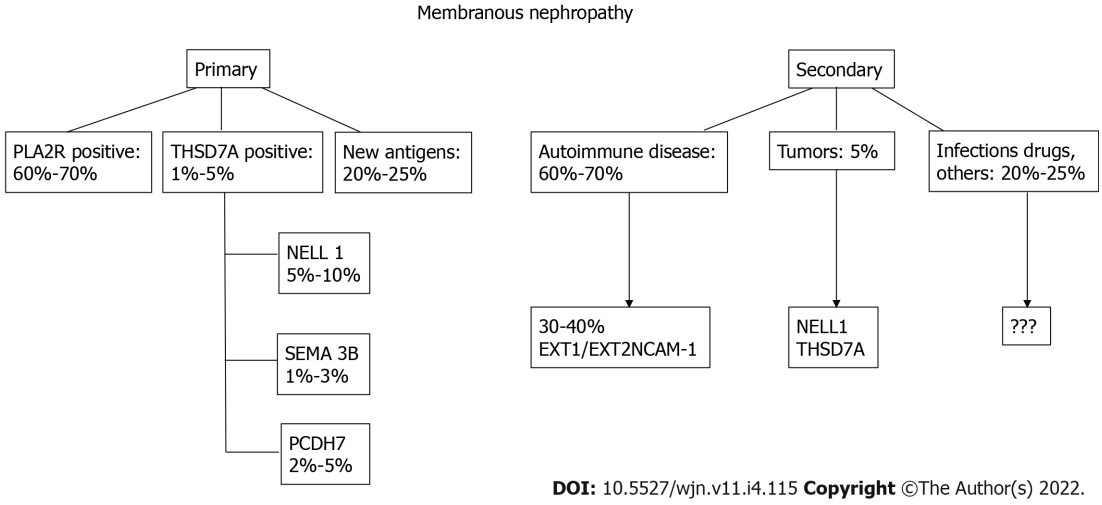

Figure 6 New proposed classification of membranous nephropathy. PLA2R: Phospholipase A2 receptor; THSD7A: Thrombospondin type 1 domain containing 7A; NELL-1: Neural epidermal growth factor-like 1 protein; Sema: Sema domain; PCDH7: Protocadherin 7; EXT: Exostosin; NCAM-1: Neural cell adhesion molecule 1.

MN, five of which were associated with the anti-CNTN1 antibody[46-49]. However, given the limited available research, no conclusions regarding a common antigen can be drawn.

In their recent study, Le Quintrec *et al*[43] looked for a novel target antigen by analyzing kidney biopsies from 5 patients positive for anti-contactin 1 antibodies who presented with MN combined with chronic inflammatory demyelinating polyneuropathy.

Western blot analysis revealed contactin 1 expression in kidney glomeruli. Confocal microscopic analysis showed the presence and colocalization of contactin 1 and IgG4 on the GBM. Eluted IgG could bind paranodal tissue and colocalized with commercial anti-contactin 1 antibody. Based on these findings, contactin 1 is a novel common antigenic target in MN associated with chronic inflammatory demyelinating polyneuropathy.

CTNT 1 is a glycosylphosphatidylinositol-anchored cell membrane protein expressed on the extracellular side.

Anti-CNTN1 predominantly comprises the IgG4 subclass. IgG4 deposits were found to colocalize with CNTN 1 or PLA2R1 in kidney biopsies. IgG4-PLA2R1-MN is considered a kidney autoimmune disease. After the formation of immune complexes, complement may be activated[50].

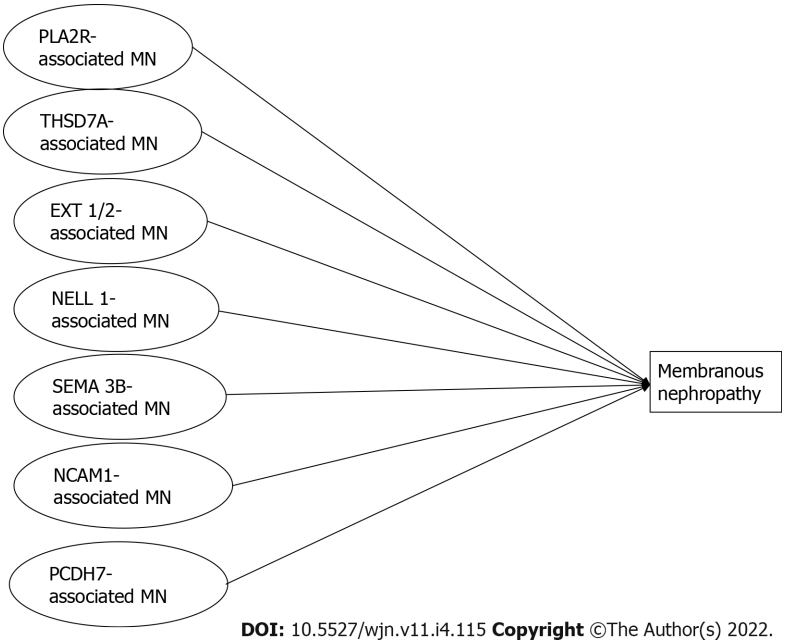

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Figure 7 Schematic classification of different conditions leading to membranous nephropathy. PLA2R: Phospholipase A2 receptor; THSD7A: Thrombospondin type 1 domain containing 7A; NELL-1: Neural epidermal growth factor-like 1 protein; Sema: Sema domain; PCDH7: Protocadherin 7; EXT: Exostosin; NCAM-1: Neural cell adhesion molecule 1; MN: Membranous nephropathy.

In conclusion, CNTN1 is the first discovered target involved in combined MN and anti-CNTN1-related CIDP.

Le Quintrec *et al*[43] detected three proteins (CNTN1, CASPR1, and NF155) in human glomerular extracts by immunoblotting and mass spectrometry. The authors were unable to show CNTN1 staining in podocytes in the normal human kidney. This finding could be ascribed to the fact that epitopes recognized by anti-CNTN1 antibodies are accessible only under pathological conditions[51].

CONCLUSIONS

MN has long been classified as primary MN (70%) with no disease association, and secondary MN (30%) with an underlying disease such as autoimmune disorders, tumors or infections. The principal known antigens involved as targets in primary MN were phospholipase A2 receptor and THSD7A. The availability of new techniques has allowed the discovery of new antigens and antibodies that are less frequently involved. Preliminary studies of patients at follow-up have shown different pathological findings and different outcomes associated with each of these new antigens. Now, it is possible that each new-type of antigen associated MN represents a distinct disease that causes the deposition of immune deposits along the GBM. The thickening of the GBM is the common result of these different diseases.

FOOTNOTES

Author contributions: Salvadori M and Tsalouchos A contributed equally to the manuscript; Salvadori M designed the study, performed the last revision and provided answers to the reviewers; Tsalouchos A collected the data from literature; Salvadori M and Tsalouchos A analyzed the collected data and wrote the manuscript.

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REFERENCES

1 **Beck LH Jr**, Bonegio RG, Lambeau G, Beck DM, Powell DW, Cummins TD, Klein JB, Salant DJ. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. *N Engl J Med* 2009; **361**: 11-21 [PMID: 19571279 DOI: 10.1056/NEJMoa0810457]
2 **Tomas NM**, Beck LH Jr, Meyer-Schwesinger C, Seitz-Polski B, Ma H, Zahner G, Dolla G, Hoxha E, Helmchen U, Dabert-Gay AS, Debayle D, Merchant M, Klein J, Salant DJ, Stahl RAK, Lambeau G. Thrombospondin type-1 domain-containing 7A in idiopathic membranous nephropathy. *N Engl J Med* 2014; **371**: 2277-2287 [PMID: 25394321 DOI: 10.1056/NEJMoa1409354]
3 **Debiec H**, Guigonis V, Mougenot B, Decobert F, Haymann JP, Bensman A, Deschênes G, Ronco PM. Antenatal membranous glomerulonephritis due to anti-neutral endopeptidase antibodies. *N Engl J Med* 2002; **346**: 2053-2060 [PMID: 12087141 DOI: 10.1056/NEJMoa012895]
4 **Debiec H**, Nauta J, Coulet F, van der Burg M, Guigonis V, Schurmans T, de Heer E, Soubrier F, Janssen F, Ronco P. Role of truncating mutations in MME gene in fetomaternal alloimmunisation and antenatal glomerulopathies. *Lancet* 2004; **364**: 1252-1259 [PMID: 15464186 DOI: 10.1016/S0140-6736(04)17142-0]
5 **Xie Q**, Li Y, Xue J, Xiong Z, Wang L, Sun Z, Ren Y, Zhu X, Hao CM. Renal phospholipase A2 receptor in hepatitis B virus-associated membranous nephropathy. *Am J Nephrol* 2015; **41**: 345-353 [PMID: 26087695 DOI: 10.1159/000431331]
6 **Berchtold L**, Zanetta G, Dahan K, Mihout F, Peltier J, Guerrot D, Brochériou I, Ronco P, Debiec H. Efficacy and Safety of Rituximab in Hepatitis B Virus-Associated PLA2R-Positive Membranous Nephropathy. *Kidney Int Rep* 2018; **3**: 486-491 [PMID: 29725654 DOI: 10.1016/j.ekir.2017.09.009]
7 **Stehlé T**, Audard V, Ronco P, Debiec H. Phospholipase A2 receptor and sarcoidosis-associated membranous nephropathy. *Nephrol Dial Transplant* 2015; **30**: 1047-1050 [PMID: 25839741 DOI: 10.1093/ndt/gfv080]
8 **Sethi S**. New 'Antigens' in Membranous Nephropathy. *J Am Soc Nephrol* 2021; **32**: 268-278 [PMID: 33380523 DOI: 10.1681/ASN.2020071082]
9 **Sethi S**, Madden BJ, Debiec H, Charlesworth MC, Gross L, Ravindran A, Hummel AM, Specks U, Fervenza FC, Ronco P. Exostosin 1/Exostosin 2-Associated Membranous Nephropathy. *J Am Soc Nephrol* 2019; **30**: 1123-1136 [PMID: 31061139 DOI: 10.1681/ASN.2018080852]
10 **Busse-Wicher M**, Wicher KB, Kusche-Gullberg M. The exostosin family: proteins with many functions. *Matrix Biol* 2014; **35**: 25-33 [PMID: 24128412 DOI: 10.1016/j.matbio.2013.10.001]
11 **Ahn J**, Lüdecke HJ, Lindow S, Horton WA, Lee B, Wagner MJ, Horsthemke B, Wells DE. Cloning of the putative tumour suppressor gene for hereditary multiple exostoses (EXT1). *Nat Genet* 1995; **11**: 137-143 [PMID: 7550340 DOI: 10.1038/ng1095-137]
12 **Paulson JC**, Colley KJ. Glycosyltransferases. Structure, localization, and control of cell type-specific glycosylation. *J Biol Chem* 1989; **264**: 17615-17618 [PMID: 2681181]
13 **Busse M**, Feta A, Presto J, Wilén M, Grønning M, Kjellén L, Kusche-Gullberg M. Contribution of EXT1, EXT2, and EXTL3 to heparan sulfate chain elongation. *J Biol Chem* 2007; **282**: 32802-32810 [PMID: 17761672 DOI: 10.1074/jbc.M703560200]
14 **Cook A**, Raskind W, Blanton SH, Pauli RM, Gregg RG, Francomano CA, Puffenberger E, Conrad EU, Schmale G, Schellenberg G. Genetic heterogeneity in families with hereditary multiple exostoses. *Am J Hum Genet* 1993; **53**: 71-79 [PMID: 8317501]
15 **Ravindran A**, Casal Moura M, Fervenza FC, Nasr SH, Alexander MP, Fidler ME, Herrera Hernandez LP, Zhang P, Grande JP, Cornell LD, Gross LA, Negron V, Jenson GE, Madden BJ, Charlesworth MC, Sethi S. In Patients with Membranous Lupus Nephritis, Exostosin-Positivity and Exostosin-Negativity Represent Two Different Phenotypes. *J Am Soc Nephrol* 2021; **32**: 695-706 [PMID: 33478971 DOI: 10.1681/ASN.2020081181]
16 **Anders HJ**. Nephropathic autoantigens in the spectrum of lupus nephritis. *Nat Rev Nephrol* 2019; **15**: 595-596 [PMID: 31197262 DOI: 10.1038/s41581-019-0168-x]
17 **Caza TN**, Hassen SI, Kuperman M, Sharma SG, Dvanajscak Z, Arthur J, Edmondson R, Storey A, Herzog C, Kenan DJ, Larsen CP. Neural cell adhesion molecule 1 is a novel autoantigen in membranous lupus nephritis. *Kidney Int* 2021; **100**: 171-181 [PMID: 33045259 DOI: 10.1016/j.kint.2020.09.016]
18 **Uhlén M**, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson Å, Kampf C, Sjöstedt E, Asplund A, Olsson I, Edlund K, Lundberg E, Navani S, Szigyarto CA, Odeberg J, Djureinovic D, Takanen JO, Hober S, Alm T, Edqvist PH, Berling H, Tegel H, Mulder J, Rockberg J, Nilsson P, Schwenk JM, Hamsten M, von Feilitzen K, Forsberg M, Persson L, Johansson F, Zwahlen M, von Heijne G, Nielsen J, Pontén F. Proteomics. Tissue-based map of the human proteome. *Science* 2015; **347**: 1260419 [PMID: 25613900 DOI: 10.1126/science.1260419]
19 **Zhang X**, Zara J, Siu RK, Ting K, Soo C. The role of NELL-1, a growth factor associated with craniosynostosis, in promoting bone regeneration. *J Dent Res* 2010; **89**: 865-878 [PMID: 20647499 DOI: 10.1177/0022034510376401]
20 **Matsuhashi S**, Noji S, Koyama E, Myokai F, Ohuchi H, Taniguchi S, Hori K. New gene, nel, encoding a M(r) 93 K protein with EGF-like repeats is strongly expressed in neural tissues of early stage chick embryos. *Dev Dyn* 1995; **203**: 212-222

[PMID: 7655083 DOI: 10.1002/aja.1002030209]
21 **Watanabe TK**, Katagiri T, Suzuki M, Shimizu F, Fujiwara T, Kanemoto N, Nakamura Y, Hirai Y, Maekawa H, Takahashi Ei. Cloning and characterization of two novel human cDNAs (NELL-1 and NELL2) encoding proteins with six EGF-like repeats. *Genomics* 1996; **38**: 273-276 [PMID: 8975702 DOI: 10.1006/geno.1996.0628]
22 The Human Protein Atlas: NELL-1. [cited 20 April 2022]. Available from: www.proteinatlas.org/ENSG00000165973-NELL-1/tissue/kidney
23 **Sethi S**, Debiec H, Madden B, Charlesworth MC, Morelle J, Gross L, Ravindran A, Buob D, Jadoul M, Fervenza FC, Ronco P. Neural epidermal growth factor-like 1 protein (NELL-1) associated membranous nephropathy. *Kidney Int* 2020; **97**: 163-174 [PMID: 31901340 DOI: 10.1016/j.kint.2019.09.014]
24 **Ahmad SB**, Appel GB. Antigens, antibodies, and membranous nephropathy: a decade of progress. *Kidney Int* 2020; **97**: 29-31 [PMID: 31901352]
25 **Kudose S**, Santoriello D, Debiec H, Canetta PA, Bomback AS, Stokes MB, Batal I, Ronco P, D'Agati VD, Markowitz GS. The clinicopathologic spectrum of segmental membranous glomerulopathy. *Kidney Int* 2021; **99**: 247-255 [PMID: 32599086 DOI: 10.1016/j.kint.2020.06.014]
26 **Caza TN**, Hassen SI, Dvanajscak Z, Kuperman M, Edmondson R, Herzog C, Storey A, Arthur J, Cossey LN, Sharma SG, Kenan DJ, Larsen CP. NELL-1 is a target antigen in malignancy-associated membranous nephropathy. *Kidney Int* 2021; **99**: 967-976 [PMID: 32828756 DOI: 10.1016/j.kint.2020.07.039]
27 **Spain RI**, Andeen NK, Gibson PC, Samuels MH, Morris CD, Solomon AJ, Solomon R, Waslo C, Avasare RS. Lipoic acid supplementation associated with neural epidermal growth factor-like 1 (NELL-1)-associated membranous nephropathy. *Kidney Int* 2021; **100**: 1208-1213 [PMID: 34662650 DOI: 10.1016/j.kint.2021.10.010]
28 **Ronco P**, Debiec H. Membranous nephropathy: current understanding of various causes in light of new target antigens. *Curr Opin Nephrol Hypertens* 2021; **30**: 287-293 [PMID: 33480620 DOI: 10.1097/MNH.0000000000000697]
29 **Sethi S**, Debiec H, Madden B, Vivarelli M, Charlesworth MC, Ravindran A, Gross L, Ulinski T, Buob D, Tran CL, Emma F, Diomedi-Camassei F, Fervenza FC, Ronco P. Semaphorin 3B-associated membranous nephropathy is a distinct type of disease predominantly present in pediatric patients. *Kidney Int* 2020; **98**: 1253-1264 [PMID: 32534052 DOI: 10.1016/j.kint.2020.05.030]
30 **Guan F**, Villegas G, Teichman J, Mundel P, Tufro A. Autocrine class 3 semaphorin system regulates slit diaphragm proteins and podocyte survival. *Kidney Int* 2006; **69**: 1564-1569 [PMID: 16541019 DOI: 10.1038/sj.ki.5000313]
31 **Alto LT**, Terman JR. Semaphorins and their Signaling Mechanisms. *Methods Mol Biol* 2017; **1493**: 1-25 [PMID: 27787839 DOI: 10.1007/978-1-4939-6448-2_1]
32 **Yazdani U**, Terman JR. The semaphorins. *Genome Biol* 2006; **7**: 211 [PMID: 16584533 DOI: 10.1186/gb-2006-7-3-211]
33 **Tapia R**, Guan F, Gershin I, Teichman J, Villegas G, Tufro A. Semaphorin3a disrupts podocyte foot processes causing acute proteinuria. *Kidney Int* 2008; **73**: 733-740 [PMID: 18075495 DOI: 10.1038/sj.ki.5002726]
34 **Fila M**, Debiec H, Perrochia H, Djouadi N, Verpont MC, Buob D, Ronco P. Recurrence of Anti-Semaphorin 3B-Mediated Membranous Nephropathy after Kidney Transplantation. *J Am Soc Nephrol* 2022; **33**: 503-509 [PMID: 35017170 DOI: 10.1681/ASN.2021101323]
35 **Chauhan V**, Anis A, Chauhan A. Effects of Starvation on the Levels of Triglycerides, Diacylglycerol, and Activity of Lipase in Male and Female Drosophila Melanogaster. *J Lipids* 2021; **2021**: 5583114 [PMID: 33833879 DOI: 10.1681/ASN.2020081165]
36 **Hanset N**, Aydin S, Demoulin N, Cosyns JP, Castanares-Zapatero D, Crott R, Cambier JF, Pochet JM, Gillerot G, Reginster F, Houssiau F, Debiec H, Ronco P, Jadoul M, Morelle J; UCLouvain Kidney Disease Network. Podocyte Antigen Staining to Identify Distinct Phenotypes and Outcomes in Membranous Nephropathy: A Retrospective Multicenter Cohort Study. *Am J Kidney Dis* 2020; **76**: 624-635 [PMID: 32668319 DOI: 10.1053/j.ajkd.2020.04.013]
37 **Brasch J**, Harrison OJ, Honig B, Shapiro L. Thinking outside the cell: how cadherins drive adhesion. *Trends Cell Biol* 2012; **22**: 299-310 [PMID: 22555008 DOI: 10.1016/j.tcb.2012.03.004]
38 **Khalili-Shirazi A**, Kaisar M, Mallinson G, Jones S, Bhelt D, Fraser C, Clarke AR, Hawke SH, Jackson GS, Collinge J. Beta-PrP form of human prion protein stimulates production of monoclonal antibodies to epitope 91-110 that recognise native PrPSc. *Biochim Biophys Acta* 2007; **1774**: 1438-1450 [PMID: 17936697 DOI: 10.1016/j.ceb.2007.09.006]
39 **Halbleib JM**, Nelson WJ. Cadherins in development: cell adhesion, sorting, and tissue morphogenesis. *Genes Dev* 2006; **20**: 3199-3214 [PMID: 17158740 DOI: 10.1101/gad.1486806]
40 **Ravindran A**, Madden B, Charlesworth MC, Sharma R, Sethi A, Debiec H, Cattran D, Fervenza FC, Smith RJ, Ronco P, Sethi S. Proteomic Analysis of Complement Proteins in Membranous Nephropathy. *Kidney Int Rep* 2020; **5**: 618-626 [PMID: 32405583 DOI: 10.1016/j.ekir.2020.01.018]
41 **Bobart SA**, Tehranian S, Sethi S, Alexander MP, Nasr SH, Moura Marta C, Vrana JA, Said S, Giesen CD, Lieske JC, Fervenza FC, De Vriese AS. A Target Antigen-Based Approach to the Classification of Membranous Nephropathy. *Mayo Clin Proc* 2021; **96**: 577-591 [PMID: 33673911 DOI: 10.1016/j.mayocp.2020.11.028]
42 **Sethi S**. Membranous nephropathy: a single disease or a pattern of injury resulting from different diseases. *Clin Kidney J* 2021; **14**: 2166-2169 [PMID: 34603694 DOI: 10.1093/ckj/sfab069]
43 **Le Quintrec M**, Teisseyre M, Bec N, Delmont E, Szwarc I, Perrochia H, Machet MC, Chauvin A, Mavroudakis N, Taieb G, Lanfranco L, Rigothier C, José B, Concetta C, Geneste C, Pernin V, Larroque C, Devaux J, Beyze A. Contactin-1 is a novel target antigen in membranous nephropathy associated with chronic inflammatory demyelinating polyneuropathy. *Kidney Int* 2021; **100**: 1240-1249 [PMID: 34600965 DOI: 10.1016/j.kint.2021.08.014]
44 **Hashimoto Y**, Ogata H, Yamasaki R, Sasaguri T, Ko S, Yamashita K, Xu Z, Matsushita T, Tateishi T, Akiyama S, Maruyama S, Yamamoto A, Kira JI. Chronic Inflammatory Demyelinating Polyneuropathy With Concurrent Membranous Nephropathy: An Anti-paranode and Podocyte Protein Antibody Study and Literature Survey. *Front Neurol* 2018; **9**: 997 [PMID: 30538665 DOI: 10.3389/fneur.2018.00997]
45 **Xu Q**, Liu S, Zhang P, Wang Z, Chang X, Liu Y, Yan J, He R, Luo X, Zou LY, Chu X, Guo Y, Huang S, Fu X, Huang Y. Characteristics of Anti-Contactin1 Antibody-Associated Autoimmune Nodopathies With Concomitant Membranous

Nephropathy. *Front Immunol* 2021; **12**: 759187 [PMID: 34675937 DOI: 10.3389/fimmu.2021.759187]

46 **Doppler K**, Appeltshauser L, Wilhelmi K, Villmann C, Dib-Hajj SD, Waxman SG, Mäurer M, Weishaupt A, Sommer C. Destruction of paranodal architecture in inflammatory neuropathy with anti-contactin-1 autoantibodies. *J Neurol Neurosurg Psychiatry* 2015; **86**: 720-728 [PMID: 25694474 DOI: 10.1136/jnnp-2014-309916]

47 **Witte AS**, Burke JF. Membranous glomerulonephritis associated with chronic progressive demyelinating neuropathy. *Neurology* 1987; **37**: 342-345 [PMID: 3027615 DOI: 10.1212/wnl.37.2.342]

48 **Kohli A**, Tandon P, Kher V. Chronic inflammatory demyelinating polyradiculoneuropathy with membranous glomerulonephritis: report of one case. *Clin Neurol Neurosurg* 1992; **94**: 31-33 [PMID: 1321695 DOI: 10.1016/0303-8467(92)90115-j]

49 **Plemel DJA**, Nazarali SA, Damji KF, Chen SH. Tube shunt surgery in pig eyes: a wet lab teaching model. *Can J Ophthalmol* 2019; **54**: 585-589 [PMID: 31564349 DOI: 10.1017/cjn.2020.46]

50 **Brglez V**, Boyer-Suavet S, Seitz-Polski B. Complement Pathways in Membranous Nephropathy: Complex and Multifactorial. *Kidney Int Rep* 2020; **5**: 572-574 [PMID: 32406418 DOI: 10.1016/j.ekir.2020.02.1033]

51 **Debiec H**, Ronco P. When contactin antibodies hit the podocyte: a new neurorenal syndrome. *Kidney Int* 2021; **100**: 1163-1165 [PMID: 34802555 DOI: 10.1016/j.kint.2021.09.022]

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ORIGINAL ARTICLE

Observational Study

Concordance between bio-impedance analysis and clinical score in fluid-status assessment of maintenance haemodialysis patients: A single centre experience

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Abstract

BACKGROUND

The burden of chronic kidney disease (CKD) is rising rapidly globally. Fluid overload (FO), an independent predictor of mortality in CKD, should be accurately assessed to guide estimation of the volume of fluid to be removed during haemodialysis (HD). Clinical score (CS) and bio-impedance analysis (BIA) have been utilized in assessment of FO and BIA has demonstrated reproducibility and accuracy in determination of fluid status in patients on HD. There is need to determine the performance of locally-developed CSs in fluid status assessment when evaluated against BIA.

AIM

To assess the hydration status of patients on maintenance HD using BIA and a CS, as well as to evaluate the performance of that CS against BIA in fluid status assessment.

METHODS

This was a single-centre, hospital-based cross-sectional study which recruited adult patients with CKD who were on maintenance HD at Kenyatta National Hospital. The patients were aged 18 years and above and had been on maintenance HD for at least 3 mo. Those with pacemakers, metallic implants, or bilateral limbs amputations were excluded. Data on the patients' clinical history, physical examination, and chest radiograph findings were collected. BIA was

performed on each of the study participants using the Quantum® II bio-impedance analyser manufactured by RJL Systems together with the BC 4® software. In evaluating the performance of the CS, BIA was considered as the gold standard test. A 2-by-2 table of the participants' fluid status at each of the CS values obtained compared to their paired BIA results was constructed (either ++, +-, -- or -+ for FO using the CS and BIA, respectively). The results from this 2-by-2 table were used to compute the sensitivity and specificity of the CS at the various reference points and subsequently plot a receiver operating characteristic (ROC) curve that was used to determine the best cut-off point. Those above and below the best CS cut-off point as determined by the ROC were classified as being positive and negative for FO, respectively. The proportions of participants diagnosed with FO by the CS and BIA, respectively, were computed and summarized in a 2-by-2 contingency table for comparison. McNemar's chi-squared test was used to assess any statistically significant difference in proportions of patients diagnosed as having FO by CS and BIA. Logistic regression analysis was conducted to assess whether the variables for the duration of dialysis, the number of missed dialysis sessions, advisement by health care professional on fluid or salt intake, actual fluid intake, the number of anti-hypertensives used, or body mass index were associated with a patient's odds of having FO as diagnosed by BIA.

RESULTS

From 100 patients on maintenance HD screened for eligibility, 80 were recruited into this study. Seventy-one (88.75%) patients were fluid overloaded when evaluated using BIA with mean extracellular volume of 3.02 ± 1.79 L as opposed to the forty-seven (58.25%) patients who had FO when evaluated using the CS. The difference was significant, with a *P* value of < 0.0001 (95% confidence interval: 0.1758-0.4242). Using CS, values above 4 were indicative of FO while values less than or equal to 4 denoted the best cut-off for no FO. The sensitivity and specificity for the CS were 63% and 78% respectively. None of the factors evaluated for association with FO showed statistical significance on the multivariable logistic regression model.

CONCLUSION

FO is very prevalent in patients on chronic HD at the Kenyatta National Hospital. CS detects FO less frequently when compared with BIA. The sensitivity and specificity for the CS were 63% and 78% respectively. None of the factors evaluated for association with FO showed statistical significance on the multivariable logistic regression model.

Key Words: Bio-impedance analysis; Clinical score; Chronic kidney disease; Maintenance haemodialysis; Fluid overload; Concordance

Core Tip: Bio-impedance analysis (BIA) has been validated as an accurate and reliable tool for determining fluid status in chronic kidney disease (CKD) patients but is not widely available in low-income settings. In this study we assess how a clinical score (CS) compares with BIA in this population for possible use as a low-cost substitute where BIA is not available. Patients with a CS score greater than 4 were considered to have fluid overload (FO), and detected using this parameter in 58.75% of patients. CSs of ≤ 4 represented no FO, and represented 41.25% of patients. The CS had a sensitivity of 63% and a specificity of 78% in making a diagnosis of FO compared with BIA, which was used as the reference in patients with CKD on maintenance haemodialysis.

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INTRODUCTION

Chronic kidney disease (CKD) refers to the abnormalities in kidney structure and function with effects on the individual's health for more than 3 mo. The indicators for kidney damage include reduced estimated glomerular filtration rate, abnormal urinalysis findings, or abnormal histologic findings on kidney biopsy[1]. The risk factors for CKD include diabetes mellitus (DM), high blood pressure, and

glomerulonephritides[2,3]. Advanced CKD is heralded by general ill-health, symptomatic anaemia, signs and symptoms of uraemia, and fluid overload (FO). End stage kidney disease (ESKD) is characterised by reduced ability to excrete enough sodium and the resultant water retention, which presents as a FO state. In this state, the patient requires kidney replacement therapy (KRT) for sustenance of bodily functions. The KRT modalities include dialysis and kidney allograft transplantation. Dialytic modalities include haemodialysis (HD) and peritoneal dialysis (PD). In Africa, less than 3% of those who require KRT receive it, with HD as the most popular modality[4].

Fluid status

The extracellular volume (ECV) varies by ± 1 L in healthy individuals and is dependent on salt intake. The ECV is severely affected by kidney disease. FO is defined as the ECV in excess of that observed in healthy individuals with normal kidney function[5]. Chronic FO increases mortality from arterial hypertension, left ventricular dilatation and hypertrophy, and congestive heart failure[6]. Oedema predisposes the patient to skin infections, especially in diabetic patients, and can result in sepsis, limb amputations, and high mortality. Oedema in the gut leads to malabsorption of nutrients and, in the lungs, it increases risk of bronchitis and pneumonia[7]. Among the patients with CKD, the state of hydration comes only second to the presence of DM in predicting mortality[8]. Greater than 15% of relative overhydration corresponds to > 2.5 L FO and is independently associated with high mortality [9]. This degree of over hydration is associated with an 8.5% increase in deaths even in stable CKD group of patients on dialysis[8]. HD removes waste products and fluids. Conversely, dehydration is associated with muscular cramps, low blood pressure, cardiac stunning, and loss of residual kidney function[6].

Several methods have been employed in assessment of fluid status in CKD patients. Varied signs and symptoms have been put together to comprise a clinical scoring system for assessment of state of hydration among patients. Clinical scores (CSs) are easy to document and can be recorded consistently and regularly. These make the scores appealing for utilization in assessment of hydration status. However, clinical scoring systems have inherent weaknesses of incompleteness, subjectivity of the observer, and lack of specificity. The specificity of CSs can be increased by scoring symptoms that manifest *de novo* and clear with correction of hydration status. Wizemann *et al*[10] utilized this approach in a study whereby signs and symptoms of dehydration and FO were grouped and scored.

There are other methods which have been employed in assessment of the fluid status. These include imaging like chest X-rays and ultrasonographic scanning, monitoring of plasma volume by dilution methods, clinical methods, and bio-impedance analysis (BIA). The BIA is based on the principle that in a cylinder, the electrical impedance varies directly with length and inversely to the product of cross-sectional area and specific sensitivity[9]. In BIA, an alternating current is passed through the body and the current passes extra- or intracellularly depending on whether it is low or high. High frequency current passes through extra- and intracellular spaces. Bio-impedance-defined overhydration (OH) independently predicts mortality due to its ability to discriminate absolute and relative extracellular fluid (ECF) volume[9].

Employment of various techniques simultaneously can achieve better results in evaluation of hydration status in patients. However, this is not practically feasible in a clinical setting. We hypothesised that the CS and BIA were equal in assessing hydration status of adult patients on maintenance HD. This study assessed the hydration status of patients on maintenance HD using BIA and CS in addition to determining of degree of concordance between the two methods in assessment of hydration status. The study determined the factors associated with FO in this population, which included the HD vintage, knowledge and practice of fluid and salt restrictions, the number of antihypertensive or diuretic medications, and body mass index (BMI). Approval was obtained from the Kenyatta National Hospital - University of Nairobi Ethics and Research Committee under proposal P822/012/2018.

MATERIALS AND METHODS

This was a single-centre hospital-based cross-sectional analytic study carried out between March and April 2019. It was performed in the Renal Department at the Kenyatta National Hospital, which is a national teaching and referral hospital located in Nairobi-Kenya. The study recruited ESKD who had been on maintenance HD. Those included were adult patients aged ≥ 18 years who had been on HD for at least 3 mo. We excluded patients who had undergone bilateral limbs amputation, had implanted metallic devices, pacemakers, or metallic intravascular devices, or who were very sick.

The sample size was estimated using the sample size formula for comparing paired proportions (McNemar's *Z* test, 2-sided equality)[11]. Using the prevalence of FO using BIA by Bajaber *et al*[12] (69%), prevalence of FO using a CS by Wizemann *et al*[10] (35%), α of 5% and β of 20%, the calculated sample size after adjusting upwards by 15% to account for non-response was 80 patients. The study employed systematic random sampling without replacement. Structured history and physical examinations were performed by one clinician. Weight was measured to the nearest 0.1 kg using a digital scale placed on a firm flat surface after the participants had removed heavy outer garments, shoes and

emptied their pockets. The weighing scale was calibrated daily. The height was taken using a stadiometer and employed a standard protocol. Two measurements were taken and the average of the two readings recorded to the nearest centimetre. Oedema was assessed using a standard scoring system for uniformity[13].

Chest radiographs were obtained to assess findings of FO. The findings assessed included dilated veins in the upper lung zones and cardiomegaly that were classified as stage 1 hypervolemia. Stage 2 hypervolemia was marked by interstitial oedema evidenced by Kerley B lines, while stage 3 was evidenced by alveolar oedema or pleural effusion as reported by two radiologists at the University of Nairobi who were blinded to study procedures. A CS that had not been previously validated was developed for the study. It entailed eliciting signs and symptoms for hypovolemia like intradialytic hypotension, muscle cramps, dizziness, or fatigue during HD session and the need for treatment of hypotension with normal saline infusion, which were scored at -1 each. Signs and symptoms scored as hypervolemia included hypertension, hypoxia noted by oxygen saturation < 90%, presence of ascites, pleural effusion, or pulmonary oedema, which were scored at +1 each. The interdialytic weight gain was determined and scored as +1 for each kilogram gained since the last session of HD. Presence of gallop rhythm was scored at +2, dyspnoea classification by New York Heart Association was scored from 0 to +3, chest radiograph features of FO scored from +1 to +3 based on stages described above, and oedema of ankles and tibia was scored from 0 to +4 as shown in Table 1.

BIA was done by placing electrodes on one side of the body either left or right upper and lower limbs after lying supine for 10 min. For patients who used arteriovenous fistulae (AVF) for HD vascular access, the side without AVF was used. Measurement of resistance and reactance were then determined based on the manufacturer's guidelines. The machine used was the Quantum® II bio-impedance analyser manufactured by RJL Systems, Inc., Clinton Township, Michigan, United States, together with the BC 4® software from the same manufacturer. Hydration status was based on Wabel *et al*[14], which classified fluid status into three categories based on ECF estimation by BIA. These included dehydration where the ECF is estimated to be less than of -1.1 L, normal hydration with ECF ± 1.1 L, and FO where ECF is > 1.1 L. FO was further stratified as mild FO, where ECF was 1.1-2.5 L, and gross FO, where ECF was > 2.5 L.

The target variable was FO, as diagnosed by the newly developed CS and BIA. The predictor variables included BMI in kg/m^2, blood pressure in mmHg, antihypertensive medications used, fluid intake, salt intake, number of HD sessions per week, adherence to HD treatment, missed HD sessions during the 2 wk preceding the study period, HD vintage, antihypertensive medications, and fluid restriction. An adherent patient was one who had not missed any HD sessions in the 2 wk that preceded the study or any doses of scheduled antihypertensive medications in the week prior to evaluation and had received education on fluid and salt restriction which the patient was following, all based on the patients' self-report.

Statistical analysis

Data were analysed using STATA® software. Continuous variables included age, duration of CKD, HD vintage, systolic and diastolic blood pressure, and BMI. Normally distributed continuous data had their means and standard deviations computed. For skewed continuous data, medians and inter-quartile ranges (IQR) were computed. Categorical variables like sex, co-morbidities, hydration status by both CS and BIA, and HD vascular access, had frequencies calculated and were presented as counts and percentages. The result for each participant's CS (positive or negative for FO) was compared to its paired BIA result. Using BIA as the gold standard test for diagnosing FO, the CS sensitivity and specificity measures together with the false positive rate (FPR) at each of the CS values obtained by the participants were computed using the formulae: Se = TP/(TP + FN), Sp = FP/(FP + TN), FPR = 1 – SP. Where Se was sensitivity, Sp was specificity, TP was true positive, FN was false negative, FP was false positive, TN was true negative.

A receiver operating characteristic (ROC) curve (graph of sensitivity *vs* FPR) was plotted for scores obtained in order to establish the best cut-off point for determining FO using the CS[15]. The point which gave the greatest area under the ROC and in which the differential positive rate (DPR) value was highest was interpreted as the optimal cut-off point for the CS. The DPR was calculated using the formula: DPR = (Se + Sp) – 1. Values of the CS that were above and below the cut-off point were established as optimal on the ROC were interpreted as positive and negative for FO respectively.

The McNemar's chi square test was used to assess any statistically significant differences in proportions of patients diagnosed as having FO by both CS and BIA. Stepwise logistic regression analysis was conducted in order to assess whether the duration of dialysis, number of missed dialysis sessions, advise on fluid intake, actual fluid intake, advise on salt intake, number of anti-hypertensives used, and BMI were significant predictors of FO in this study population. Univariable logistic regression models between each of the predictor variables and FO was conducted at a liberal *P* value of 0.20. The variables having a *P* value of < 0.20 in the univariable models were added to the multivariable model where their association with the odds of FO was tested at a 5% significance level. Non-significant variables were eliminated from the multivariable model if they did not result in > 30% change in the coefficient of the significant variables[16]. The Hosmer-Lemeshow goodness of fit was computed to evaluate how well the final logistic regression model fit the data with a *P* value > 0.05, indicating a well-

Table 1 Clinical score parameters

Symptoms		Score
Scored as dehydration	Intradialytic hypotension	-1
	Muscle cramps, dizziness or fatigue during current session of dialysis	-1
	Symptomatic dialysis hypotension treated by NaCl (0.9%) infusion	-1
Scored as normohydration	Absence of symptoms given in this table	0
Scored as fluid overload	Hypertension	+1
	SPO_2 less than 90%	+1
	Presence of ascites	+1
	Presence of pleural effusion or pulmonary oedema on clinical examination	+1
	Inter dialytic weight gain - per 1 kg gained	+1
	Presence of gallop rhythm	+2
	Dyspnoea based on NYHA class	0 to +3
	Chest radiography features based on stage	+1 to +3
	Oedema (ankles, tibial, graded)	0 to +4

NaCl: Sodium chloride; NYHA: New York Heart Association; SPO_2: Oxygen saturation.

fitting model[15].

RESULTS

Demographic and clinical profiles of study participants

Altogether, there were about 100 patients who were on maintenance HD at Kenyatta National Hospital renal unit between March and April 2019. All 100 patients were screened for eligibility. Eleven patients were excluded because they had been on HD for less than 3 mo, two had metallic implants, two declined to participate, two were below 18 years of age, and one patient was critically ill. The excluded patients were aged between 15 years and 70 years with a median age of 40 years and were predominantly male (60%). Eighty-two patients met the inclusion criteria and were recruited into the study. However, at the time of analysis, it was noted that two participants had incomplete data (missing chest radiographs) and were excluded from the final analysis. The two excluded were a male and female patient, aged 53 years and 27 years, on HD for 28 and 3 mo, respectively. By BIA, their hydration statuses were normohydrated and gross OH, respectively. The final analyses included 80 participants (Figure 1).

Table 2 summarizes demographic and clinical characteristics of the study participants. The study participants were aged between 18 years and 75 years with a median age of 45 years with an IQR of 20.5 years. Forty-six (57.5%) were males. Most (63.75%) of the patients had secondary level education or higher. Fifty-seven (71.25%) of the participants were married and the majority (93.75%) had medical insurance that covered the costs of their HD, for a maximum of two HD sessions per week.

The median duration since diagnosis of CKD was 12.5 mo (IQR 24.5) and the median dialysis duration was 9 mo (IQR 15). Twenty-six (32.5%) patients had AVF while 54 (67.5%) were using venous catheters for HD vascular access. Seventy-seven (96.25%) patients had some residual kidney function while three participants were anuric. The comorbidities that preceded CKD as per their medical records included hypertension in 41 (51.25%) patients, glomerulonephritis in 22 (27.5%) patients, DM in 14 (17.5%) patients, obstructive uropathy in 8 (10%) patients, human immunodeficiency virus infection in 7 (8.75%) patients, kidney allograft failure in 2 (2.5%) patients, and cystic kidney disease in 1 (1.25%) patient. Seventy-six (95%) patients attended HD sessions twice per week as per institutional protocol with one of the patients on daily dialysis because he was scheduled for a kidney allograft transplantation during the week of assessment. Three (3.75%) patients were on once weekly HD. Sixty-six (82.5%) patients reported full adherence to attendance of their HD sessions and had not missed any sessions in the 2 wk preceding the study. Seventy-two (90%) patients had received education on fluid intake with the average actual fluid intake being 1010 mL in the interdialytic period with a range of 200-2800 mL. Seventy (87.5%) patients had received education on salt intake.

Table 2 Demographic and clinical characteristics of study participants

Characteristic		Statistic
Age (yr)	Median (IQR)	45 (20.5)
Sex	Male, *n* (%)	46 (57.5)
CKD duration (mo)	Median (IQR)	12.5 (24.5)
Dialysis vintage	Median (IQR)	9 (15)
Blood pressure (mmHg)	Systolic mean ± SD	150 ± 31
	Diastolic mean ± SD	91 ± 19
Body mass index (kg/m^2)	Median (IQR)	21.94 (5.13)
Comorbidities	Hypertension, *n* (%)	41 (51.25)
	Glomerulonephritis, *n* (%)	22 (27.5)
	Diabetes mellitus, *n* (%)	14 (17.5)
	Obstructive uropathy, *n* (%)	8 (10)
	HIV, *n* (%)	7 (8.75)
	Malignancy, *n* (%)	2 (2.5)
	Graft failure post-transplant, *n* (%)	2 (2.5)
	Cystic kidney disease, *n* (%)	1 (1.25)
Dialysis access	Arterio-venous fistula, *n* (%)	26 (32.5)
Clinical score	Hypovolemia, *n* (%)	0 (0.00)
	Normovolemia, *n* (%)	33 (41.25)
	Hypervolemia, *n* (%)	47 (58.25)
BIA	Dehydration, *n* (%)	0 (0.00)
	Normal hydration, *n* (%)	9 (11.25)
	Fluid overload, *n* (%)	71 (88.75)
	Mild FO, *n* (%)	25 (31.25)
	Gross FO, *n* (%)	46 (57.50)

BIA: Bio-impedance analysis; CKD: Chronic kidney disease; FO: Fluid overload; HIV: Human immunodeficiency virus; IQR: Interquartile range.

Fifty (62.5%) of the patients had systolic blood pressure (SBP) > 140 mmHg with mean SBP of 150 ± 31 mmHg. The mean diastolic blood pressure (DBP) was 91 ± 19 mmHg with 41 (51.25%) patients having DBP > 90 mmHg. Four patients (5%) were hypotensive with either SBP less than 90 mmHg or DBP less than 60 mmHg. Forty-four (55%) patients were on two or three antihypertensive agents. The median BMI was 21.94 kg/m^2 (19.50-25.63). Seventy (87.5%) patients had received health education on salt intake as part of their management prior to enrolment on the study. Sixty-seven (83.75%) patients self-reported strict adherence to their anti-hypertensive medications and had not missed any of the prescribed dose in the week prior to evaluation. Forty-eight (60%) patients reported complete adherence to all the specific aspects ESKD management, which this study sought. The patients had received all the prescribed number of HD sessions in the previous 2 wk and had adhered to dietary, salt, and fluid intake restrictions, as well as having not skipped any of the prescribed doses of anti-hypertensive medications in the week that preceded this study.

Volume status as determined by BIA

The participants had volumes in the range of -0.53 L to 8.23 L and a median of 2.76 L (IQR 2.22 L). Fluid overload was found in 71 (88.75%) patients of which 46 (57.50%) were grossly overloaded with ECV of 2.5 L above the normal volume. Nine (11.25%) patients had normal volume, and none were dehydrated according to evaluation by BIA. On average, the study participants had 3.02 ± 1.79 L of ECV.

Volume status as determined by CS

Symptoms scored as dehydration were scored in the negative. There were 2 (2.5%) with intradialytic hypotension, 1 (1.25%) with muscle cramps, dizziness, or fatigue during current session of dialysis, and

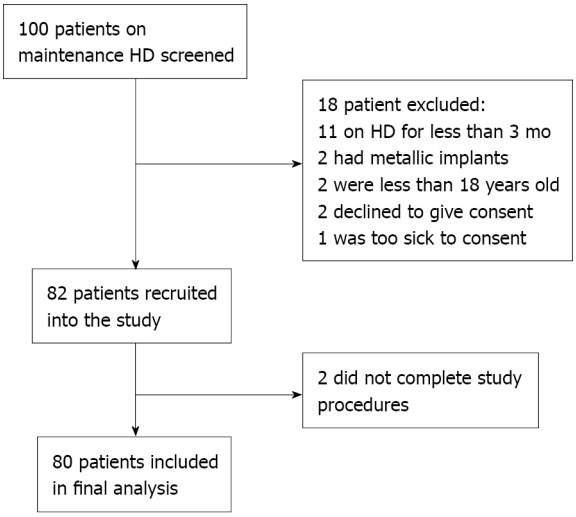

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Figure 1 Study flow diagram for screening and recruitment. HD: Haemodialysis.

1 (1.25%) with symptomatic intradialytic hypotension treated by normal saline infusion. Absence of the above symptoms were scored as normohydration at zero score. Signs and symptoms of FO scored from +1 to +4. There were 54 (67.5%) with hypertension, 8 (10%) with oxygen saturation less than 90% by digital pulse oximeter, 26 (32.5%) with ascites, and 15 (18.75%) with pleural effusion or pulmonary oedema, each scored at +1. Interdialtyic weight gain was scored at +1 per kilogram gained. Five (6.25%) of the patients had gallop rhythm, which was scored at +2. Dyspnoea was scored using New York Heart Association classification with 54 (67.5%) patients at class I, scored at zero, 17 (21.25%) at class II scored at +1, 9 (11.25%) at class III scored at +2, while none were at class IV, which would have scored at +3. Chest radiography features were scored between +1 and +3 based on the stages. Oedema was graded and scored between 0 and +4. CSs obtained varied from -2 to 16 with a mean of 5.46 ± 3.68. Each of the results obtained from the CS was used to generate sensitivity and FPR that were used to plot a ROC curve. As shown in Figure 2, the optimal cut-off point for the CS obtained from the ROC was 4. This gave the score 63% and 78% sensitivity and specificity respectively. At this cut-off point, fluid overload was picked in 58.75% while no overload was in 41.25% of the patients.

Level of agreement between BIA and CS

BIA showed that 9 (11.25%) of the patients had normal hydration, 71 (88.75%) were fluid overloaded, and none was dehydrated. The CS showed that 33 (41.25%) had normal hydration, 47 (58.25%) were fluid overloaded, and none was dehydrated (Table 2). Fifty-two (65%) patients had similar results by both BIA and CS, and consisted of 45 patients with FO and 7 patients without FO. Twenty-eight (35%) patients had differing results by the 2 methods. Bio-impedance diagnosed 26 patients to have FO, but these same patients were not picked by CS as having FO. CS picked two patients as having FO who were picked by BIA as not having FO. The calculated McNemar's chi-squared was 20.57 ($P < 0.0001$, 95% confidence interval: 0.1758-0.4242). The BIA detected significantly more patients with FO than CS. The true difference of the percentage of patients on HD picked with FO by CS and BIA was 17.58%-42.42% (Table 3).

Factors associated with FO

Duration of HD, number of missed HD sessions, whether the advice on fluid and salt restrictions against the fluid and salt was taken by the patient, as well as the number of antihypertensives medications each patient was using, and BMI were assessed for association with FO. Univariable logistic regression model of these factors associated with FO status was assessed. Using a liberal P value of 0.20, HD vintage in months, BMI and fluid intake were significantly associated with FO diagnosed by BIA (Table 4). However, from the multivariable model, using a significance level of 0.05, all these factors were not significantly associated with FO (Table 5).

DISCUSSION

The majority of the patients in this study were relatively young with a mean age of 45.6 years when compared with studies done in Europe, where the mean age was greater than 60 years[17]. The median duration of CKD was about 1 year and the median HD vintage was less than 1 year. There was a slight

Table 3 2-by-2 table assessing association between bio-impedance analysis and the clinical score

		Positive	Negative	Total	*P* value	95%CI
Clinical score	Positive	45	2	47		
	Negative	26	7	33		
	Total	71	9	80		
McNemar's Chi Square (20.57)					< 0.0001	0.1758-0.4242

CI: Confidence interval.

Table 4 Univariable analysis of factors associated with fluid overload

Variable	Values	FO+ (*n* = 71)	FO- (*n* = 9)	OR	95%CI	*P* value
Duration of dialysis[1] (mo)	3-76	71	9	1.05	0.967-1.147	0.13
No of missed dialysis sessions	0	59	7			
	≥ 1	12	2	0.712	0.131-3.856	0.70
Advised on fluid restriction	No	6	2			
	Yes	65	7	3.095	0.522-18.357	0.25
Actual fluid intake[1] (mL)	200-2800	71	9	0.998	0.997-1.000	0.082
Advised on salt intake	No	9	1			
	Yes	62	8	0.8611	0.096-7.719	0.89
Number of anti-hypertensives used	0	11	0			
	1	13	2			
	2	23	3			
	3	14	4			
	4	7	0			
	5	2	0			
	6	1	0	0.903	0.537-1.517	0.70
Patient's BMI[1] (kg/m^2)	15.82-32.53	71	9	1.196	0.942-1.520	0.11

[1]Variables eligible for inclusion in the multivariable model at a liberal *P* value of 0.20.
BMI: Body mass index; CI: Confidence interval; FO+: Positive fluid overload; FO-: Negative fluid overload; OR: Odds ratio.

Table 5 Multivariable analysis of factors associated with fluid overload

Variable	Values	OR	95%CI	*P* value
Duration of dialysis (mo)	3-76	1.054	0.962-1.154	0.258[a]
Actual fluid intake (mL)	200-2800	0.999	0.997-1.000	0.099[a]
BMI (kg/m^2)	15.82-32.53	1.191	0.934-1.519	0.159[a]

[a]*P* values are non-significant.
BMI: Body mass index; CI: Confidence interval; OR: Odds ratio.

male predominance. In a study performed elsewhere in Kenya[12], the age distribution, male predominance, and BMI were comparable to those of our study. The majority of the patients in this study also suffered from hypertension (67.5%). One of the contributors to sustained hypertension in this population is likely to be FO. The majority of the patients reported to have been counselled on diet and fluid and salt intake as a way of controlling blood pressure and FO, and they reported adherence to the recommendations. Educating patients on dietary salt and fluid restrictions are important components of

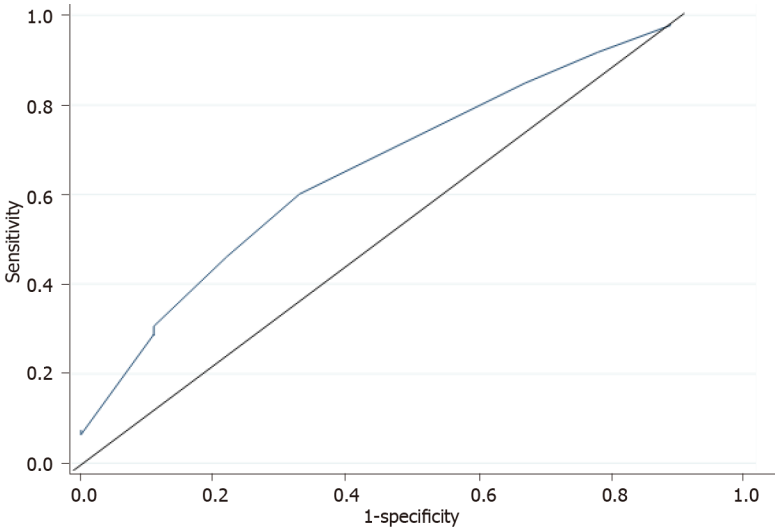

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Figure 2 Receiver operating characteristic curve for the clinical score.

the management of ESKD. Previous studies have shown that patients who fail to adhere to dietary salt and fluid intake restrictions are more likely to have FO[18]. Less than a fifth of the patients suffered from DM (17.5%). This is a lower proportion than that reported in other parts of the world, where prevalence rates of 29.7%[19] and as high as 50%[17] have been reported in some studies in Europe.

Almost all the patients (95%) were receiving 4-h HD sessions twice a week as per the Kenyatta National Hospital Renal Department protocol, partially because the health insurance could only reimburse two HD sessions per week and the patients could not afford to pay for an extra sessions out of pocket. This treatment frequency is less than the recommended thrice weekly[20]. The longer interdialytic period could lead to fluid accumulation and may contribute to the higher proportion of FO in our population. Assessment of fluid status by CS and BIA revealed that the majority of the patients were fluid overloaded. Slightly more than a half of the patients were fluid overloaded when accessed by CS while almost 90% of them were fluid overloaded when assessed by BIA. The BIA was more sensitive in picking FO status. More patients were noted to be fluid overloaded in this study compared to studies done elsewhere in South Africa and Europe[17,21,22]. A study from South Africa among patients on dialysis reported almost two in every three patients as being fluid overloaded[21]. In Germany, Passauer *et al*[17] reported similar proportion of patients on dialysis as being fluid overloaded. Almost 60% of patients in this study had more than 2.5 L of excess ECV; this is in contrast to an analysis of 1500 European HD patients of whom only 25% had gross FO[22]. A plausible explanation why studies done elsewhere have reported lower proportions of FO could be due to the fact that elsewhere, patients have HD sessions more than twice per week. The longer interdialytic period could result in more interdialytic fluid accumulation.

The proportion of patients with FO in our study was higher than that reported in a study at Moi Teaching and Referral Hospital in Kenya, where 7 in every 10 patients were reported to have FO[12]. This study excluded patients who had not attained dry weight, and this could explain why the proportion with FO was lower than that in our study. The study assessed the level of agreement between the FO status diagnosed by CS and by BIA. The difference between the numbers of patients picked as having FO by the two methods was significant. BIA was more sensitive in detecting FO when compared with CS. Similar observations had been made by Kalainy *et al*[23], and were attributed to an inherent low sensitivity and specificity of clinical parameters in picking the fluid volume before dialysis. In contrast, Wizemann *et al*[10] reported a better concordance between symptom score and BIA, with more agreement towards over hydration than dehydration. Vasko *et al*[24] compared BIA with history, signs and symptoms, laboratory evaluation, and routine imaging with chest X-rays, lung ultrasound scanning, and cardiac evaluation with echocardiograph. They concluded that clinical judgment was the most important in assessing pre-dialysis OH. Use of patient history and examination, as well as chest radiograph data, compared favourably with BIA in guiding clinical decisions. At a cut-off point of 4, the CS resulted in 63% and 78% sensitivity and specificity respectively. The BIA was more sensitive in picking patients with FO than CS and the true difference of the percentage of patients on HD picked with FO by CS and BIA was 17.58%-42.42%.

Our study was limited by the small sample size and being done in a single centre. Some tests, which could aid in assessing FO, like echocardiography, were not performed in our study population due to financial constraints. The study relied on the recall by the patients and was subject to recall bias, especially on adherence to diet and fluid intake. Some aspects, which were purely self-reported by the patients, were not verifiable.

CONCLUSION

FO is very prevalent in patients on chronic HD at the Kenyatta National Hospital. CS picks less FO when compared with BIA. However, CS could still pick more than 6 in 10 patients with FO as picked by BIA with a specificity of almost 80%. In settings where BIA is not available, CS can be utilized as a low-cost alternative to assess fluid status of patients on HD and interpreted with the knowledge that CS identifies fewer patients with FO than does BIA.

Use of BIA should be incorporated into the routine care of patients on maintenance HD. CS should also be utilized in assessment of FO, especially in places where BIA is not available. Further studies are needed to evaluate how CS compares with BIA in bigger and heterogeneous populations. It is plausible to try and increase the HD sessions to thrice per week in attempt to reduce the proportion of patients who present with FO in our setting. In addition, future studies can evaluate the validity of the CS where patients have attained their dry weight at the baseline since this may improve both the sensitivity and the specificity.

ARTICLE HIGHLIGHTS

Research background

Assessment of fluid status in patients with chronic kidney disease (CKD) on haemodialysis (HD) is important to guide treatment. Objective methods of assessment fluid status in this population of patients are needed. In CKD patients on HD, bio-impedance analysis (BIA) is reliable in assessment of fluid status though not available in many clinical situations. Clinical assessments for fluid overload (FO) are more popular in practice, though the individual elements are imprecise and may underestimate FO. There is need to determine the performance of a locally-developed clinical score (CS) in fluid status assessment when evaluated against BIA.

Research motivation

This study was motivated by the need to derive a local method of assessing fluid status in patients on HD and determine how this method compares with the BIA.

Research objectives

The objectives of this study were to assess the hydration status of patients on maintenance HD using BIA and a CS, as well as to evaluate the performance of that CS against BIA in fluid status assessment.

Research methods

This was a single-centre, hospital-based cross-sectional study which recruited adult patients with CKD who were on maintenance HD. The patients were aged 18 years and above and had been on maintenance HD for at least 3 mo. Those with pacemakers, metallic implants, or bilateral limb amputations were excluded. Data on the participants' clinical history, physical examination, and chest radiograph findings were collected. BIA was performed on each of the study participants using the Quantum® II bio-impedance analyser manufactured by RJL Systems together with the BC 4® software. In evaluating the performance of the CS, BIA was considered as the gold standard test.

Research results

From 100 patients on maintenance HD screened for eligibility, 80 were recruited into this study. Seventy-one (88.75%) patients were fluid overloaded when evaluated using BIA with mean extracellular volume of 3.02 ± 1.79 L as opposed to the forty-seven (58.25%) patients who had FO when evaluated using the CS ($P < 0.0001$, 95% confidence interval: 0.1758-0.4242). The best cut-off point identified for the CS was four with values > 4 indicating FO and values 4 indicating no FO. At this cut-off point, the CS had 63% and 78% sensitivity and specificity respectively. None of the factors evaluated for association with FO showed statistical significance on the multivariable logistic regression model.

Research conclusions

Fluid overload is very prevalent in patients on chronic HD at the Kenyatta National Hospital Clinical score detects less FO when compared with BIA. The sensitivity and specificity for the CS were 63% and 78% respectively. None of the factors evaluated for association with FO showed statistical significance on the multivariable logistic regression model.

Research perspectives

Almost 90% of the patients had FO by BIA, and 57.5% had gross FO. BIA diagnosed significantly more patients with FO than the CS. The CS had a sensitivity if 63% and a specificity of 78% at a cut-off of 4.

FOOTNOTES

Author contributions: Muchiri K, Kayima JK, Ogola EN, McLigeyo S, and Kabinga SK designed and coordinated the study; Muchiri K performed all the study procedures; Ndung'u SW analysed and interpreted the data; Muchiri K, Kabinga SK, and Ndung'u SW wrote the manuscript; and all authors approved the final version of this article.

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REFERENCES

1 **Levin A**, Stevens PE, Bilious RW, Coresh J, De Francisco ALM, De Jong PE, Griffith KE, Hemmelgarn BR, Iseki K, Lamb EJ, Levey AS, Riella MC, Shlipak MG, Wang H, White CT, Winearls CG. Improving global outcomes (KDIGO) CKD work group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int Supp* 2013; **3**: 1-150 [DOI: 10.1038/kisup.2012.73]
2 **Gansevoort RT**, Correa-Rotter R, Hemmelgarn BR, Jafar TH, Heerspink HJ, Mann JF, Matsushita K, Wen CP. Chronic kidney disease and cardiovascular risk: epidemiology, mechanisms, and prevention. *Lancet* 2013; **382**: 339-352 [PMID: 23727170 DOI: 10.1016/S0140-6736(13)60595-4]
3 **GBD 2015 Mortality and Causes of Death Collaborators**. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016; **388**: 1459-1544 [PMID: 27733281 DOI: 10.1016/S0140-6736(16)31012-1]
4 **Liyanage T**, Ninomiya T, Jha V, Neal B, Patrice HM, Okpechi I, Zhao MH, Lv J, Garg AX, Knight J, Rodgers A, Gallagher M, Kotwal S, Cass A, Perkovic V. Worldwide access to treatment for end-stage kidney disease: a systematic review. *Lancet* 2015; **385**: 1975-1982 [PMID: 25777665 DOI: 10.1016/S0140-6736(14)61601-9]
5 **Dou Y**, Zhu F, Kotanko P. Assessment of extracellular fluid volume and fluid status in hemodialysis patients: current status and technical advances. *Semin Dial* 2012; **25**: 377-387 [PMID: 22686593 DOI: 10.1111/j.1525-139X.2012.01095.x]
6 **Moissl U**, Arias-Guillén M, Wabel P, Fontseré N, Carrera M, Campistol JM, Maduell F. Bioimpedance-guided fluid management in hemodialysis patients. *Clin J Am Soc Nephrol* 2013; **8**: 1575-1582 [PMID: 23949235 DOI: 10.2215/CJN.12411212]
7 **Kraemer M**, Rode C, Wizemann V. Detection limit of methods to assess fluid status changes in dialysis patients. *Kidney Int* 2006; **69**: 1609-1620 [PMID: 16501488 DOI: 10.1038/sj.ki.5000286]
8 **Wizemann V**, Wabel P, Chamney P, Zaluska W, Moissl U, Rode C, Malecka-Masalska T, Marcelli D. The mortality risk of overhydration in haemodialysis patients. *Nephrol Dial Transplant* 2009; **24**: 1574-1579 [PMID: 19131355 DOI: 10.1093/ndt/gfn707]
9 **Tabinor M**, Elphick E, Dudson M, Kwok CS, Lambie M, Davies SJ. Bioimpedance-defined overhydration predicts survival in end stage kidney failure (ESKF): systematic review and subgroup meta-analysis. *Sci Rep* 2018; **8**: 4441 [PMID: 29535377 DOI: 10.1038/s41598-018-21226-y]
10 **Wizemann V**, Schilling M. Dilemma of assessing volume state--the use and the limitations of a clinical score. *Nephrol Dial Transplant* 1995; **10**: 2114-2117 [PMID: 8643179]

11 **Connor RJ**. Sample size for testing differences in proportions for the paired-sample design. *Biometrics* 1987; **43**: 207-211 [PMID: 3567305]

12 **Bajaber AA**. Body water distribution and nutrition status of end stage renal disease patients undergoing haemodialysis at Moi teaching and referral hospital. Kenya: Moi University, 2014

13 **Nieman J**, Patten A, Chung ES. A Standardized Method for Assessing Edema. *J Card Fail* 2013; **19**: S86 [DOI: 10.1016/j.cardfail.2013.06.276]

14 **Wabel P**, Moissl U, Chamney P, Jirka T, Machek P, Ponce P, Taborsky P, Tetta C, Velasco N, Vlasak J, Zaluska W, Wizemann V. Towards improved cardiovascular management: the necessity of combining blood pressure and fluid overload. *Nephrol Dial Transplant* 2008; **23**: 2965-2971 [PMID: 18458032 DOI: 10.1093/ndt/gfn228]

15 **Hosmer DW**, Lemeshow S. Multiple Logistic Regression. In: Shewhart WA, Wilks SS. Applied Logistic Regression. 2nd ed. New York: John Wiley & Sons, 2000: 31-46

16 **Dohoo IR**, Martin SW, Stryhn H. Methods in epidemiologic research. 3rd ed. Florida: VER Inc, 2012: 201-222

17 **Passauer J**, Petrov H, Schleser A, Leicht J, Pucalka K. Evaluation of clinical dry weight assessment in haemodialysis patients using bioimpedance spectroscopy: a cross-sectional study. *Nephrol Dial Transplant* 2010; **25**: 545-551 [PMID: 19808949 DOI: 10.1093/ndt/gfp517]

18 **Kugler C**, Vlaminck H, Haverich A, Maes B. Nonadherence with diet and fluid restrictions among adults having hemodialysis. *J Nurs Scholarsh* 2005; **37**: 25-29 [PMID: 15813583 DOI: 10.1111/j.1547-5069.2005.00009.x]

19 **Cheng HT**, Xu X, Lim PS, Hung KY. Worldwide Epidemiology of Diabetes-Related End-Stage Renal Disease, 2000-2015. *Diabetes Care* 2021; **44**: 89-97 [PMID: 33203706 DOI: 10.2337/dc20-1913]

20 **National Kidney Foundation**. KDOQI Clinical Practice Guideline for Hemodialysis Adequacy: 2015 update. *Am J Kidney Dis* 2015; **66**: 884-930 [PMID: 26498416 DOI: 10.1053/j.ajkd.2015.07.015]

21 **Hassan MO**, Duarte R, Dix-Peek T, Vachiat A, Dickens C, Grinter S, Naidoo S, Manga P, Naicker S. Volume overload and its risk factors in South African chronic kidney disease patients: an appraisal of bioimpedance spectroscopy and inferior vena cava measurements. *Clin Nephrol* 2016; **86**: 27-34 [PMID: 27285312 DOI: 10.5414/CN108778]

22 **Wabel P**, Chamney P, Moissl U, Jirka T. Importance of whole-body bioimpedance spectroscopy for the management of fluid balance. *Blood Purif* 2009; **27**: 75-80 [PMID: 19169022 DOI: 10.1159/000167013]

23 **Kalainy S**, Reid R, Jindal K, Pannu N, Braam B. Fluid volume expansion and depletion in hemodialysis patients lack association with clinical parameters. *Can J Kidney Health Dis* 2015; **2**: 54 [PMID: 26702360 DOI: 10.1186/s40697-015-0090-5]

24 **Vasko R**, Müller GA, Ratliff BB, Jung K, Gauczinski S, Koziolek MJ. Clinical judgment is the most important element in overhydration assessment of chronic hemodialysis patients. *Clin Exp Nephrol* 2013; **17**: 563-568 [PMID: 23192771 DOI: 10.1007/s10157-012-0745-9]

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